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Application note

Batch culture of CHO cells in the Multifors Cell

ZHAW, IBT, CH-Wädenswil, and INFORS HT, CH-Bottmingen, www.infors-ht.com

1. Introduction

The Multifors Cell can be used to easily optimise development processes by parallel cultivation of animal cell cultures. Cultivation of the CHO (Chinese hamster ovary) cell line in the Multifors Cell bioreactor (INFORS HT, CH-Bottmingen) is described in the following as an example of batch cultivation of parallel samples.

CHO cells are used quite commonly in biotechnology in the production of recombinant proteins. Cultures of the CHO XM-111 clone, transfected with an expression vector from the research group of Prof. Dr. M. Fussenegger at ETH Zürich, were carried out successfully in the Multifors Cell.

These cells contain the code for the gene of the recombinant SEAP protein (secreted alkaline phosphatase) which is controlled by the tetracycline-regulated PhCMV-1 promoter. The use of the expression vectors enables the expression of two genes by means of a single promoter that can be regulated. Accordingly, the SEAP production process consists of a non-productive growth phase, followed by a proliferation-inhibited production phase based on the depletion of tetracycline through a change of medium.

2. Technical specifications of the Multifors Cell

- Culture vessels: 2 x 1 L total volume/TV (0.7 L TV versions available as well)
- Regulation of pO_2 by supply of a 3-gas mixture of air, O_2, and N_2
- Regulation of pH by means of CO_2
- Exit gas cooling as standard
- 2 x 4 autoclavable peristaltic pumps
- Temperature regulation by means of a heating block
- Detection of online parameters by means of Iris software
- Aseptic sampling as standard
- Regulation of Antifoam or Level feasible
- Various spargers and impellers available

3. Experimental specifications

The CHO XM-111 cells were cultivated for several days in the 1 L Multifors Cell parallel bioreactor (INFORS HT, CH-Bottmingen).

a) Medium

Serum- and protein-free HP-1 medium (Cell Culture Technologies GmbH) was used for analysis of the growth of CHO cells. Supplements included 10 mL/L Pluronic F-68 and 2.5 mL/L tetracycline.

b) Parameter settings in the Multifors Cell

Both culture vessels of the Multifors Cell were readied for cell culture and the pH and pO_2 sensors were calibrated. A temperature of 37°C was selected to provide optimal growth conditions. The pO_2 content of 40% was regulated with the 3-gas mixture at up to 0.06 vvm directly via the ring sparger. Additional air was supplied into the headspace at up to 0.7 vvm. The pH value of 7.2 was maintained by adding CO_2 as part of the gas supplied through the sparger. Optimal distribution of the cell suspension was attained at a stirring rate of 75 rpm.

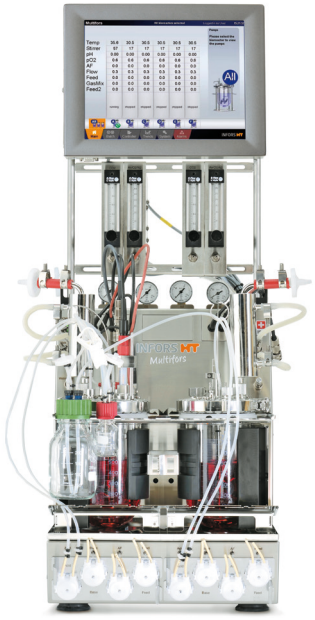

Fig. 1:
Parallel cultures in the Multifors Cell

4. Batch cultivation

Batch cultivation of parallel samples is used, for example, in order to test the growth of cell cultures under specific conditions or to rapidly achieve reproducibility of cell cultures.

Both culture vessels of the Multifors Cell parallel bioreactor were inoculated with shaker pre-cultures of the CHO XM-111 cell line, cultivated in the shaker incubator (Multitron Cell, INFORS HT) at 125 rpm, 37°C and a relative humidity of 85% rH. The viable cell concentration of the freshly inoculated bioreactor vessels was 0.5 x 10^6 cells per mL at a viability of 93.7% in bioreactor 1 (BR1) and 0.35 x 10^6 cells per mL at a viability of 97.1% in bioreactor 2 (BR2). A working volume of 700 mL was selected for each vessel such that no additional medium was added during cultivation. The CHO cells were allowed to grow at the selected conditions for a period of 4 days. The daily samples taken were used to determine cell growth as well as measure substrate consumption or other metabolite concentrations.

5. Analysis

a) Parameter analysis

The viable cell concentration was assayed daily using the Cedex device (Innovatis). The analysis of the growth and production substrates was carried out using the Bioprofile Analyzer 100 Plus (Nova Biomedical).

b) Formulas

The following formulas were used to calculate the maximum growth rate μ_{max} and doubling time t_d.

$$\mu_{max} = \frac{\ln(x2) - \ln(x1)}{t2 - t1} \left[\frac{1}{h}\right] \qquad t_d = \frac{\ln(2)}{\mu_{max}} [h]$$

6. Analysis of results

The growth experiments on CHO XM-111 cells were carried out in parallel culture vessels using a single Multifors Cell bioreactor unit.

