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BIOLYTICA

Large Scale Harvesting of Cells

Objective

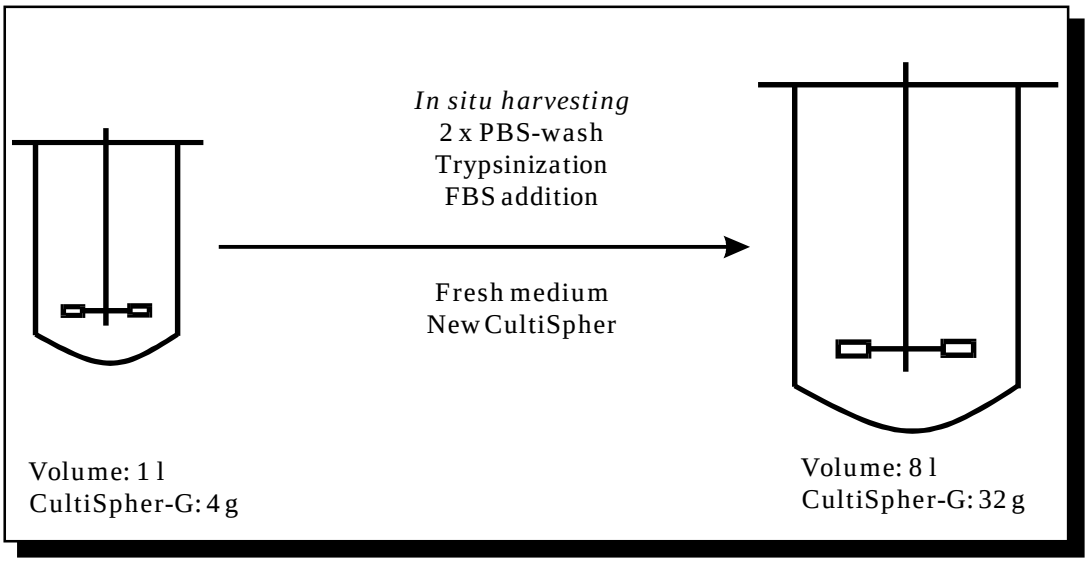
The recovery of cells from CultiSpher-G cultivations is routinely performed when monitoring cell growth and harvesting from small spinner cultures. This application note describes the technique used for large-scale harvesting of cells.

Culture Conditions

Vessel: 1 liter and 10 liter fermenters (SGI)
Cell line: r-CHO (Transgene S.A.)
Medium: MEM α2000 supplemented with 10% dialysed FBS ,30 nM Methotrexate, 100 U/ml penicillin G and 100 µg/ml streptomycin. pH was maintained at 7.2.
Microcarriers: CultiSpher-G 4 g/l prepared according to instructions.
Agitation speed: 30-40 RPM
Oxygen supply: Bubble free through 2 m silicone tubing/l culture volume with pure oxygen. Dissolved oxygen was maintained at 50% of air saturation.

Harvesting

Cells were detached in situ with 0.05% Trypsin in PBS(100 ml) containing Ca^{2+}, Mg^{2+}, after 2 washes of the confluent beads with Ca^{2+}, Mg^{2+} free PBS (750 ml/ 4 g dry weight of beads). After 15 minutes at 37 °C with stirring at 30 RPM, residual trypsin was inhibited by adding dialysed FBS to a concentration of 10% (v/v) before diluting the cells in fresh medium containing new beads (4 g/L). Cell counts were performed with a Malassez chamber using Trypan Blue exclusion method.



Discussion

More than 90% of the cells were recovered from the beads by trypsinization. Part of the yield of $16.5 \cdot 10^9$ cells was used for inoculation of the 10 liter fermenter.
The cells recovered from the 1 liter fermenter would have been enough to inoculate a 50 liter fermentor (if $0.3 \cdot 10^6$ cells/ml are used as inoculum), a potential scale-up of 50 times.

Reference

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

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