Application note

CHO cell culture in the Multitron Cell with shaker flasks

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

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 shaker flasks shows that the Multitron Cell incubation shaker (INFORS HT, CH-Bottmingen) is very well suited for the production of seed cultures.

The CHO cell line is frequently used in biotechnology. For the production of seed cultures for inoculation and for the production of the SEAP protein, the CHO XM-111 clone was used. This clone was transfected by the group of Professor Dr. M. Fussenegger of the ETH Zurich with an expression vector which codes for the gene of the recombinant protein SEAP (Secreted Alkaline Phosphatase) and 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 non-productive growth phase followed by a proliferation-inhibited production phase based on the media exchange without tetracycline.

2. Technical specifications of the Multitron Cell

- 50 mm shaking throw (option for 25 mm)
- Aeration with air and CO_2 (0–10% as standard, optional 0–20%)
- Hygienic Direct Steam Humidification
- Different trays available (for disposable bags, with «Sticky Stuff», with flask clamps, etc.)
- Further options (cooling, decontamination, ShakerBag, etc.)

3. Experimental specifications

For the production of seed culture as an inoculum for later cultivation and SEAP production in bioreactors, three shaker flask cultures with the CHO XM-111 cell line were set up. Culture took place in 250 mL, 500 mL and one 1000 mL shaker flask (Corning) in each case, using the serum- and protein-free HP-1 medium (Cell Culture Technologies GmbH). 2 g/L Pluronic F-68 (Sigma) and 2.5 mg/L tetracycline (Sigma) were added to the medium. For optimal cultivation conditions, a temperature of 37°C, a humidity of 85% and a CO_2 saturation of 5% were selected. All flasks were shaken in a Multitron Cell incubator (INFORS HT, CH-Bottmingen) at a constant 122 rpm over several days for cultivation.

4. Fed-batch shaking cultures

The shaker cultures were set up with different volumes and a correspondingly adapted feeding strategy.

a) Process of cultivation in a 250 mL shaker flask took place according to the following pattern:

day/hours – step	volume in mL	viable cell concentration per mL	viability in %
0 d/0 h – inoculation	50	3.0×10^5	86.9
1 d/21 h – feed	50 (+ 30 mL)	6.6×10^5	91
2 d/43 h	80 (max. 32%)	1.95×10^6	97.4
3 d/67 h	80	**2.25×10^6**	98.3
4 d/91 h	80	1.95×10^6	98.7

The maximum growth rate μ_{max} which could be achieved was 0.048 h^{-1} and a doubling time t_d = 14.4 h was reached.

Fig. 1: Multitron Cell

b) Process of cultivation in a 500 mL shaker flask took place according to the following pattern:

day/hours – step	volume in mL	viable cell concentration per mL	viability in %
0 d/0 h – inoculation	100	7.8×10^5	95.5
1 d/24 h – feed	100 (+ 50 mL)	1.88×10^6	98.7
2 d/47 h – feed	150 (+ 50 mL)	2.18×10^6	99.1
3 d/67 h – media exchange	250 (+ 50 mL)	2.33×10^6	99.3
4 d/91 h	300 (max. 60%)	2.63×10^6	99.7
5 d/115 h	300	**4.65×10^6**	99.4
6 d/139 h	300	3.90×10^6	98.1
7 d/163 h	300	2.55×10^6	81.8

The maximum growth rate μ_{max} which could be achieved was 0.024 h^{-1} and a doubling time t_d = 28.9 h was reached.

c) Process of cultivation in a 1000 mL shaker flask took place according to the following pattern:

day/hours – step	volume in mL	viable cell concentration per mL	viability in %
0 d/0 h – inoculation	150	6.6×10^5	98.9
1 d/23 h – feed	150 (+ 50 mL)	9.6×10^5	98.9
2 d/45 h – feed	200 (+ 50 mL)	1.95×10^6	99
3 d/66 h – feed	250 (+ 50 mL)	1.10×10^6	99.3
4 d/93 h	300	2.18×10^6	99.4
5 d/117 h – 15-fold dilution	400 (max. 40%)	**2.25×10^6**	98.5
8 d/189 h	400	1.40×10^6	66.9

The maximum growth rate μ_{max} which could be achieved was 0.044 h^{-1} and a doubling time t_d = 15.65 h was reached.

d) Evaporation with Direct Steam Humidification

To examinate the daily fluid loss, 125 mL shaker flasks were used with 15 mL, 20 mL and 25 mL working volume at 37°C and 85% rH humidity. Before and after the cultivation the total volume was measured.

5. Analysis

a) Parameter analysis

The daily determination of the viable cell concentration was performed using the NucleoCounter YC 100 (Chemotec). 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 the results

For the optimisation of CHO seed culture, three different sizes of shaker flasks (250 mL, 500 mL and 1000 mL) were set up 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.

A comparison of the maximum cell concentration showed the 500 mL shaker flask culture produced up to 4.65 x 10^6 cells per mL. In all cultures a viability could be achieved of over 98% up to 6 days. Through an optimised feeding strategy, the cultivation of seed culture is possible after 2 days up to a maximum of 5 days (Fig. 2).

