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# Batch cultivation of CHO-K1 cells in Labfors 5 Cell

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# 1. Introduction

Laboratory-scale bioreactors are widely applied for the cultivation of animal and human cells, e.g. for the production of small amounts of cells and products, in process optimization or as an intermediate step in the seed train. In the following the successful batch cultivation of a CHO-K1 (Chinese hamster ovary) cell line adapted for growth in suspension in the lab-scale bioreactor Labfors Cell 5 (INFORS HT, CH-Bottmingen) is discussed.

The CHO cell line is widely used in academia and industry as an expression system for pharmaceuticals. The clone used here was kindly provided by Prof. Dr. Thomas Noll, University of Bielefeld, Germany.

# 2. Technical specification

- Culture vessel: 3.6 L total volume with round bottom
- Magnetic stirrer (30–300 rpm)
- Pitched blade impeller
- pO<sub>2</sub> control via aeration with 3-gasmix air, O<sub>2</sub> and N<sub>2</sub>
- pH control via CO<sub>2</sub>
- Air cooler as standard
- Temperature control via heating mat on the outer vessel wall
- $\bullet$  Sparger: ring sparger with  $\mu$  pores at the bottom
- Aseptic sampling
- Control of antifoam and level optional
- Determination of online parameters via Iris Control Software

# 3. Methods

## a) Medium

CHO-K1 cells were cultivated in the serum- and protein- free CHOMACS CD medium (Miltenyi Biotec GmbH, Germany). Furthermore, 4 mmol/L L-glutamine (PAA Laboratories, Germany) were added to the medium.

#### b) Parameter setting

The cultivation vessel of the Labfors 5 Cell was set up for cell culture, pH and pO<sub>2</sub> probes were calibrated. The working volume was 2 L, temperature was controlled at 36.9°C. Stirrer speed was set to 156 rpm during cultivation, corresponding to a mixing time of approx.  $\Theta_{95} = 5$  s (determined by colour change method – solution of starch/iodine-potassium/ sodium thiosulfate) and a stirrer tip speed of 0.7 m/s. These values are within the process window determined by Platas et al. (2012) for lab-scale bioreactors.

The pO<sub>2</sub> of 30% air saturation was controlled by a 3-gasmix of air, oxygen and nitrogen by direct sparging up with an aeration rate up to 100 mL/min (0.05 vvm). pH was controlled at 7.15 via sparging with CO<sub>2</sub> or addition of 0.5 M NaCO<sub>3</sub> via peristaltic pumps.

#### c) Batch cultivation

Precultures were performed in shake flask. The initial concentration of viable cells after inoculation was set to  $3 \times 10^5$  cells/mL. The cultivation lasted 6 days and was judged with respect to cell growth, substrate uptake and metabolite production by means of aseptically taken samples.



Fig. 1: Labfors 5 Cell bioreactor

#### d) Analytics

The cell concentration was determined by a haemocytometer (Neubauer Improved, Blue Brand, Deutschland), viability by the trypan blue exclusion method. Glucose, lactate and glutamine were analysed by means of a YSI 7100 MBA (Yellow Springs Instruments, USA), ammonia by a spectrophotometer (Bio-Rad, München, Germany) and the Spectroquant kit 1.14752.0001 (Merck, Darmstadt, Germany) at a wave length of 690 nm.

## 4. Results

The batch cultivation of CHO-K1 cells was successfully run for 6 days. Time course of viable and total cell concentration is shown in fig. 2.



Fig. 2: Time course of viable and total cell density

The maximal viable cell concentration was 1.27 x  $10^7$  cells/mL at day 5. This corresponds to a multiplication factor of 36 (ratio of maximal to initial cell density). During exponential growth (up to day 3) the average viability was 98%. During this phase the growth rate  $\mu$  was 0.049/h, the doubling time 14.1 h (fig. 3).



Fig. 3: Determination of maximal growth rate  $\mu_{\text{max}}$  and doubling time  $t_{\text{d}}$ 

The time course of the main substrates (glucose and glutamine) as well as the main metabolites (lactate and ammonia) are shown in the following figures. Glucose was totally consumed at the end of the cultivation. At low glucose concentrations (from day 3 on) cells started to consume lactate. Glutamine was consumed totally after 3 days. This corresponds with the end of exponential growth. Lactate and ammonia did not reach inhibiting concentrations.



Fig. 4: Time course of glucose and lactate concentration



Fig. 5: Time course of glutamine and ammonia concentration

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### 5. Summary

- A batch culture of suspendable CHO-K1 cells was successfully carried out over a period of 6 days. The growth behavior of CHO-K1 cells was comparable to previous experiments in different cultivation systems.
- The maximal cell concentration was 1.27 x 10<sup>7</sup> cells/mL at day 5.
- During exponential growth the mean viability was 98%.
- Growth rate and doubling time were as expected.
- Oxygen supply was sufficient even at these very high cell densities.

#### References

Platas OB, Jandt U, Phan LdM, Villanueva ME, Schaletzky M, Rath A, Freund S, Reichl U, Skerhutt E, Scholz S, Noll Th, Sandig V, Pörtner R, Zeng AP: Evaluation of criteria for bioreactor comparison and operation standardisation for mammalian cell culture. Engineering in Life Sciences, 12(5): 518–528, 2012, DOI: 10.1002/elsc.201100163

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