INFORS HT

Application note

Culturing of *Sf*9 insect cells with the baculovirus expression vector system (BEVS), and SEAP production

1. Introduction

The BEVS (baculovirus expression vector system) has become a well-liked expression system for the production of recombinant proteins in insect cells. It is common to use insect cell lines of *Spodoptera frugiperda* for this purpose and to infect them with baculoviruses of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) or modified variants thereof.

The following application examples involved the culturing of the *Sf*9 insect cell line (Invitrogen, USA), which had been infected with a baculovirus expressing SEAP (secreted alkaline phosphatase) developed by the group of Prof. M. Sievers (Zurich University of Applied Sciences). On the one hand the growth behavior and on the other hand the productivity within different orbitally shaken culture vessels were compared.

In order to optimise the growth and production behaviour, preliminary experiments were performed to determine and then apply not only suitable infection and harvesting conditions for the virus, but also corresponding culturing parameters for different orbitally shaken culturing systems by methods of process engineering (data not shown).

2. Technical specifications

Multitron Cell incubator shaker (50 mm) with ShakerBag Option (INFORS HT, Switzerland)

- Trays: «StickyStuff» tray and ShakerBag Option tray
- Direct gas feed (air or air/CO_2; 20–2000 mL/min)
- CO_2 control 0–20 %
- Mobile Pt100

3. Materials and methods

Preliminary experiments

• Parallel growth experiments in shake flasks

For the parallel growth experiments, *Sf*9 suspension cells from maintenance culture were expanded in three 250 mL shake flasks. The cells were cultured at an inoculation density of approx. 0.6 x 10^6 cells mL^{-1} at a working volume of 100 mL at 27 °C, 100 rpm in Sf-900™ III SFM medium (Gibco Invitrogen).

• Cell growth with ShakerBag Option in an orbitally shaken Cultibag RM

For the growth experiment applying the ShakerBag Option and Multitron Cell (INFORS HT) in an orbitally shaken 2 L Cultibag RM (Sartorius Stedim Biotech), *Sf*9 suspension cells from maintenance culture were expanded in a working volume of 1 L. The viable cell density was 0.62 x 10^6 cells mL^{-1}. The cells were cultured at 27 °C, 38–46 rpm in Sf-900™ III SFM medium (Gibco Invitrogen).

Inoculum for production experiments

To produce inoculum, *Sf*9 suspension cells from maintenance culture were expanded in three parallel 250 mL shake flasks (Corning). In the first two days, the culturing was done at an inoculation density of 1 x 10^6 cells mL^{-1} each in 100 mL Sf-900™ III SFM medium (Gibco Invitrogen) at 27 °C and 100 rpm in the Multitron Cell (INFORS HT). Then, on day 2, the batches were scaled to 200 mL culture volume each in 500 mL shake flasks.

The cell density was determined using a Cedex HiRes (Roche Diagnostics, Switzerland).

Fig. 1: Multitron Cell incubator shaker

Comparison of SEAP production in orbitally shaken culturing systems

• Cell cultivation

For the production experiments using the *Sf*9 insect cell line, three different orbitally shaken culturing vessels were used in the Multitron Cell. Aside from the Cultiflask 50 (Sartorius Stedim Biotech, discontinued; identical to TubeSpin bioreactor 50 from TPP) and the 500 mL disposable shake flask (Corning), the orbitally shaken disposable culturing bag (Cultibag RM basic 2 L, Sartorius Stedim Biotech) featuring the ShakerBag Option (INFORS HT) was used. SEAP (secreted alkaline phosphatase) was produced as a model protein.

The culturing conditions of the three systems are summarised in Table 1.

Parameter	500 mL shake flask	Cultiflask 50	ShakerBag Option 2 L
Amplitude	50 mm	50 mm	50 mm
Temperature	27 °C	27 °C	27 °C
pH	~6.2	~6.2	~6.2
Starting volume for growth	50 mL	12 mL	500 mL
Starting viable cell density	1.06 x 10^6 mL^{-1}	1.02 x 10^6 mL^{-1}	1 x 10^6 mL^{-1}
Gassing	passive through membrane	passive through membrane	0.2 vvm active
Shaking speed	60 rpm	175 rpm	35 rpm

Table 1: Parameter settings for the different culturing vessels

• Virus infection

Following the growth phase of two days, on day 0 p.i. (post injection), the cells were infected with the virus. For this purpose, the culturing systems were adjusted to the desired working volume and the calculated viable cell density.

For all three culturing runs, a viral titre of 1.12 x 10^9 pfu mL^{-1}, a multiplicity of infection (MOI) of 0.01 pfu $cell^{-1}$ and a cell density and/or time of infection (TOI), of 2 x 10^6 cells mL^{-1} were determined. The two parameters as well as the time of harvesting (TOH) were defined individually for each virus and were determined beforehand in preliminary experiments (not

shown) for the virus used herein. Following the SEAP production experiments, the samples from days 4 and 5 p.i. were tested for SEAP activity enzymatically using the Multiskan Spectrum photometer.

Appropriate revolution speeds (rpm) for these culturing systems in the Multitron Cell were determined beforehand based on k_La values and mixing time determinations (results not shown). The settings as used are summarised in Table 2.

