Application note

Batch cultivation of a human neuronal cell line 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 human neuronal cell line adapted for growth in suspension in the lab -scale bioreactor Labfors Cell 5 (INFORS HT, CH-Bottmingen) is discussed. The cell line AGE1.HN$_{AAT}$ was kindly provided by ProBioGen AG, Berlin, Germany. Application of human neuronal cell lines for production of biopharmaceuticals promise to produce specific and complex glyco structures, which plays an important role, especially in the expression of human proteins or antibodies.

2. Technical specification

- Culture vessel: 3.6 L total volume with round bottom
- Magnetic stirrer (30–300 rpm)
- Pitched blade impeller
- pO_2 control via aeration with 3-gasmix air, O_2 and N_2
- pH control via CO_2
- Air cooler as standard
- Temperature control via heating mat
- Sparger: ring sparger with µ pores at the bottom
- Aseptic sampling
- Control of antifoam and level optional
- Determination of online parameters via Iris Control Software

3. Methods

a) Medium

The cells were cultivated in the serum- and protein- free 42-Max-UB medium (Teutocell AG, Germany). Furthermore, 5 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_2 probes were calibrated. The working volume was 2.5 L, temperature was controlled at 36.9°C. Stirrer speed was set to 156 rpm during cultivation, corresponding to a mixing time of approx. Θ_{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_2 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 30 mL/min (0.012 vvm). pH was controlled at 7.15 via sparging with CO_2 or addition of 0.5 M $NaCO_3$ via peristaltic pumps.

c) Batch cultivation

Precultures were performed in shake flask. The initial concentration of viable cells after inoculation was set to 1 x 10^6 cells/mL. The cultivation lasted 5 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 AGE1.HN cells was successfully run for 5 days. Time course of viable and total cell concentration is shown in fig. 2.

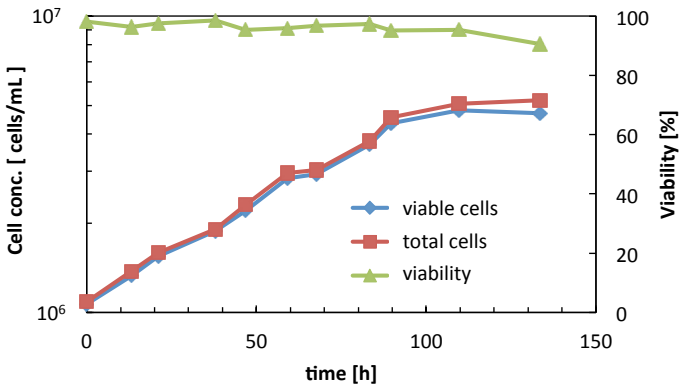

Fig. 2: Time course of viable and total cell density

The maximal viable cell concentration was 4.8 x 10^6 cells/mL at day 4. During exponential growth (up to day 3) the average viability was 95%. During this phase the growth rate *μ was* 0.016/h, the doubling time 44.1 h (fig. 3).

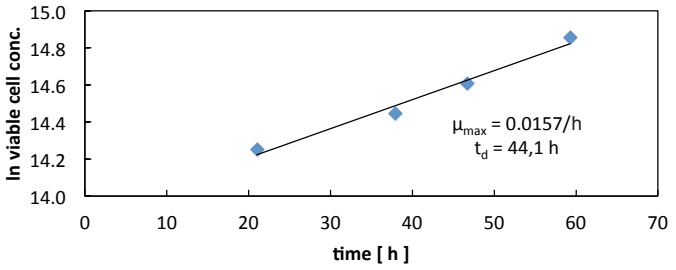

Fig. 3: Determination of maximal growth rate μ_{max} and doubling time t_d

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 and glutamine were totally consumed at the end of the cultivation. This corresponds with the end of exponential growth. Lactate and ammonia did not reach inhibiting concentrations.

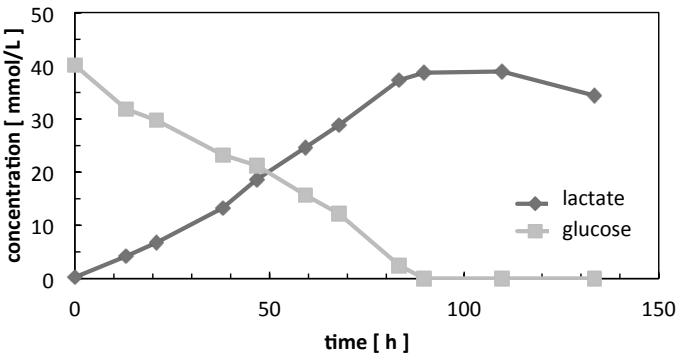

Fig. 4: Time course of glucose and lactate concentration

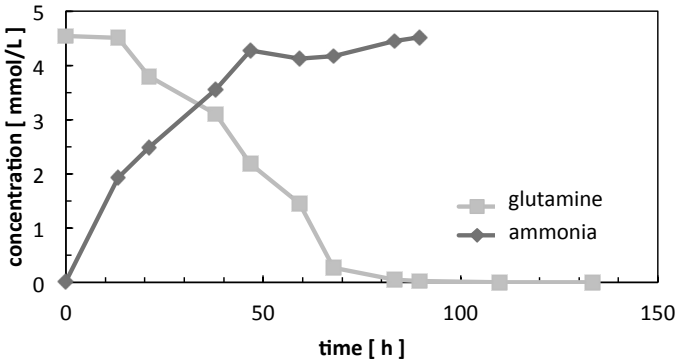

Fig. 5: Time course of glutamine and ammonia concentration

5. Summary

- A batch culture of suspendable AGE1.HN_{AAT} cells was successfully carried out over a period of 5 days. The growth behavior was comparable to previous experiments in different cultivation systems.
- The maximal cell concentration was 4.8 x 10^6 cells/mL at day 4.
- During exponential growth the mean viability was 95%.
- Growth rate and doubling time were as expected.
- Oxygen supply was sufficient even at these very high cell densities.

References

Niklas J, Schräder E, Sandig V, Noll T, Heinzle E: Quantitative characterization of metabolism and metabolic shifts during growth of the new human cell line age1.hn using time- resolved metabolic flux analysis. Bioprocess and biosystems engineering, 34(5):533–545, 2011. ISSN 1615-7605. DOI: 10.1007/s00449-010-0502-y

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