INFORS HT

Application note

Fed-batch cultivation of CHO cells culture in Minifors Cell

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1. Introduction

The use of bench-scale bioreactors allows the simple optimisation of development processes for the cultivation of animal cell cultures. The following example of the cultivation of CHO (Chinese Hamster Ovary) cells in the Minifors Cell bench-scale bioreactor (INFORS HT, CH-Bottmingen) illustrates the advantages of a fed-batch strategy for the optimisation of the SEAP production.

The CHO cell line is frequently used in biotechnology. For this experimental work, the CHO XM-111 clone was used. This clone was transfected by the group of Professor Dr. M. Fussenegger at the ETH Zurich with an expression vector which codes the gene of the recombinant protein SEAP (Secreted Alkaline Phosphatase) and is controlled by tetracycline using the promoter PhCMV-1. The use of the expression vectors makes the selectable expression of two genes possible by means of a promoter. This allows the production of SEAP as a process comprising of a non-productive growth phase followed by a proliferation-inhibited production phase based on the media exchange without tetracycline.

2. Technical specifications of the Minifors Cell

- Vessel: 2.5 L total volume (also 5 L available)
- Gassing with O_2, air and CO_2
- Exit gas cooler as standard
- 4 peristaltic pumps with autoclavable pump heads
- Temperature control via a thermal block for heating and cooling
- pO_2 and pH control
- Data logging of online parameters with Iris software
- Sampling as standard
- Antifoam/level control possible
- Various spargers and impellors are available

3. Experimental specifications

For production of SEAP over several days when culturing CHO XM-111 cells in a 2.5 L Minifors Cell bioreactor (INFORS HT, CH-Bottmingen).

a) Medium

For the growth phase of the CHO cell the serum- and protein-free HP-1 medium (Cell Culture Technologies GmbH) was used and for the production phase, HP-5 medium was used. The growth medium was supplemented with 2 g/L Pluronic F-68 and 2.5 g/L tetracycline. The HP-5 medium had 2 g/L Pluronic F-68 added.

b) Parameter settings for Minifors Cell

For the optimal growth conditions, a temperature of 37°C was selected. For the production of the SEAP protein, a temperature shift to 30°C took place on the fourth day. For the regulation of pO_2, control was set to 50% with gas sparging at a rate of up to 0.03 vvm. Additionally, the head space was also gassed with air at a rate of 0.3 vvm. The pH value of 7.2 could be regulated with the addition of CO_2 via the sparger. Optimal distribution of the cell suspension was achieved with a stirrer speed of 100 rpm.

4. Fed-batch cultures

Fed-batch cultivation was used to reach higher cell concentrations. Fresh medium is regularly introduced into the culture to prevent nutrient limitation. The initial volume of the CHO culture in the Minifors Cell was 600 mL and the culture was fed with 300 mL HP-1 during the first day (30 h), and also on the second day (46 h). On the second day (54 h), an additional 400 mL was fed. Medium exchange from the growth medium HP-1 to the HP-5 medium for the production phase took place on the third day (65 h). Additionally, a temperature shift from 37°C to 30°C was also made at this time to inhibit proliferation.

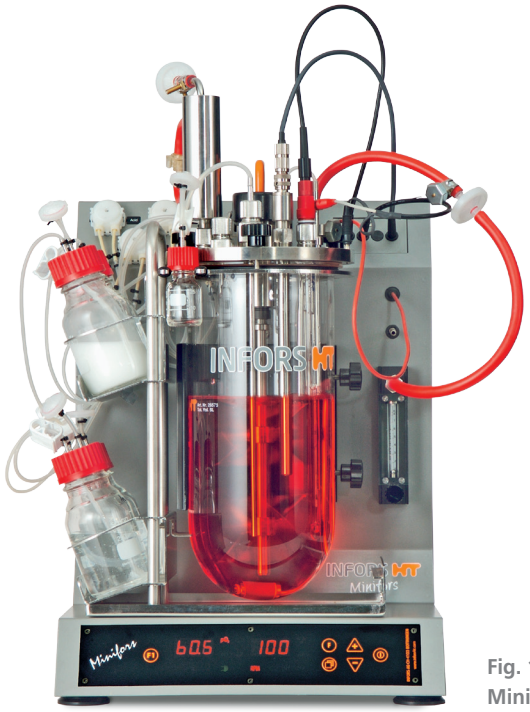

Fig. 1: Minifors Cell

5. Analysis

a) Parameter analysis

The daily determination of the viable cell concentration was performed using the NucleoCounter YC 100 (Chemometec). The analysis of growth and production substrates was accomplished using the Bioprofile Analyzer 100 Plus (Nova Biomedical).

b) Formulas

For calculation of the maximum growth rate μ_{max} and the doubling time t_d, the formulae shown below were used.

$$\mu_{max} = \frac{\ln(x_2) - \ln(x_1)}{(t_2 - t_1)}\ [h^{-1}] \qquad t_d = \frac{\ln(2)}{\mu_{max}}\ [h]$$

Analysis of the SEAP activity was achieved by following the increasing slope for substrate conversion photometrically. The SEAP activity per time unit is calculated according to the following equation:

$$SEAP\ Activity = \frac{\Delta_{405nm} / \min}{\varepsilon \cdot d \cdot V} = 2.4 \cdot \frac{\Delta A_{405nm}}{\min}$$

x = cell concentration per mL
T = process time in h
ΔA_{405nm} = absorption at 405 nm
ε = extinktion coefficient
V = sample volume
d = diameter chamber

6. Analysis of results

For the optimisation of the SEAP production in CHO XM-111 cells, a fed-batch culture was performed using the Multifors Cell. During the cultivation of the CHO XM-111 cells, daily sampling took place for the determination of the cell concentrations, substrate consumption and the production/activity of the SEAP protein.

