Single-Use Bioreactors for the production of a heterologous peroxidase by *Aspergilli*.

'an alternative for stirred tank reactors?'

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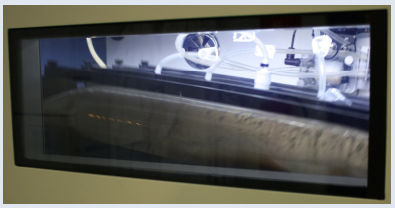
Introduction



The application of single-use equipment becomes more and more common in the biopharmaceutical industry. Compared to the traditional glass or stainless steel stirred tanks reactors (CSTR), the single-use bioreactor offers clear advantages with respect to: turnaround time (no SIP or CIP required), cost reduction, lowered cross contamination (safety), reduced validation requirements.

Due to its two-dimensional movement profile expressed as rpm ("rocks" per minute), the CELL-tainer (see photo) single-use bioreactor creates a superior gas-liquid mass-transfer compared to other single-use bioreactors presently available. This offers the opportunity to reach high cell-densities and therefore high volumetric productivities. With the CELL-tainer k_La values of 300 h^{-1} and above are measured, making the system extremely suitable for microbial, yeast and mycelial cultures.

A comparison is made between the CELL-tainer and the standard stirred bioreactor during the production of *Arthromyces ramosus* peroxidase (ArP) in *Aspergillus niger* (inducible *inu*E-promotor) and in *Aspergillus awamori* (inducible *exl*A-promotor) during fed-batch fermentations.

Results

At low rpm (30) fed-batch cultivation of *Aspergillus* resulted in a pellet morphology. In comparison with a CSTR 50% less total protein was produced and no ArP activity was detected (Table 1). SDS-PAGE of EOF samples showed no presence of ArP in the medium (Figure 1). This marginal ArP activity under these fermentation conditions needs to be investigated further.

	[protein] (mg/l)	[ArP] (ΔA/min)
CELL-tainer	73	3,9
CSTR	126	170

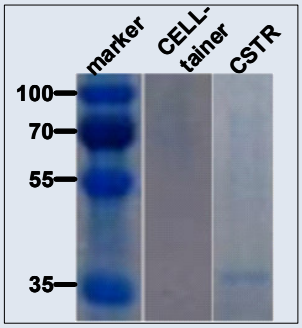


Table 1 / Figure 1: EOF results and SDS-PAGE of ***A. niger***

	[protein] (mg/g)	[ArP] (ΔA/g)
CELL-tainer	40	50
CSTR	113,6	153

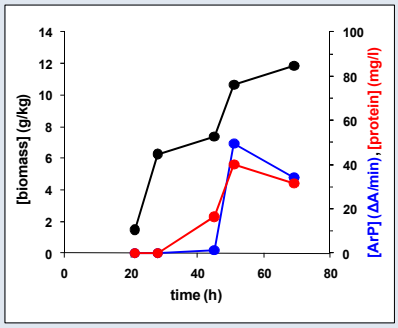


Table 2 / Figure 2: EOF results and biomass and ArP profile of ***A. awamori***.

To Investigate the potential further of the CELL-tainer a fed-batch fermentation with *A. awamori* was performed at maximum rpm (50). With this procedure only filamentous morphology was obtained, causing a viscous medium which tends to stick to the walls of the CELL-tainer. Nevertheless a substantial ArP activity was produced with this strain under oxygen non-limited conditions (see Table 2 / Figure 2).

Conclusion / Future Plans

- Initial experiments showed that the CELL-tainer is a useful type of bioreactor to perform fungal fermentations. Compared to a fully optimised CSTR process approx. 3 times less ArP was produced by equal amounts of biomass with *A. awamori*.
- Variation in rpm generates a mild condition to perform fungal fermentations under different morphology regimes.
- The CELL-tainer was tested under harsh fermentation conditions, but showed good performance in these first experiments. Nevertheless aerobically performed fed-batch fermentations are possible to carry out in the CELL-tainer.
- Although some optimisations on the CELL-tainer have to be carried out, these experiments show that by using the CELL-tainer system, single-use technology is applicable for viscous filamentous fermentations as well.