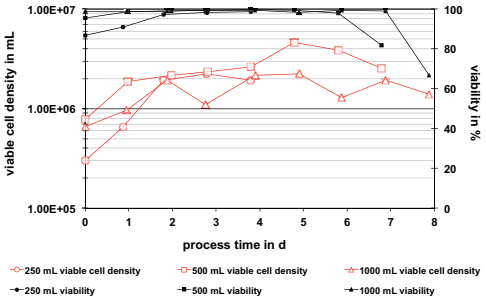

Fig. 2: Comparison of the cell concentration and viability

Glucose and glutamine consumption, particularly in the 500 mL shaker flask, showed that substrate was available in adequate quantities up to the fifth day, so the feeding method was preventing substrate limitation (Fig. 3).

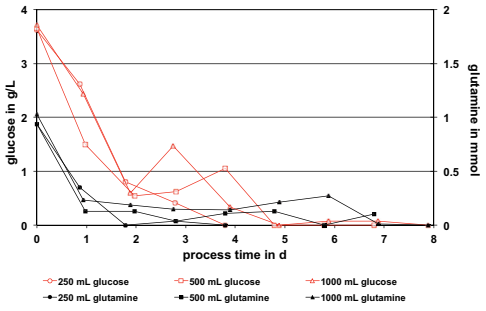

Fig. 3: Comparison of glucose and glutamine

The simultaneous formation of ammonium, lactate and glutamate is known to reduce the rate of cell growth. A subcritical concentration of ammonium was likewise observed up to the fifth day of incubation in all cultures. Also, the aeration and continuous supply of 5% CO_2 makes optimal buffering possible for the culture system and, therefore, suitable pH conditions. (Fig. 4).

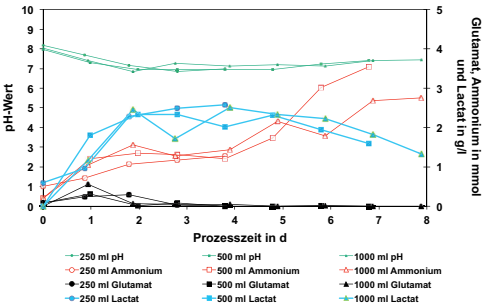

Fig. 4: Comparison of ammonium, glutamate, lactate and pH

The Direct Steam Humidification has an important influence on fluid loss and also changes the osmolarity. Especially in small working volumes could be shown, that the maximal evaporation is less than 0.4% in one day.

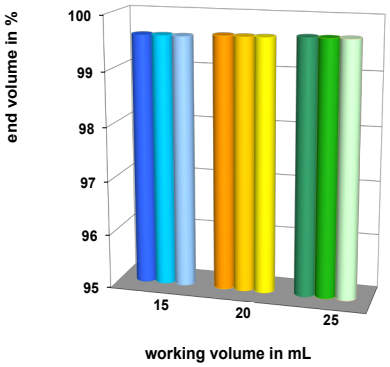

Fig. 5: Evaporation in 125 mL shaker flask

The working volume in the shaker flask has a significant influence on oxygen transfer and should not be exceeded.

7. Summary

- The shaker flask cultures reach a total number of cells between 1.8 x 10^8 and 1.4 x 10^9 after 2 to 5 days. This can be used as an inoculum for a 2.5 L bioreactor with a viable cell concentration of approx. 5 x 10^5 per mL.
- The higher the cell densityand cell viability during the inoculation, the higher cell density can be reached during the cultivation.
- Early exchange of medium is preferred.
- A small inoculum volume and an optimal feeding strategy permit, that additional cell culture nutrients could be supplied. On the other hand the culture is diluted and so toxic products are reduced.
- The low evaporation of less than 0.5% at 85% relative humidity results in a stable osmolarity in the culture and reproducibility of processes.

The shaker culture is available for further process steps, e.g. inoculation of a bioreactor (Minifors Cell, Multifors Cell, Labfors 4 Cell) or of disposable bags (in a Multitron Cell).

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Infors AG
Headoffice, Switzerland
Rittergasse 27
CH-4103 Bottmingen
T +41 (0)61 425 77 00
F +41 (0)61 425 77 01
headoffice@infors-ht.com

Infors GmbH
Dachauer Str. 6
D-85254 Einsbach
T +49 (0)8135 8333
F +49 (0)8135 8320
infors.de@infors-ht.com

Infors UK Ltd
The Courtyard Business Centre, Dovers Farm, Lonesome Lane, Reigate, Surrey, RH2 7QT, UK
T +44 (0)1737 22 31 00
F +44 (0)1737 24 72 13
infors.uk@infors-ht.com

Infors Sarl
2, rue du Buisson aux Fraises
Bâtiment D13
FR-91300 Massy
T +33 (0)1 69 30 95 04
F +33 (0)1 69 30 95 05
infors.fr@infors-ht.com

Infors Benelux BV
Fabriekstraat 38
NL-7005 AR Doetinchem
P.O. Box 613
NL-7000 AP Doetinchem
T +31 (0)314 364 450
F +31 (0)314 378 076
infors.bnl@infors-ht.com

Infors Canada
8350 rue Bombardier
Anjou, Quebec
Canada H1J 1A6
T +1 514 352 50 95
F +1 514 352 56 10
infors.ca@infors-ht.com

Infors Biotechnology (China) Co., Ltd.
Room 505C, Building 106
Lize Zhongyuan
Wangjing New Industrial Zone
Chaoyang District, Beijing
100102 P. R. of China
T +86 (0)10 51 65 20 68
F +86 (0)10 64 39 05 85
info@infors-ht.com.cn

Reference: ZHAW Zurich University of Applied Sciences, Life Sciences and Facility Managment, IBT Institut of Biotechnology