The cell concentration increased during the first day on the basis of an inoculum cell concentration of 5.1 x 10^5 per mL to 1.1 x 10^6 per mL. The cell concentration rose to 2.55 x 10^6 per mL, up to the point of medium exchange on the third day. After the medium exchange on the fifth day, the maximum cell concentration of 3.15 x 10^6 per mL was reached. After this time the decline phase began. The cell viability fell from 99.2% to a value of 94.7% at the time of the medium change. Altogether, cultivation was continued to a viability level of 18.2% and a cell concentration of 3.9 x 10^5 per mL (Fig. 2).

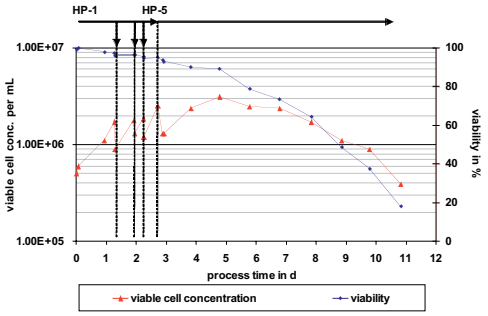

Fig. 2: Cell concentration and viability

For calculation of the maximum growth rate μ_{max} and the doubling rate t_d estimations were made during the growth phase between 54 h (2nd day) and 65 h (3rd day). A growth rate of 0.040 per hour and a doubling time of 17.43 h were determined.

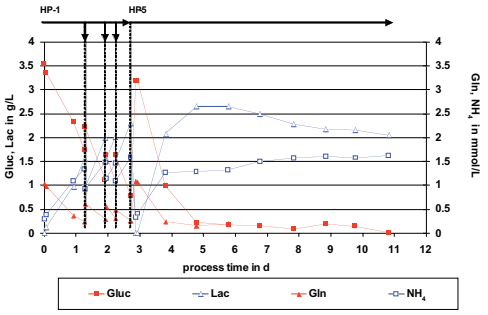

Fig. 3: Comparison of glucose and glutamine with ammonia and lactate concentrations

The glucose and glutamine concentration is shown in Figure 3. Both substrates serve as energy sources to the CHO cells and are consumed during growth.

Moving in the opposite directions to the decrease in glucose and glutamine concentrations, both ammonia and lactate are formed. The data indicates that feeding strategy was able to optimise the most important metabolic parameters for the cultivation of CHO cells in a 2.5 L vessel of the Minifors Cell (Fig. 3).

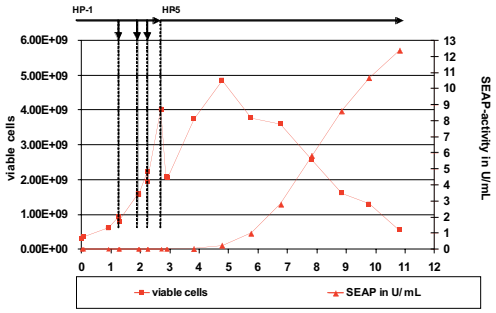

Fig. 4: SEAP activity

Following the change to the HP-5 medium, the SEAP product formation began on the third day and reached a maximum activity by day 11 of 12.36 U/mL (Fig. 4).

The recording of online parameters with the Iris software makes the monitoring of the control parameters such as the temperature, pH and the pO_2 saturation possible. This monitoring of key parameters makes it possible to control the complete cultivation and shows all values deviating during the process. Possible deviations of the temperature and the pO_2 measurement could result from the addition of fresh medium (feeding) (Fig. 5).

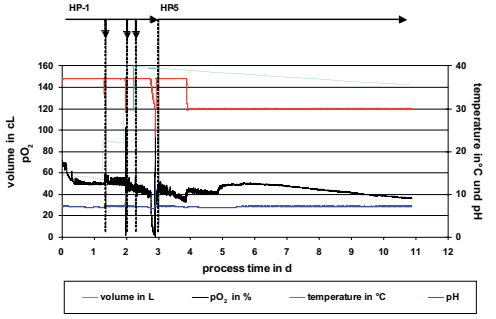

Fig. 5: Volume, temperature, pH and pO_2 values against process time in days

The regulation of pO_2 was achieved by gassing the culture with a 2-gas mix of air and oxygen and could be kept between 40% and 50%. The parallel gassing with CO_2 provided pH stability at pH = 7.2 and so maintained well-adapted culture conditions for the cells for growth and then production of SEAP (Fig. 6).

7. Summary

- Fed-batch cultivation of CHO XM-111 was accomplished over 11 days.
- A maximum cell concentration of 3.15 x 10^6 per mL (4.85 x 10^9 total cells) with 90% viability was reached on the fifth day of cultivation.
- Conversion to production medium and a temperature shift makes optimisation of the SEAP production possible.
- SEAP activity was observed on the 11th day at 12.36 U per mL.
- Recording of most important metabolic parameters i.e. pH, pO_2, stirrer speed and temperature allowed for process control by optimisation of cultivation parameters as standard.

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