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Use of a biphasic perfusion process based on mild hypothermia for recombinant glucocerebrosidase (GBA) production

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Introduction

• Recombinant glucocerebrosidase (GBA): enzyme used for replacement therapy of Type I Gaucher disease, a lysosomal storage disorder
• Exposure of terminal mannose residues is important to increase GBA delivery to pathological macrophages: enzymatic deglycosylation of the commercially available CHO-derived Cerezyme® (Genzyme)
• Previous work at our laboratory (Gutierrez, 2010) developed GBA-producing clones derived from different CHO parental cell lines, including glycosylation mutants
• Focus of the present study: upstream process development based on evaluation of temperature reduction, supplementation of the culture medium with a productivity enhancer and perfusion
  - DOE to investigate mild hypothermic conditions and valeric acid supplementation for two cell lines (CHO-GBA-36K, CHO-GBA-65P)
  - Perfusion operation under the selected conditions, with the aim to maximize process productivity and product quality

Materials and Methods

• Cell Culture
  - CHO-GBA-36K and CHO-GBA-65P were cultivated in TC-LECC (Kell AG, Germany), a customized CD, ADCF medium
  - A 2² DOE was performed in spinner flasks for each cell line (Table 1)

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

<table>
<thead>
<tr>
<th>Runs and replicates</th>
<th>Valeric Acid</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] (n=2)</td>
<td>-1 (0 mM)</td>
<td>-1 (31 °C)</td>
</tr>
<tr>
<td>[2] (n=2)</td>
<td>-1 (0 mM)</td>
<td>+1 (37 °C)</td>
</tr>
<tr>
<td>[3] (n=2)</td>
<td>+1 (2 mM)</td>
<td>-1 (31 °C)</td>
</tr>
<tr>
<td>[4] (n=2)</td>
<td>+1 (2 mM)</td>
<td>+1 (37 °C)</td>
</tr>
<tr>
<td>[5] (n=3)</td>
<td>0 (1 mM)</td>
<td>0 (34 °C)</td>
</tr>
</tbody>
</table>

• Perfusion process
  - Stirred tank bioreactor (RALF, BioEngineering AG)
  - pH 7.1, 150 rpm, 40% air saturation, 37°C with shift to 31°C (d7)
  - CS-10 inclined settler (Biotechnology Solutions)
  - Perfusion start when target viable cell concentration reached
  - Dilution rate in increasing steps: 0.5, 0.75, 1 and 2 vvd

• Analytical
  - Viable cell density (VCD) and cell viability were determined by trypan blue exclusion method using a Neubauer chamber in an optical microscope (Eclipse TS100, Nikon) or using an automatic counter equipment (Vi-Cell XR, Beckman Coulter)
  - GBA enzyme activity in the samples was determined by measuring the release of the fluorescent compound 4-methylumbelliferone (4-MU) upon hydrolysis of the synthetic substrate 4-methyl umbelliferyl-β-D-glucopyranoside (4-MUD, Sigma, M3633). The fluorescent product was quantified with a fluorescence microplate reader (Vıctor III, Perkin Elmer) using excitation and emission filters of 355 nm and 460 nm, respectively
  - Glucose and lactate were measured using a YSI2700 analyser (Yellow Springs Instruments)

Conclusions

○ For both cell clones, a subphysiological temperature of 31°C led to an increase in the specific productivity (qP), but the effect of valeric acid supplementation was clone dependent.
○ The best overall performance regarding both cell growth and productivity was obtained for the CHO-GBA-65P cell clone at 31°C without valeric acid.
○ The maximum product titer achieved in perfusion was 9.5-fold higher compared to batch at 31°C and 22-fold higher than the control batch (at 37°C).
○ Perfusion typically results in higher volumetric productivities but lower titers. However, for this enzyme, our results show that a biphasic perfusion strategy including a temperature downshift can significantly enhance also GBA titer in the harvest, which is an advantage for subsequent downstream processing.

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