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Application note

CHO cell culture in the Multitron Cell with ShakerBag option

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1. Introduction

The use of incubation shakers is gaining ever more significance for the cultivation of animal cells. The following example of the cultivation of CHO (Chinese Hamster Ovary) cells in a variety of large **disposable bags** shows that the Multitron Cell incubation shaker (INFORS HT, CH-Bottmingen) is very well suited for this application in the areas of pharmaceutical research and development, and for the actual production of active substances in large disposable bags.

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 for 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 a nonproductive 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 Multitron Cell

- ShakerBag option
- 50 mm shaker stroke
- Aeration with air and CO_2 (0–10% as standard, optionally 0–20%)
- Different trays available (for disposable bags, with «Sticky Stuff», with flask clamps, etc.)
- Further options (cooling, humidification, decontamination, etc.)

3. Experimental specifications

The CHO cells were cultivated in disposable bags of 2 L (1 L working volume / WV) and a 20 L (10 L WV). The inoculation concentration for both sizes came within the range of 0.5 x 10^6 cells per mL. The minimum volume for fed- batch cultures in large disposable bags amounted to 25% of the maximum working volume. The culture conditions were selected with a temperature of 37°C and a pH about 7. The supply of the CHO cells with oxygen and the maintenance of a suitable pH value were made using the gassing station.

4. Fed-batch cultures

The two chosen sizes of large disposable bags were set up with different volumes and a correspondingly adapted feeding strategy of serum- and protein-free HP-1 medium.

a) 2 L disposable bag

The initial inoculation conditions for the 2 L disposable bag used a cell concentration of 6 x 10^5 cells per mL with a viability of 99% in 250 mL HP-1 medium. Cultivation was carried out over a period of five days altogether. On the first day, feeding with 100 mL took place and so leading to an increase in the final volume to 350 mL. Additionally, on the second day 200 mL were added and on the third day a further 450 ml of the HP-1 medium were introduced to reach the working volume of 1 L. The maximum growth rate μ_{max} which could be achieved was 0.046 h^{-1} and the doubling time t_d was 15.1 h. The maximum cell concentration was measured at 2.4 x 10^6 cells per mL and had a viability of 98%.

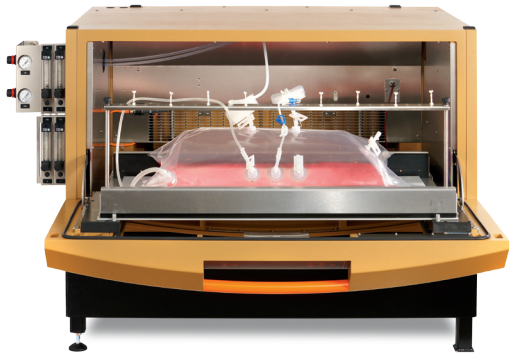

Fig. 1: Multitron Cell with ShakerBag option

For the supply of oxygen, air was continuously gassed up to 1 vvm. The CO_2 supplied at the beginning was 10% and was reduced to 5% with increasing cell concentration. The shaking frequency was selected between 40 and 70 rpm.

b) 20 L disposable bag

The initial inoculation conditions for the 20 L disposable bag used a cell concentration of 7.0 x 10^5 cells per mL and a viability of 99%. The inoculum was added to 2 L HP-1 medium in the 20 L disposable bag. On the first day, feeding with 1 L fresh medium took place. Additionally, 2 L medium was introduced on the second day and further 2.5 L on each of the third and fourth day. Altogether, the 10 L maximum working volume had a maximum cell concentration on the fifth day of 3.0 x 10^6 cells per mL with a viability of 98%. The maximum growth rate μ_{max} was determined as 0.035 h^{-1} and the doubling time t_d was 19.8 h.

For the supply of oxygen, air was continuously gassed up to 1 vvm. The CO_2 supplied at the beginning was 10% and was reduced to 5% with increasing cell concentration. The shaking frequency was maintained at a constant 30 rpm.

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) Formulae

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

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

6. Analysis of results

For the process development for the production of SEAP, CHO XM-111 cells were incubated in two different sizes of large disposable bags (2 L and 20 L) under comparable conditions for several days in the Multitron Cell incubation shaker. While the culture of the CHO XM-111 cells took place, a daily sample made measurements possible for the optimum comparison of cell concentrations, substrate consumption and buffering.

The initial cultivation of the CHO cells took place in a 2 L disposable bag with a maximum working volume of 1 L. Daily feeding with fresh medium permitted cell growth of the CHO cells with a maximum viable cell concentration of 2.40 x 10^6 per mL with a viability of 98% by the third day of cultivation. Glucose consumption was observed, which was proportional to cell growth and completely exhausted by the fifth day (Fig. 2).

