

Oxygen Limitations in Small Spinner Vessels

Objective

It has been shown previously that high cell densities can be achieved in spinner vessels when cultivating CHO-K1 cells in the porous matrix of CultiSpher. Normal cell concentrations are in the range of $3\text{-}5 \cdot 10^6$ cells/ml in a working volume of 50-60 ml. How will an increase in the volume of the spinner flask affect final cell concentrations?

Culture conditions

Vessels: 50 ml spinner (Techne), 250 ml spinner (Bellco) and 500 ml spinner (Techne)

Microcarrier: 1 g/L CultiSpher-G prepared according to instructions.

Cell line: CHO-K1 (PHLS).

Agitation speed: 45 rpm

Media: DME supplemented with 10 % FBS, Penicillin(50 U/ml)/Streptomycin(50 µg/ml). pH was controlled through CO₂ atmosphere(5%)

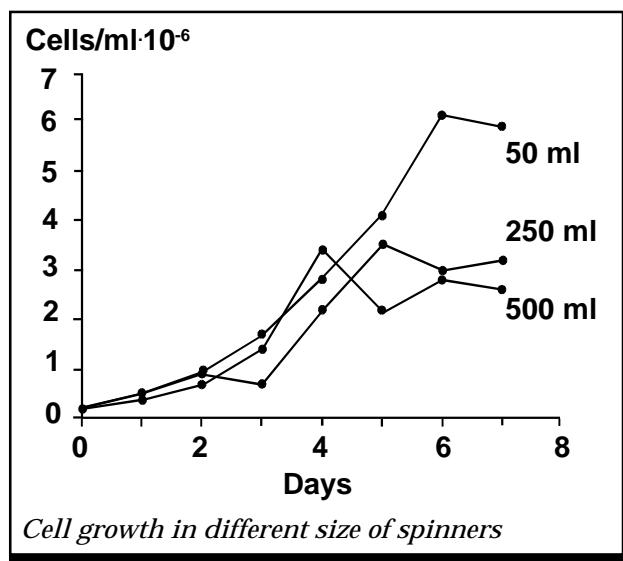
Harvesting and Transfer conditions: The media was reduced and the confluent carriers were prewashed twice for 5 min with 100 ml PBS-EDTA/g dry weight of beads. Beads were dissolved using 0.05 % Trypsin in PBS containing Ca²⁺ and Mg²⁺ for 15-30 min at 37 °C. The cells were collected by centrifugation and resuspended with fresh media supplemented with serum in order to inhibit residual trypsin.

Results

Due to a reduction in the liquid surface/volume (S/V) relationship the concentration of dissolved oxygen is decreased and lower cell densities are obtained in the larger vessels. 70-90% of the media was replaced daily, starting day 3-4 to eliminate nutrient depletion.

Vessel(ml)	S/V	Cells/ml
50	0.56	$6.0 \cdot 10^6$
250	0.15	$3.1 \cdot 10^6$
500	0.13	$2.6 \cdot 10^6$

Relation between spinner size and final cell yield



Discussion

As the volume of the culture is increased, the surface for active oxygen uptake is decreased compared to the culture volume. Lower cell densities are therefore obtained. Still, $2.5\text{-}3.5 \cdot 10^6$ cells/ml can be considered to be satisfactory values in a 500 ml spinner vessel without any specific oxygen supply system. In order to improve the yields the dissolved oxygen concentration must be increased. Performance of CultiSpher must be evaluated under non-limiting conditions. Especially important is to supply the culture with an excess of oxygen. This condition can be obtained either by using CultiSpher at 1 g/l in 50 ml spinners or reducing CultiSpher concentration to 0.5 g/l in 250 ml spinner.