The viable cell concentration in both cultures was more than doubled after one day, reaching 1.09 x 10^6 per mL in the first bioreactor and 1.04 x 10^6 per mL in the second bioreactor. Continued culture of the CHO XM-111 cells in the first bioreactor produced growth up to a maximum viable cell concentration of 3.34 x 10^6 per mL on day 3 of the culture. Steady growth of the parallel culture in the second bioreactor was observed as well leading to a maximum viable cell concentration of 3.44 x 10^6 per mL on day 3 of cultivation. The declining phase commenced from this time. The viability decreased from an average of 95% to less than 70% in bioreactor 1 (BR1) and less than 30% in bioreactor 2 (BR2) by the time the culture was discontinued. Both cultures were discontinued on day 4 after the substrates had been consumed and the viability of the cells had dropped off (Fig. 2).

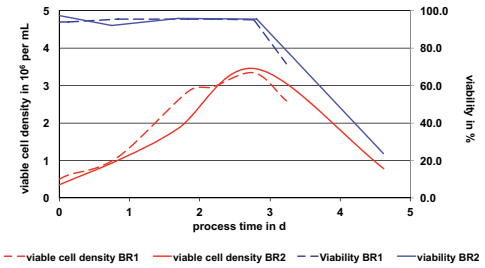

Fig. 2: Comparison of CHO XM-111 cell cultures in bioreactor 1 and bioreactor 2

The calculation of the maximum growth rate μ_{max} and doubling rate t_d was based on the growth phase between 0 h and 52 h (BR1) or 67 h (BR2). The CHO cells in bioreactor 1 attained a growth rate of 0.8447 per day and a doubling time of 19.7 h, whereas the cells in the second bioreactor grew at a growth rate of 0.8194 per day and a doubling time of 20.3 h (Fig. 3).

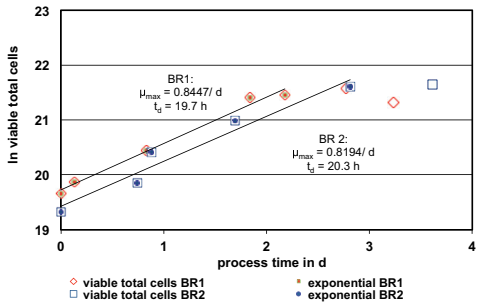

Fig. 3: Comparison of growth rate and doubling time in bioreactor 1 and bioreactor 2

The consumption of glucose and glutamine is shown in Figure 4. Both substrates are used as energy sources by the CHO cells and are consumed during growth. Ammonium and lactate are generated while the glucose and glutamine levels drop off. The major metabolic parameters for the cultivation of CHO cells were recorded in the 1 L bioreactor vessel (BR2) for graphic presentation. The glucose consumption decreases during the cultivation in BR2 while the cells are growing which is indicative of the essential need of the CHO cells in this regard. The decrease in glucose and glutamine is observed to be associated with an increase in lactate and ammonium which are considered to be toxic metabolites at elevated concentrations (Fig. 4).

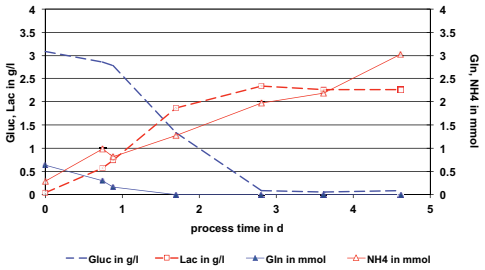

Fig. 4: Metabolite concentrations in CHO cell cultures in bioreactor 2

For regulation of the pO_2, a 3-gas mixture of air, oxygen, and nitrogen was supplied to the culture which allows the pO_2 saturation to be maintained at approx. 40%. Simultaneous CO_2 gassing ensured the pH to be stable at pH = 7.2 and thus established culture conditions that were well-adapted to the growth of the cells (Fig. 5).

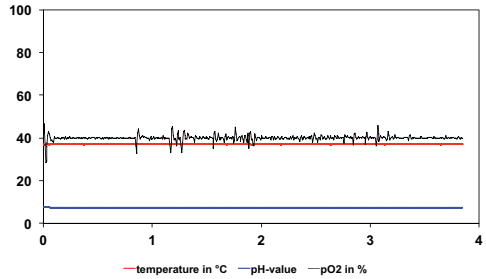

Fig. 5: Process parameters in bioreactor 1

The recorded metabolite concentrations of the culture in bioreactor 1 and the time course of the process parameters of the culture in bioreactor 2 are not shown here. Accordingly, Figure 4 and Figure 5 showing the cultures in bioreactor 2 and bioreactor 1, respectively, are used for the assessment of the experiment in exemplary fashion.

7. Summary

- Parallel batch cultivation of CHO XM-111 cells has been completed successfully in just 4 days.
- On day 3 of cultivation, the maximum viable cell concentration reached 3.34 x 10^6 per mL (2.33 x 10^9 total viable cell count; BR1) and 3.44 x 10^6 per mL (2.41 x 10^9 total viable cell count; BR2) and the viability was 95% in both bioreactor vessels.
- The growth rate and doubling time of the cultures were compared successfully.
- The major metabolic parameters that were recorded showed similar patterns.
- Successful comparison of culture parameters during parallel cultivation is feasible.
- pH, pO_2, stirrer and temperature regulation for process control and optimal setting of the culture parameters are provided as standard.
- Parallel cultures with up to 6 cultures vessels can be run with a single control unit of the Multifors Cell.

AP005_en_0114

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