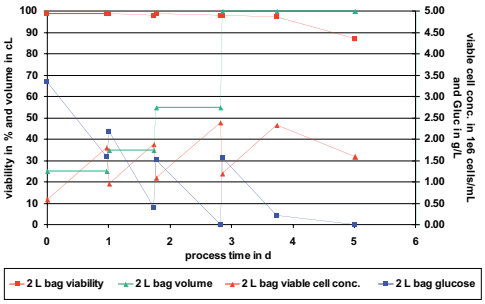

Fig. 2: 2 L disposable bag: Comparison of the viable cell concentration, viability, glucose and volume

Glucose and glutamine concentration in the 2 L disposable bag are shown in Figure 3. Both substrates serve as energy sources for the CHO cells and are consumed during growth. Moving in the opposite directions to the decrease of glucose and glutamine, concentrations of ammonium and lactate increase. Recording of the relationships between these most important metabolic parameters allows the optimisation of the cultivation of the CHO cells in a disposable bag.

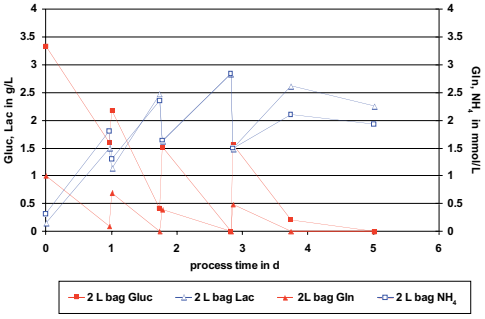

Fig. 3: 2 L disposable bag: Comparison of glucose, glutamine, ammonium and lactate

A comparison to 2 L disposable bag and a simultaneous scale-up of the CHO cultivation were observed in a large 20 L disposable bag. The optimised feeding with fresh medium permitted a cell growth on the fifth day up to a maximum cell concentration of 3.0 x 10^6 per mL with viability of over 97%. Also, in the 20 L disposable bag glucose consumption was observed to be proportional to cell growth (Fig. 4).

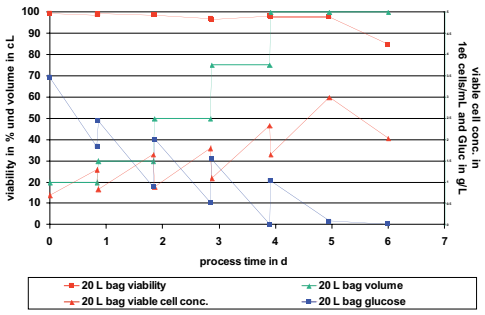

Fig. 4: 20 L disposable bag: Comparison of the viable cell concentration, viability, glucose and volume

Nutrient consumption in the second culture in 10 L working volume showed a higher glutamine consumption. Glucose limitation could be avoided to a large extent by using an adapted feeding strategy. Additionally, less ammonium formed than in 1 L working volume and a lactate concentration of under 2.5 g/L was observed (Fig. 5).

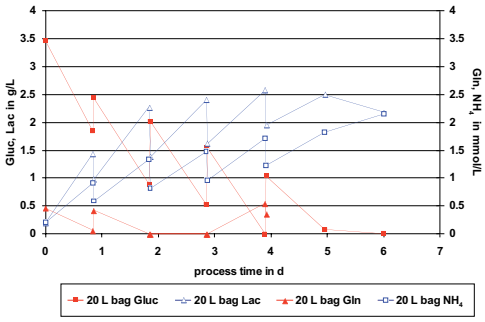

Fig. 5: 20 L disposable bag: Comparison of glucose, glutamine, ammonium and lactate

Altogether, a good oxygen supply with minimal foaming could be observed and antifoam reagent was not required.

7. Summary

- The maximum cell concentration achieved of 3 x 10^6 per mL with a viability of 97% was found in the large 20 L disposable bag with 10 L working volume.
- By using feeding strategies adapted for the 1 L and 10 L working volume sizes, comparable results were obtained.
- Optimisation and scale-up is possible.
- An optimised substrate supply can be achieved with a suitable feeding strategy.
- The shaker throw of 50 mm permits a gentle shaking of the cell culture.
- The results of cultivation in large disposable bags of 2 L and 20 L in the Multitron Cell are comparable with other cell culture systems.
- A single unit of Multitron Cell can be used for cultures varying from 10 mL to 10 L working volume. Thus, expansion from the starter cultures to small-scale production in only one equipment becomes possible.

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