New Option for Incubator Shaker:
Cell cultivation with orbital waves in flexible single-use shaker bags up to 10 L

Iris Poggendorf[1], Lidija Lisica[1], Nadia Wohlwend[1], Johanna Olownia[2], Regine Eibl[1]

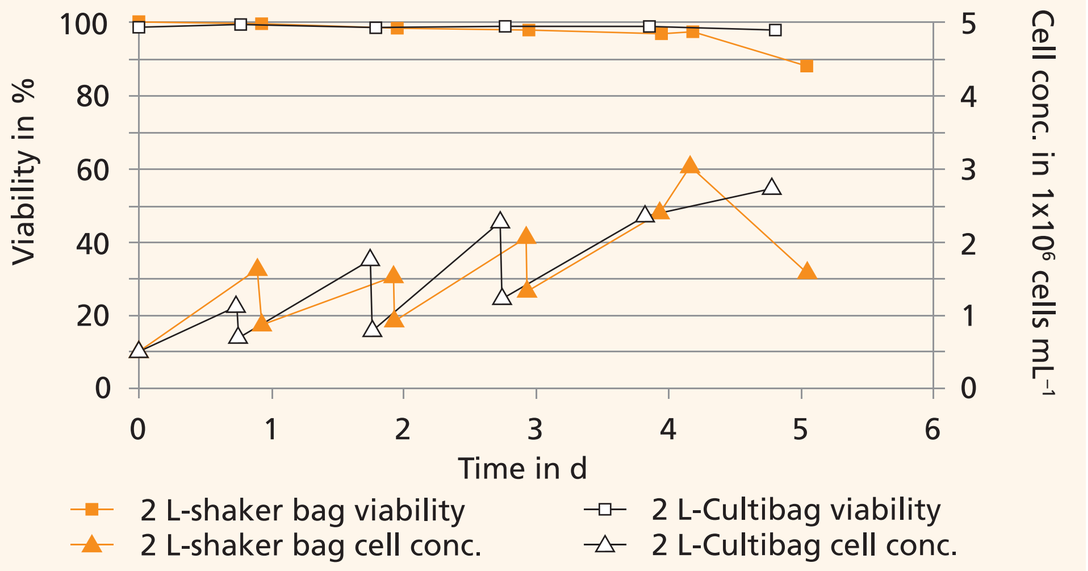
Zurich University of Applied Sciences
Life Sciences and Facility Management

Introduction

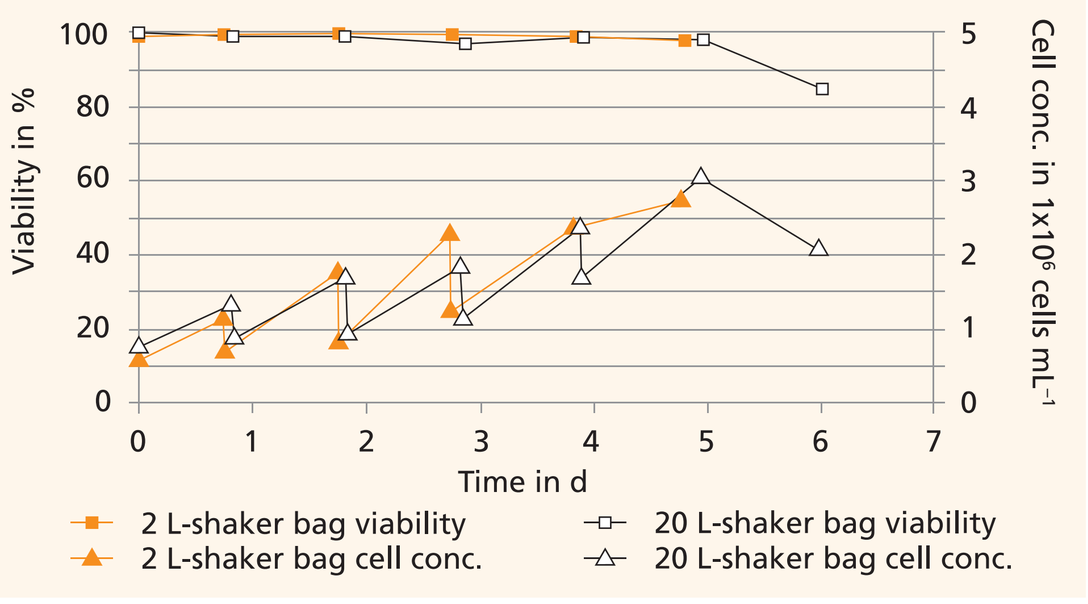
Orbitally shaken, single-use bags represent a new method for the production of seed cultures or protein production on the lab scale.

