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Production of recombinant human growth hormone

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1. Introduction

The production of recombinant proteins for clinical use often requires mammalian cell cultures in order to ensure of the correct folding and glycosilation of the desired protein. Due to long and expensive cultivation processes it is necessary to optimise the process as well as the productivity of the host cells. This can be achieved by the choice of bioreactor or by the optimisation of the cultivation parameters like stirring speed, temperature etc.
The aim of the study was to examine growth and productivity of the recombinant CHO^{SFS} hGH cells in different cell culture systems which shall result in higher cell densities and product concentration. Furthermore the influence of temperature on cell growth and product formation was examined.

2. Material and Methods

Cell line:
CHO^{SFS} hGH: Chinese hamster ovary

Product:
Cells were transfected to produce hGH (human growth hormone)

Medium:
ProCHO4-CDM (BioWhittaker, USA), serum free and low protein concentration supplemented with 4 mM L-Glutamin.

Parameters beeing observed
- cell count was carried out with the trypan blue method
- cell densities in BelloCell 500 were calculated from glucose uptake rates
- the amount of hGH was measured with a specific sandwich ELISA

A. Cultivation in different culture systems

In the first part several cultivation systems were tested towards their suitability for batch-cultivation. All cultures were grown at 37 °C and 5 % CO_2. While cells are agitated by stirrers in spinner flasks and Biostat B, the whole medium is revolved in BelloCell 500, RCCS-D and miniPerm. Only the BelloCell 500 retains the CHO cells in a matrix.

B. Temperature experiments

Furthermore the influence of temperature on growth and productivity was determined. The experiment was carried out in 250 ml spinner flask. The suspension batch cultures were grown at 37 °C, 34 °C and 31 °C, in an incubator at 5 % CO_2 and 20 rpm.

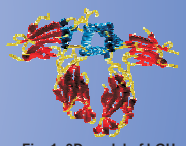

Fig. 1: 3D model of hGH

3. Used cultivation systems

Spinner flask

miniPERM

BelloCell 500

Biostat B

RCCS-D

Spinner flask (Techne Coorperation, England): 50 ml medium, 20 rpm
miniPerm (Vivascience, Germany): 35 ml medium, 30 rpm
RCCS-D (Synthecon, USA): 45 ml medium, 8 rpm
Bioststst B (Sartorius, BBI Systems, Germany): 1.5 l medium, 100 rpm
BelloCell 500 (CESCO Bioengineering Co, Taiwan): 300 ml medium, up/down 1mm/s, top/bottom delay 10 s

4. Results A.

Effect of cultivation system on cell growth

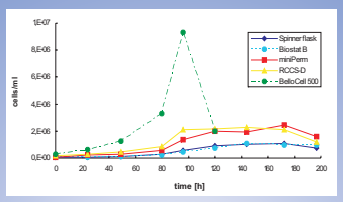

Fig. 2: Number of living cells during the cultivation at 37 °C and at an atmosphere of 5 % CO_2 in differend cultivation systems.

Effect of cultivation system on hGH production

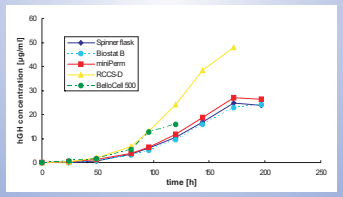

Fig. 3: hGH concentation during the cultivation in different cultivation systems.

The results show an increase of living cells in the BelloCell 500. The highest hGH concentration is achieved in the RCCS-D. A maximum concentration of hGH of 49.0 µg/ml is achieved.

4. Results B.

Effect of temperature on cell growth

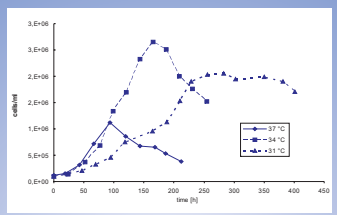

Fig. 4: Number of living cells during the cultivation in spinner flasks at 37 °C, 34 °C and 31 °C at an atmosphere of 5 % CO_2 and 20 rpm.

Effect of temperature on hGH production

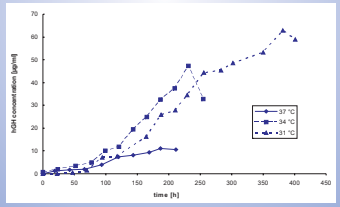

Fig. 5: hGH concentation during the cultivation in spinner flasks at 37 °C, 34 °C and 31 °C measured with a sandwich ELISA.

The results show a significant increase in cell growth at a temperature of 34 °C. The maximum of hGH concentration is achieved at a cultivation temperature of 31 °C. A maximum concentration of hGH of 62.8 µg/ml is achieved.

5. Summary

Both experiments show that cell growth and productivity is influenced by the cultivation system as well as the temperature. Compared to traditional cultivation systems like spinner flask an Biostat B the highest cell number is achieved in the BelloCell 500 but the maximum of hGH amount is achieved in the RCCS-D. The reduction of cultivation temperature in CHO batch cultures from 37 °C to 34 °C and 31 °C has a positive influence of growth and productivity.

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