

# Influence of Dissolved Oxygen Concentration on Cell Growth

## Objective

The importance of dissolved oxygen on the cell yield in a given culture system is shown by the following experiments:

- Decreasing the liquid surface/volume(S/V) relationship when stationary growth phase is reached
- Active oxygen supply (in the form of air) through a membrane in the culture vessel.

## Culture Conditions

**Vessel:** 1000 ml spinner vessel (Bellco)

**Oxygen supply:** Air was supplied through a membrane loop(Biott) by a peristaltic pump(type 550 Watson-Marlow). Pump flow rates: day 1-4 (105 ml/min), day 4-6 (162 ml/min) and day 6-9 (225 ml/min).

**Microcarriers:** CultiSpher-G 1 g/l, prepared according to instructions.

**Cell line:** CHO-K1 (PHLS)

**Agitation speed:** 45 RPM

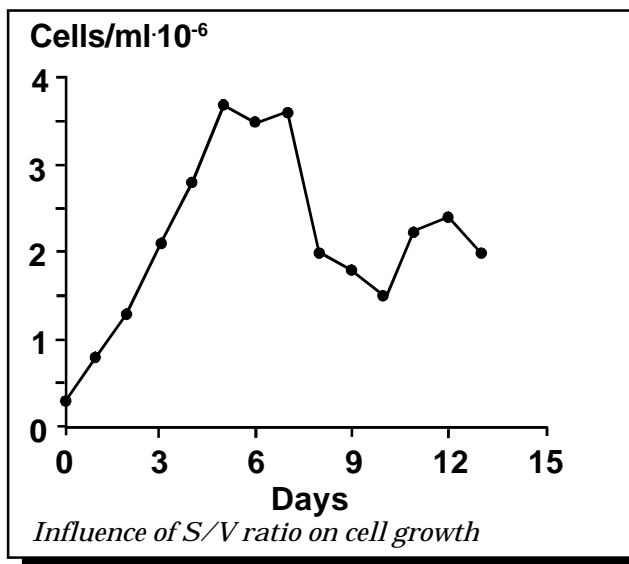
**Media:** DME supplemented with 10 % FBS, penicillin G(50 U/ml) and streptomycin(50 µg/ml). pH was controlled through CO<sub>2</sub> atmosphere(5%). 90-95 % of the media, starting from day 3, was exchanged every day.

## Results

### Increase of S/V

Oxygen was only supplied to the culture through surface aeration.

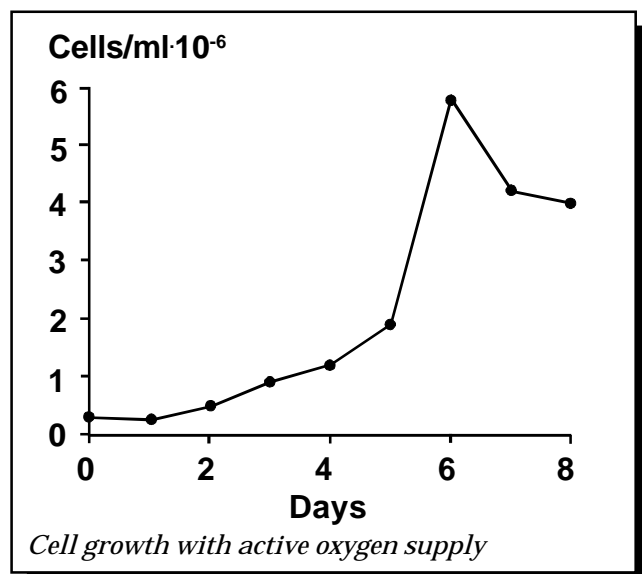
Frequent media changes and a microcarrier concentration of 1 g/l eliminates all limiting factors except dissolved oxygen. When stationary growth phase is obtained, the S/V is decreased from 0.23 to 0.11 cm<sup>-1</sup>, by addition of 500 ml new medium. The cell concentration decreased from 3.5·10<sup>6</sup> to 2.0·10<sup>6</sup> cells/ml.



### Active oxygen supply

Active supply of oxygen to the culture medium through a porous loop immersed in the liquid is superior to passive supply.

The cell concentration increased 2-fold from 3.5 to 6.0·10<sup>6</sup> cells/ml. The decrease in cell concentration observed at day 7 is most likely due to lack of pH control. Buffering capacity of the bicarbonate system is not enough to neutralize lactic acid produced by 6·10<sup>6</sup> cells/ml.



## Discussion

A given liquid surface area can only adsorb a limited amount of oxygen at a specific agitation rate. Concentration of dissolved oxygen will determine the maximal cell concentration. The system must be optimized after this limiting factor in terms of microcarrier concentration, media volume, media exchange, etc.

When the culture volume in a given vessel was increased 2-fold, the cell yield/ml decreased 1.5-fold. This is due to the reduction in dissolved oxygen concentration.

The cell yield increased 2-fold when oxygen was supplied through a porous membrane loop.