

Optimization of Microcarrier Concentration

Objective

In order to find the optimum CultiSpher concentration in a given system all other parameters, like oxygen and nutrient supply have to be supplied in excess. When this is done the only factor that influences maximal cell yield is the available surface area of CultiSpher.

Culture conditions

Vessel: 50 ml spinner(Techno).

Microcarrier: CultiSpher-G(at indicated concentrations) prepared according to instructions.

Microcarrier concentration: The culture was started at a microcarrier concentration of 1 g/l. When cell growth ceased 50% of the culture (containing microcarriers) was removed and the same volume of new media was added. The procedure was repeated until no further increase in cell growth was obtained.

Cell line: CHO-K1(PHLS).

Agitation speed: 45 RPM.

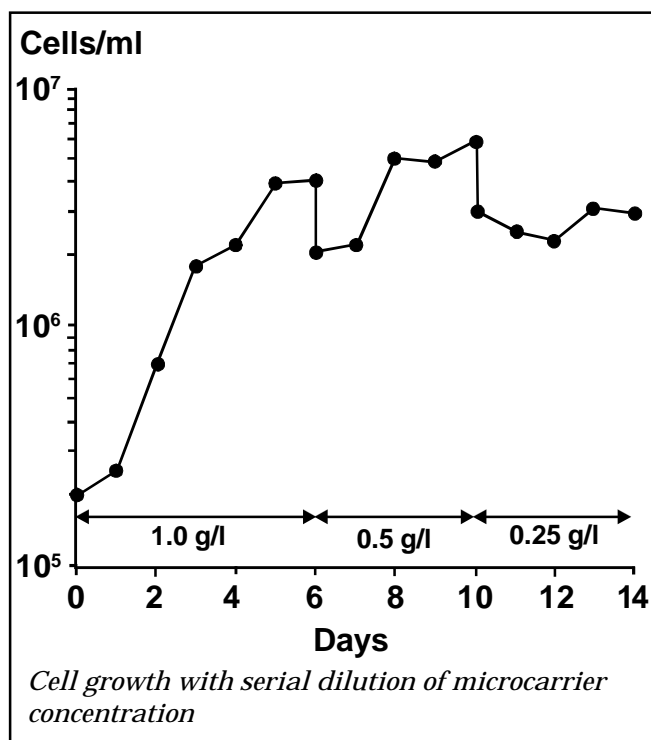
Media: DMEM supplemented with 10% FBS, penicillin(100 U/ml) and streptomycin(100 µg/ml). 80% of the medium was exchanged daily starting from day 3. pH was controlled by CO₂ atmosphere(5%).

Results

The serial dilution resulted in a maximal cell concentration of $6 \cdot 10^6$ cells/ml at a microcarrier concentration of 0.5 g/l. When the culture was diluted further, a concentration of $3.4 \cdot 10^6$ cells/ml was obtained. At this stage the available surface area is the limiting factor. Maximum number of CHO-K1 cells that can be grown on CultiSpher-G can thus be calculated to $136 \cdot 10^8$ cells/g dry weight. As each gram of CultiSpher-G contains $4 \cdot 10^6$ microcarriers, the maximum number of cells on each microcarrier can be calculated to 3,500.

CultiSpher(g/l)	Cells/microcarrier
1.0	1,000
0.5	3,000
0.25	3,500

Number of cells on each microcarrier as a function of CultiSpher concentration



Discussion

Cells can rarely be grown to higher densities than $10 \cdot 10^6$ cells/ml without specially designed systems for oxygenation and nutrient supply. Based on these results CultiSpher-G should not be used at higher concentration than 0.8 g/l in such systems. We also recommend to supply surface area in slight excess, as the cells often grow out from the microcarriers when the surface area is limiting. We also recommend to perform an experiment according to this application note to determine the characteristics of your system.