

THE WAVE BIOREACTOR WITH PERFUSION TO PRODUCE RECOMBINANT SEAP WITH CHO XM111.10 IN PROTEIN-FREE MEDIA

Abstract

The Wave Bioreactor was chosen to propagate the tetracycline responsible, SEAP (= Secreted alkaline phosphatase) producing CHO XM111.10¹⁾ in fedbatch and perfusion cultures. The cells grew in the protein-free, chemically defined medium ChoMaster. The maximal cell density in the fedbatch was $3.4 \cdot 10^6$ cells/mL and in the perfusion system $5.5 \cdot 10^6$ cells/mL. The average SEAP productivity was given by the enzyme activity with $1.07 \mu\text{M}$ Nitrophenol (NP) formed/min-day·mL for the perfusion culture, $0.39 \mu\text{M}$ NP/min-day·mL for the fedbatch.

Culture conditions and reactor configuration

The bioreactor consisted of a 2 liter plastic Cellbag, which was inoculated with 400 mL cell suspension, containing $4.2 \cdot 10^5$ viable cells/mL in ChoMaster HP1 or 5 (Cell Culture Technologies, Zürich) and 0.3% Pluronic-F68. The cells were fed periodically during the first eight days. Until the batch volume of 1 liter was obtained, the continuous cultivation was started. The culture conditions were 37°C , 9% CO_2 and 12 rocks per minute.

In the perfusion system, a cell-free harvest was guaranteed by a dialysis membrane filter. The wave perfusion system was equipped with two timer-controlled pumps: the one for feed addition and waste elimination in a synchronized loop, the other for the membrane-wash steps.

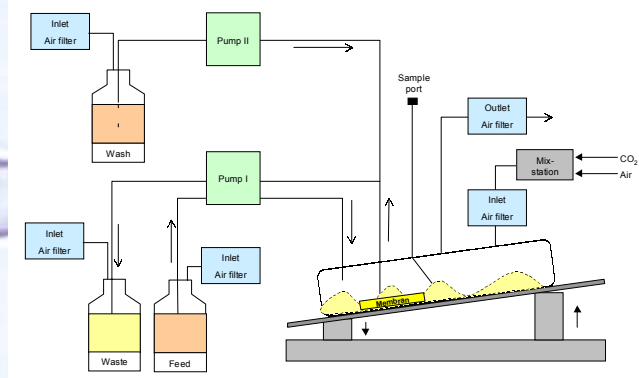


Figure 1: Cultivation of CHO XM111.10 cells in Wave with perfusion.

Results

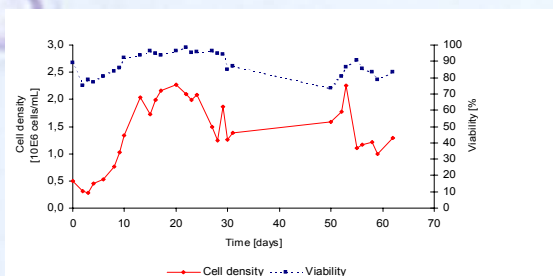


Figure 2: Fedbatch cultivation of CHO XM111.10 with medium exchange during 60 days in Wave in the presence of tetracycline in ChoMaster HP5.

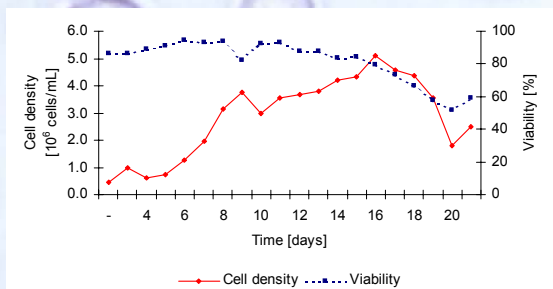


Figure 3: Growth behaviour of SEAP producing CHO XM111.10 in ChoMaster HP5 (without tetracycline) in the Wave perfusion system.

Conclusion

The perfusion system of the Wave reactor is suited to culture genetically engineered CHO cells in protein-free media. The achieved cell density and the product yield were high, compared to the results of the fedbatch cultures in the Wave and Biostat B, respectively.

The CHO XM111.10 were propagated in the tetracycline containing culture medium ChoMaster HP1, until a cell density of $6 \cdot 10^5$ to $8 \cdot 10^5$ cells/mL was achieved. In the absence of tetracycline the expression of SEAP was induced and the production phase initiated. At this time, the regulated medium exchange was started in the fedbatch and in the perfusion culture.

Daily removal of cell suspension in the fedbatch culture and replacing the same volume of fresh culture medium enabled the cells to grow for 60 days at an average cell density of $1.7 \cdot 10^6$ cells/mL in ChoMaster HP5. The cell viability ranged between 85 and 95%.

The perfusion cultivation was performed during 21 days. The feeding was coupled with elimination of the cell-free medium containing the recombinant SEAP. The maximal cell density was $5.5 \cdot 10^6$ cells/mL. The SEAP production with $1.07 \mu\text{M}$ NP/min-day·mL was high in comparison to the fedbatch in Wave and Biostat B (Table 1).

The lactat concentration, accumulated during the first 8 days, was reduced from 2.4 gram per liter to levels between 0.8 and 1.8 gram/L during the exchange mode.

Table 1: Growth parameters of different bioreactor systems

*The yield is given by $\mu\text{Mol NPformed/min}\cdot\text{mL}$.

	Wave		Biostat B
Cultivation mode	Fedbatch	Perfusion	Fedbatch
Maximal cell density (cells/mL)	$3.4 \cdot 10^6$	$5.5 \cdot 10^6$	$2.2 \cdot 10^6$
Cell density average (cells/mL)	$2 \cdot 10^6$	$3.6 \cdot 10^6$	$1.5 \cdot 10^6$
Cultivation time (d)	60	21	24
Av. yield/day*	0.39 ± 0.16 (n=19)	1.07 ± 0.22 (n=14)	0.59 ± 0.50 (n=20)

1) M. Fussenegger et al., Biotech. Bioeng. 55: 927-933, 1997.