

Growth of r-CHO in 1, 4 and 10 liter Fermenters

Objective

No data have previously been reported concerning the use of porous microcarriers in a long term serum-free production of a recombinant protein by mammalian cells. This application note describes the cultivation of r-CHO cells in 1, 4 & 10 liter fermenters.

Culture Conditions

Vessels: 1,4 & 10 liter (SGI).

Cell line: r-CHO cells secreting human von Willebrand factor (Transgene S.A.).

Microcarrier: CultiSpher-G 4 g/l, prewashed and sterilized according to instructions.

Oxygen supply: Pure oxygen through 2 m silicone tubing/l culture volume.

Agitation speed: Stirring speed was maintained at 30-40 RPM initially and then increased to 60 RPM.

Medium: Iscove/Ham F12 (50/50) supplemented with 6 mg/l of bovine insulin. Initially (day 1-4) cells were grown in MEM α 2000 supplemented with 10% dialysed FBS. All the media contained 100 units/ml of penicillin G and 100 μ g/ml of streptomycin. pH was maintained at 7.20 \pm 0.20 by supplying air and CO₂ in the head space of the fermenter.

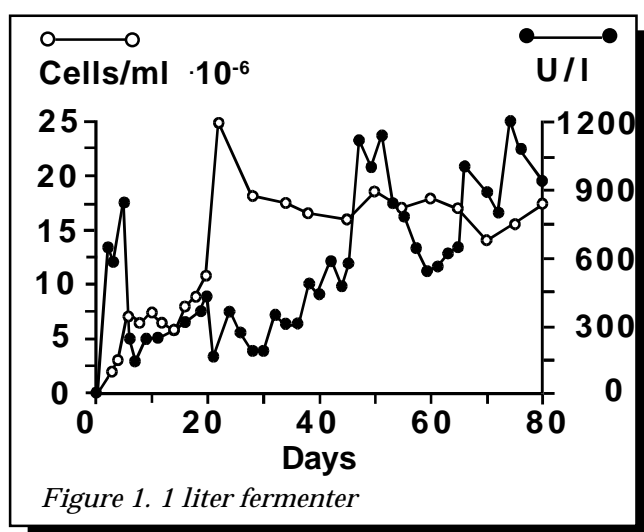


Figure 1. 1 liter fermenter

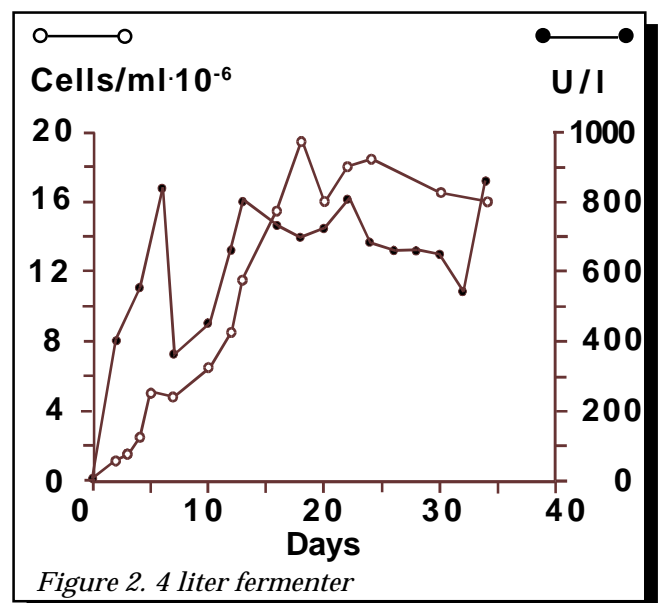


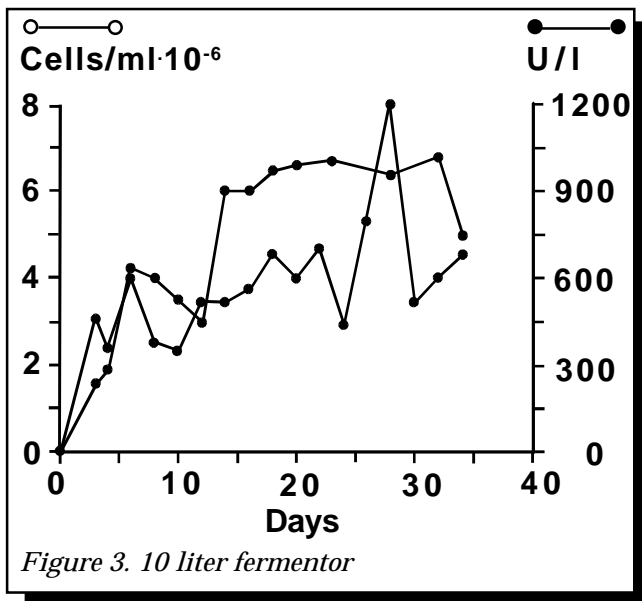
Figure 2. 4 liter fermenter

Inoculum: All fermenters were seeded with $3.0 \cdot 10^5$ cells/ml. The 1 and 4 liter fermenters were seeded with cells from 175 cm² flasks and the 10 liter fermenter with cells amplified in a 1 liter fermenter with CultiSpher-G (Application note 101).

Results

More than 90 % of the cells attached to the beads within the first 24 hours. Cell densities remained between $13.8 - 18.4 \cdot 10^6$ cells/ml for a period of 80 days in the 1 liter fermenter. These cells were utilized as inoculum for the 10 liter fermenter. More than 90 % of the cells were recovered from the beads by trypsinization. Cell densities in the 10 liter fermenter were $6-7 \cdot 10^6$ cells/ml during 35 days. Although a lower cell density, the volumetric productivity of von Willebrand factor was similar in both fermenters (365 and 297 U/l/day for 1 & 10 liter respectively). The 4 liter fermenter gave similar yields as the 1 liter; $16-18 \cdot 10^6$ cells/ml during 35 days and a recombinant protein production of 339 U/l/day.

Growth of r-CHO in 1, 4 and 10 liters Fermenters



Discussion

Recombinant CHO cells were grown and maintained for long-term culture exceeding two months in CultiSpher-G. Three different fermenters were utilized and a quasi-linear scale-up was easily performed from 1 liter to 10 liter fermentors. During the serum-free production phase, cells were protected from shear forces by the sponge-like bead structure and the stirring rate could be increased from 40 to 60 RPM which decreased bead aggregation.

Reference

Mignot, G. et al. (1990) "Production of recombinant Von Willebrand factor by CHO cells cultured in macroporous microcarriers". *Cytotechnology* 4, 163-171