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BIOLYTICA

Critical Parameters for the Cultivation of r-CHO Cells

Objective

The growth of CHO cells and its expression of recombinant protein is influenced of the concentration of Disolved Oxygen(D.O.) Concentrated solutions of important factors and nutrients added to supplement the medium does not enhance recombinant protein production.

Culture conditions

Vessel: 1 liter fermentor (SGI)
Cell line: CHO expressing von Willebrand factor (Transgene)
Microcarrier: CultiSpher-G 4 g/L prepared according to instructions.
Medium: day 1-4, MEM α2000 supplemented with 10% dialysed FBS and 30 nM methotrexate, day 4-80 Iscove/Hams F12 (50/50) supplemented with 6 mg/L of bovine insulin. Serum-free medium was changed daily or every other day. All media was supplemented with 100 units/ml of penicillin G and 100 µg/ml of streptomycin. pH was maintained 7.20±0.20 by supplying air and CO_2 in the fermentor headspace.
Inoculum: Microcarriers were seeded with $3.0 \cdot 10^5$ cells/ml.
Oxygen supply: Efficient bubble free oxygenation was performed through 2 m silicone tubing/l with pure oxygen. Oxygen was regulated at 20% of air saturation from day 1 to 30. On day 30 the D.O. was increased to 50% of air saturation.
Agitation: 30-40 rpm.

Results

The mean volumetric von Willebrand production during the initial period when D.O. was 20% of air saturation, was 145 ±39 units/l/day. Increasing the D.O. to 50% of air saturation improved production up to 365 ±140 units/L/day (2.5-fold). The higher specific productivity was maintained from day 50 to day 80 ($3\text{-}4 \cdot 10^{-2}$ units/ 10^6 cells/day) while cell densities remained between 13.8 and $18.4 \cdot 10^6$ cells/ml (eqivalent to $2.5\text{-}3 \cdot 10^8$ cells/ml beads). A further increase of D.O. up to 75% did not improve von Willebrand production (data not shown).
In order to increase specific recombinant von Willbrand production, concentrated solutions of glucose, glutamine, essential amino-acids and bovine insulin were added 24 hours after medium change to supplement the medium. None of these supplements, alone or in combination, had a significant effect on von Willebrand production (data not shown). The expression of this recombinant protein was not limited by a nutritonal deficiency.

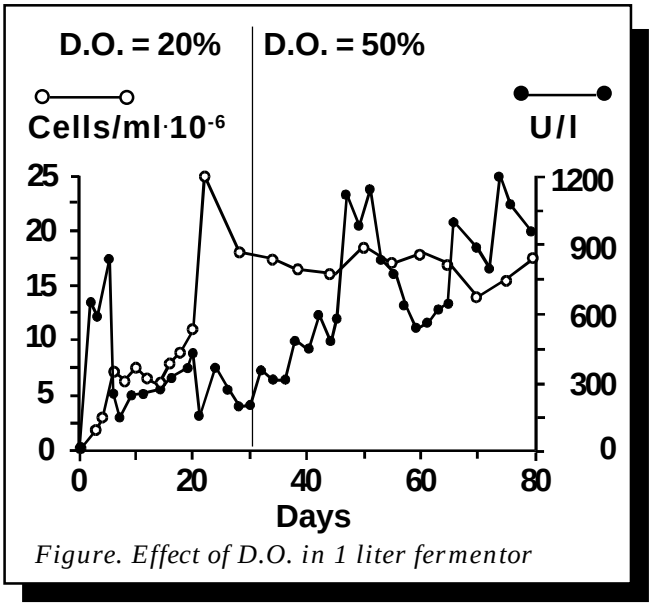

Figure. Effect of D.O. in 1 liter fermentor

Discussion

When cells are cultivated in macroporous microcarriers, oxygen tension is a critical parameter. The optimum value in these experiments was about 50% of air saturation.

If the oxygen concentration around the beads is 50% of air saturation, at the center of a bead, oxygen concentration is about 2.0-2.5 µg/ml. This is based on oxygen profiles within spheres and the characteristics of the above described system (max. cell density $3.3 \cdot 10^8$ cells/ml beads, O_2 uptake rate of 3.0 ng $O_2/10^6$ cells/sec., bead mean diameter of 220 µm). But, if the O_2 concentration around the beads is 20% of air saturation at the center it is about 0.5 µg/ml (i.e. less than 10% of saturation by air) which is sub-optimal for most cell lines in monolayer cultures.

The specific recombinant protein production rate was not improved by adding supplements alone or in combination.

Reference

Mignot, G. et al. (1990) "Production of recombinant Von Willebrand factor by CHO cells cultured in macroporous microcarriers". Cytotechnology 4, 163-171

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