Percell
BIOLYTICA

Serial Cultivation of CHO-K1 Cells

Objective

A high recovery of the cultured cells and a large growth span is a perequisite for a successful scale-up of a production process based on animal cell technology. CultiSpher's enzymatically degradable matrix together with its large cell growth potential makes it an ideal microcarrier for large scale cell culture.

Culture conditions

Vessels: 50 ml spinners(Techne).
Microcarrier: 2 g/l CultiSpher-G prepared according to instructions.
Cell line: CHO-K1(PHLS).
Agitation speed: 45 RPM.
Media: DME supplemented with 10% FBS, penicillin G(100 U/ml) and streptomycin(100 µg/ml). pH was controlled through CO_2 atmosphere. Media volume was varied according to the following scheme; day 0: 30 ml, day 1-3: 50 ml and day 4-6: 60 ml.
Harvesting: Cells were grown for 6 days(1st). The collected beads were washed twice with PBS-EDTA(0.25 mM EDTA, 50 ml/g dry weight of beads). The matrix was dissolved by trypsin(5 mg/ml in PBS containing Ca^{2+} and Mg^{2+}, pH 8.0, 30 ml/g dry weight of beads). CultiSpher-G was complete dissolved after 30 minutes treatment at 37°C. Residual trypsin activity was inhibited by addition of serum containing media. A part of this recovered cell population was used for innoculation on new beads(2nd).

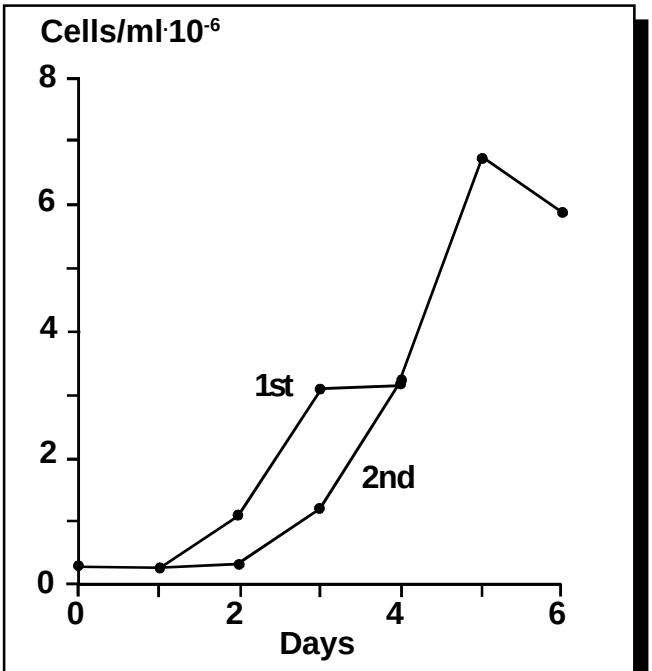

Figure: Growth of original cells(1st) and harvested(2nd) cell population

Results:

After 6 days of growth a cell concentration of almost $7 \cdot 10^6$ cells/ml. Harvesting with trypsin resulted in $370 \cdot 10^6$ cells with a 98% viability (Trypan Blue exclusion). A small part of this population, $10 \cdot 10^6$ cells was used for innoculation on new beads. When compared to the original growth characteristics, this population showed a slightly prolonged (1 day) lag phase.
Growth rate at exponential growth were identical.

Discussion

CultiSpher-G's gelatin matrix may be dissolved by different enzymes. Certain enzymes, like Dispase(Boehringer-Mannheim) act very specific on gelatin and collagen. The harvested cells will be practically uneffected after treatment. However it may be very difficult to obtain a single cell suspensions. This is especially true for cell lines that are "sticky" like Vero and MDCK. Trypsin on the other hand will dissolves the matrix and also act on cell surface proteins. We therefore recommend an optimized treatment with trypsin when the cells are to be used in a scale-up procedure.
This optimized treatment starts with a washing with PBS-EDTA for removal of divalent ions. Trypsin is dissolved in PBS containing Ca^{2+} and Mg^{2+} as these ions are required for maximum activity. pH is also slightly higher(8.0) than normal culture pH to be close to trypsin's pH optimum.
Cell yields using this optimized procedure give results that are indistinguishable from routine counting procedures.The recovered cell population showed only a slightly prolonged lag phase and growth in exponential phase was equal to the original cell population.
The number of cells that can be recovered from 1 g of CultiSpher in this experiment was $3.7 \cdot 10^9$ cells. This will be enough for inoculation of a 40 liter fermenter at a cell concentration of 100 000 cells/ml.

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