USE OF A BIPHASIC PERFUSION PROCESS BASED ON MILD HYPOTHERMIA FOR RECOMBINANT GLUCOCEREBROSIDASE (GBA) PRODUCTION

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The main goal of this study was to develop an innovative CHO-based process for the production of glucocerebrosidase (GBA), an enzyme used for the replacement therapy of Type 1 Gaucher disease. The focus of the present study was on the development of a perfusion process, combining strategies that are commonly used for process optimization: temperature reduction, and supplementation of the culture medium with productivity enhancers, such as short chain fatty acids.
The effects of mild hypothermic conditions combined with valeric acid supplementation were first studied in batch shake flasks for two clones (CHO-GBA-36K and CHO-GBA-65P), developed previously using as host the cell lines CHO.K1 (ATCC CCL-61) and CHO.PRO5 (a glycosylation mutant developed by Stanley et al. Cell 6:121, 1975), respectively. A DOE approach was used (Table 1) to select the most promising cultivation conditions to be further applied to a perfusion process. The best performance regarding both cell growth and GBA production was obtained for the CHO-GBA-65P clone under condition [1], at 31ºC with no valeric acid (Table 1). Under this condition, CHO-GBA-65P achieved a maximum qP of 58.4 mU/10^6 cells/d, which is 4.2 fold higher than qP at the control condition [2] and 2.7 fold higher than the maximum qP obtained for the CHO-GBA-36K clone, which was achieved at 31ºC with 2 mM valeric acid supplementation (condition [3]).

Table 1: DOE for evaluation of the effects of valeric acid supplementation and mild hypothermia.

Condition	Valeric Acid (mM)	Temperature (°C)	q_P (mU/10^6 cells/d)	
			CHO-GBA-36K	CHO-GBA-65P
[1] (n=2)	0	31	3.2 ± 0.2	58.4 ± 4.7
[2] (n=2)	0	37	3.4 ± 1.3	13.8 ± 3.0
[3] (n=2)	2	31	21.9 ± 4.1	14.9 ± 2.7
[4] (n=2)	2	37	3.1 ± 1.7	5.0 ± 0.2
[5] (n=3)	1	34	4.7 ± 1.1	17.7 ± 1.4

Subsequently, a biphasic perfusion process using CHO-GBA-65P was investigated in a stirred tank bioreactor. Perfusion was started on day 3, and a temperature downshift to 31°C was applied on day 7 (Fig 1-A). The application of a perfusion rate of 2 vvd enabled cell densities up to 50x10^6 cells/mL to be achieved. The high cell densities combined with the low residence time of the enzyme product in the bioreactor, avoiding its degradation, were probably related to the high GBA activities achieved (Fig 1-B), which were 9.5-fold higher than the maximum activity achieved in batch culture at 31°C. Perfusion typically results in higher volumetric productivities at lower titers, but for this enzyme product our results show that a biphasic perfusion strategy containing a temperature downshift can significantly enhance also GBA titer in the harvest, which is an advantage for subsequent downstream processing.

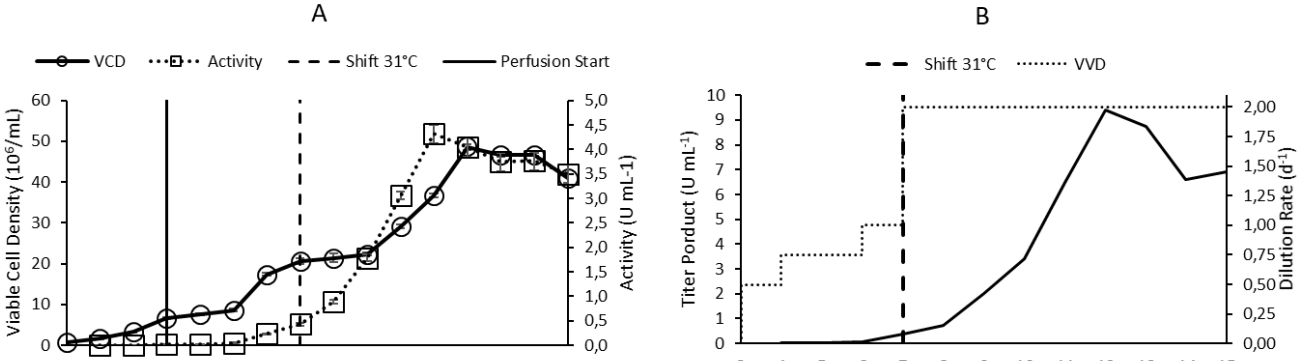

Fig 1: Perfusion process using CHO-GBA-65P clone: (A) Viable Cell Density (VCD) and GBA activity. (B) Product Titer.