Using this new technology, successful cultivation of the cell lines CHO XM 111 (Chinese Hamster Ovary) and Sf-21 (Sf-9) (*Spodoptera frugipedra*) was accomplished. Also, scale-up of the CHO cell cultivation from 1 L to 10 L working volume is possible using orbital-shaken, single-use bags. For these cell cultures a serum- and protein-free chemically defined media was used, which needs sophisticated system efforts for the cell growth, but is a requirement for the production of therapeutic products.

CHO XM 111 fed-batch cultivation in 2 L shaker bag vs 2 L Cultibag



CHO XM 111 fed-batch cultivation scale-up from 1 L to 10 L



Details[3]

The CHO and Sf-9 cell lines are frequently used in biotechnology. For this experimental work, the CHO XM 111 clone was used. This clone was transformed 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 controlled by tetracycline using the promoter Ph-CMV-1. The Sf-9 is a cell clone of the cell line Sf-21. These insect cells, developed from ovaries of the moth species *Spodoptera frugipedra*, are well known for virus infection and production of recombinant proteins.

The Multitron Cell for shaker bag was used with a 50 mm shaker stroke and aeration with maximal 2 vvm air and CO_2 (0–10% only for CHO cells). The cultivation of CHO XM 111 were done at 37°C, pH 7.2 and shaker speed between 30 and 40 rpm. For the cultivation of Sf-9 cells a temperature of 27°C, shaker speed between 25 and 35 rpm and pH 6 were used. The oxygen transfer was determined by amperometric method.

Result

The fed-batch cultivation strategy was used in chemical defined media:

- CHO (protein- and serum-free):
  HP-1 (Cell Culture Technologies, Invitrogen) with 2.5 mL L^{-1} Tetracyclin and 10 mL L^{-1} Pluronic F-68
- Sf-9 (serum-free):
  Sf-900 II SFM (Cell Culture Technologies)

Cell growth results in maximal cell densities for

- CHO XM 111 about $3x10^6$ viable cells/ml with >98% viability
- Sf-21 (Sf-9) > $1x10^7$ viable cells/ml with >90% viability (data not shown)

An Oxygen Transfer Coefficient (k_La) about 31 h^{-1} with 2 vvm could be achieved.

Oxygen Transfer

Gassing	WV	Shaker speed	k_La	Saturation
[L min^{-1}]	[L]	[rpm]	[h^{-1}]	
2	1	40	30.34	yes
2	1	40	30.96	no

Summary

Cell growth was comparable to existing single-use bags and cultivation systems already on the market.

- 2 L shaker bag comparable to 2 L Cultibag
- Upscale from 1 L to 10 L WV in shaker bag is possible

Additionally, k_La measurements proved the oxygen transfer to be optimal for cell culture.

Reference

[1] ZHAW Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Biotechnology, Campus Grüental, CH-8820 Wädenswil, Switzerland
[2] Infors AG, Rittergasse 27, CH-4103 Bottmingen, Switzerland
[3] Development and cell cultivation result from projects by students and scientists of the ZHAW, Wädenswil. The INFORS HT provides Infors equipment and cooperates with the ZHAW in projects.