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

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Application note

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).

Technical specifications

Multitron incubator shaker (50 mm) (INFORS HT, Switzerland)

- «StickyStuff» tray
- CO_2 control 0–20 %

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).

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 (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 incubator shaker

Comparison of SEAP production in orbitally shaken culturing systems

Cell cultivation

For the production experiments using the *Sf*9 insect cell line, two different orbitally shaken culturing vessels were used in the Multitron. The Cultiflask 50 (Sartorius Stedim Biotech, discontinued; identical to TubeSpin bioreactor 50 from TPP) and the 500 mL disposable shake flask (Corning), 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
Amplitude	50 mm	50 mm
Temperature	27 °C	27 °C
pH	~6.2	~6.2
Starting volume for growth	50 mL	12 mL
Starting viable cell density	1.06 x 10^6 mL–1	1.02 x 10^6 mL–1
Gassing	passive through membrane	passive through membrane
Shaking speed	60 rpm	175 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 were determined beforehand based on k_La values and mixing time determinations (results not shown). The settings as used are summarised in Table 2.

Parameter	500 mL shake flask	Cultiflask 50
Starting volume for production	100 mL	20 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)

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

Results

Preliminary experiments in shake flasks

The culturing runs of the S*f*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.

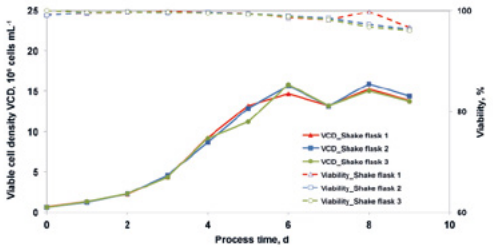

Fig. 2: Parallel culturing runs of the S*f*9 insect cell line in shake flasks

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). 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.

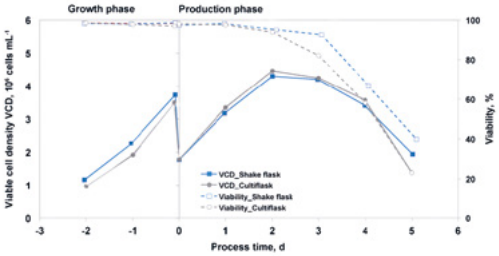

Fig. 3: Comparison of the SEAP production experiments using baculovirus-infected S*f*9 insect cells

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 36.5 ± 1.5 U mL-1 was determined for day 5 p.i.

SEAP in U/mL	500 mL shake flask	Cultiflask 50	Control
Day 4 p.i.	29.2	22.3	0.04
Day 5 p.i.	36.5	30.9	0.04

Table 3: SEAP analysis on days 4 and 5

Conclusion

- A maximal viable cell concentration of the S*f*9 insect cell line of 15.9 x 10^6 cells mL^{-1} was attained using the shake flask.
- A comparison of the production experiments in two different orbitally shaken culturing vessels showed similar growth behaviour.
- The maximal SEAP production of 36.5 U mL^{-1} was attained in the 500 mL shake flask.

- 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

- We would like to thank the CTI for funding project 12599.1 PFLS-LS