

Effect of Inoculum Size on Growth Span

Objective

The growth span (ratio between final cell density and inoculum density) indicates size of the scale-up steps. Solid microcarriers, with their restricted surface area, have a theoretical growth span of 16. Maximum scale-up step will thus be 16 times, for instance from a 1 liter fermenter to a 16 liter fermenter. In practice this figure is often reduced to 1/4 or 1/8 of the theoretical value. How much will it be possible to increase the growth span by using CultiSpher?

Culture Conditions

Vessels: 50 ml spinners (Techne).

Microcarrier: 2 g/l CultiSpher-G prepared according to instructions.

Cell line: CHO-K1 (PHLS).

Cell concentrations: Cultures were inoculated at 0.1, 0.25 or 1.0 · 10⁸ cells/g dry weight of CultiSpher-G.

Agitation speed: 45 RPM.

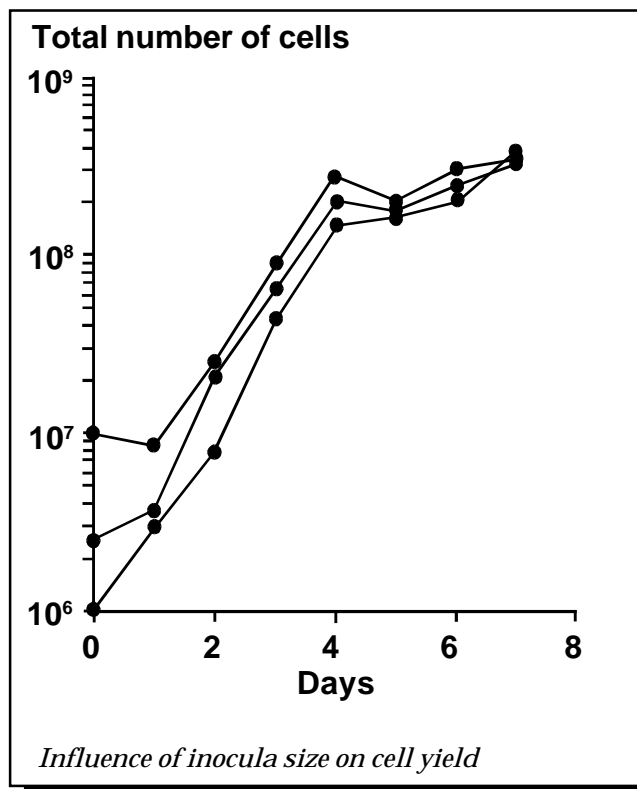
Media: DME supplemented with 10% FBS, penicillin G (100 U/ml) and streptomycin (100 µg/ml). pH was controlled through CO₂ atmosphere. Media volume was varied according to the following scheme; day 0: 30 ml, day 1-3: 50 ml and day 4-7: 60 ml.

Results

Despite a 10-fold difference in initial inoculum size, nearly identical final cell yields were obtained for the different cultures. Final cell yields were in the range of 35-40 · 10⁷ cells. This corresponds to a cell concentration of 6 · 10⁶ cells/ml. No lag phase was seen at the lowest inoculum which correspond to a cell concentration of 33,000 cells/ml.

Inocula Cells/Bead	Growth Span
2.5	390
12.5	132
25	24

Growth span as a function of inoculum size



Discussion

When reducing the inoculum size, two parameters are of utmost importance: number of cells/microcarrier and cell concentration. If the number of cells inoculated per microcarrier is below a critical value, normally 5-10, a portion of the microcarriers will be without cells throughout the culture.

If the cell concentration is below a certain critical value, pronounced lag phases and massive cell death will occur. This value varies for different cell lines and has to be determined empirically.

When CultiSpher-G is used at 2 g/l a large excess of surface area is provided for the cells. As a consequence of this maximum number of cells on each microcarrier were calculated to 1,000 (compare Application Note 108). The largest growth span obtained was 390 times. This indicates the possibility of performing scale-up in steps of almost 400 times. This will greatly reduce the problems previously encountered with microcarrier culture in large scale.