	500 mL shake flask	Cultiflask 50	ShakerBag Option 2 L
Starting volume for production	100 mL	20 mL	1000 mL
Shaking speed	60 rpm (Day –2/–1) 70 rpm (Day 0–3) 80 rpm (Day 4–5)	175 rpm (Day –2 to 2) 180 rpm (Day 3–5)	35/36 rpm (Day –2/–1) 37/38 rpm (Day 0/1) 40/42 rpm (Day 2/3) 44/46 rpm (Day 4/5)

Table 2: Adaptation of the shaking speed according to culturing vessel

4. Results

Preliminary experiments in shake flasks

The culturing runs of the *Sf*9 suspension cell line in three parallel batches of shake flasks show an identical course of growth, which is also evident from the viability of the cultures. In all samples, maximal viable cell densities in excess of 15 x 10^6 cells mL^{-1} at a viability >96 % were attained.

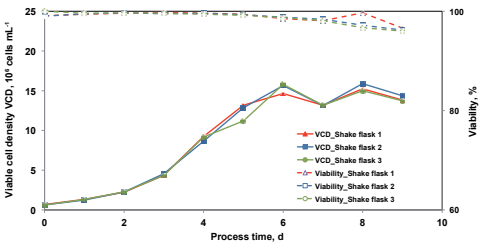

Fig. 2: Parallel culturing runs of the *Sf*9 insect cell line in shake flasks

Preliminary experiments with the ShakerBag Option

A successful test culturing run was also verified in the orbitally shaken Cultibag RM with a working volume of 1 L. The maximal viable cell density was determined to be 14 x 10^6 cells mL^{-1} at a viability of 96 %.

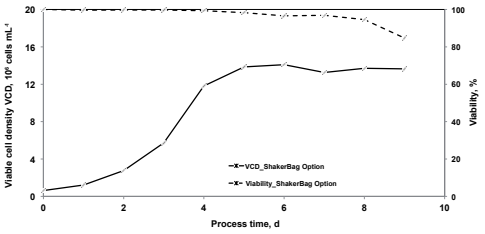

Fig. 3: Culturing of the *Sf*9 insect cell line with ShakerBag Option

Comparison of SEAP production in orbitally shaken culturing systems

For comparison of the cell cultures, the results of preliminary experiments for purposes of process engineering (results not shown) were used and optimal culturing conditions were defined for three different culturing systems. SEAP (secreted alkaline phosphatase) is produced as a model protein.

Figure 4 shows the time profiles of the viable cell density (VCD) and the viability of the three culturing systems. Since all cell cultures show similar behaviour in the growth phase, the infection with virus was done at the same point in time.

Until day 2 p.i., the cell density increased to a maximum of 4.3 x 10^6 cells mL^{-1} (Cultiflask 50) or 4.6 x 10^6 cells mL^{-1} (ShakerBag option with 2 L Cultibag RM). This was followed by a continuous decrease all the way to the production stop on day 5 p.i. It is to be presumed that the production ceased on day 2 p.i. The viability was between 98.5 and 96 % in the first few days of culturing and began to decrease more strongly on day 4 p.i. At the time production was stopped, i.e. on day 5 p.i., the viability was 66.4 %. Looking at the cell diameter, which is an indicator of the degree of infection of the SEAP production by BEVS, behaviour in line with the low degree of infection was observed, which was similar to the two culturing systems Cultiflask and shake flask.

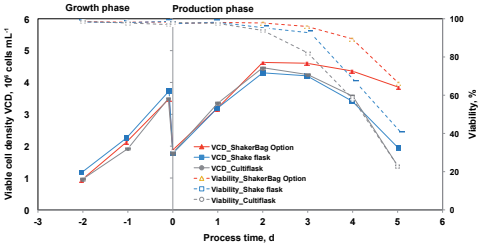

Fig. 4: Comparison of the SEAP production experiments using baculovirus-infected *Sf*9 insect cells

The values from the SEAP assay of the cell suspension on days 4 and 5 p.i. are shown in Table 3 for all culturing vessels. The highest yield of 37.7 ± 1.5 U mL^{-1} was determined for day 5 p.i. and the ShakerBag Option in the orbitally shaken disposable culturing bag.

SEAP in U/mL	500 mL Shake flask	Cultiflask 50	ShakerBag Option 2 L	Control
Day 4 p.i.	29.2	22.3	29.7	0.04
Day 5 p.i.	36.5	30.9	37.7	0.04

Table 3: SEAP analysis on days 4 and 5

5. Conclusion

- A maximal viable cell concentration of the *Sf*9 insect cell line of 15.9 x 10^6 cells mL^{-1} was attained using the shake flask.
- The maximal viable cell concentration for culturing in a 2 L disposable culturing bag was determined to be 14.1 x 10^6 cells mL^{-1}.
- A comparison of the production experiments in three different orbitally shaken culturing vessels showed similar growth behaviour while the cells with ShakerBag Option stay significantly longer in vital status during the production phase.
- The maximal SEAP production of 37.7 U mL^{-1} was attained in the orbitally shaken disposable culturing bag with ShakerBag Option.

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• Werner et al.: An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors. Chemie Ingenieur Technik 2013, 85, No. 1–2, 118–126; DOI: 10.1002/cite.201200153
ZHAW Zürcher Hochschule für Angewandte Wissenschaften, Life Sciences und Facility Management, IBT Institut für Biotechnologie
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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