Cultivation of *Sf-9* insect cells and SEAP expression in the Finesse «SmartGlass» bioreactor

Stephan C. Kaiser, Ina Dittler, Nina Steiger and Dieter Eibl

Zurich University of Applied Sciences, School of Life Science and Facility Management, Institute of Biotechnology, Biochemical Engineering and Cell Cultivation Techniques, Switzerland

Summary

This application note describes the cultivation of *Sf-9* suspension cells in the Finesse «SmartGlass» vessel bioreactor having a maximum working volume of 2.0 L. Using serum-free medium without components of animal origin viable cell densities of up to $4.964 \cdot 10^6$ cells·mL^{-1} were achieved. The SEAP expression was induced by the baculovirus expression vector system, which provided maximum SEAP activities of 56.4 U·mL^{-1}.

Introduction

Although single-use bioreactors are increasingly introduced in the biopharmaceutical production, multiple-use bioreactors made of glass and/or stainless steel are still of major importance. Most benchtop scale bioreactors, which are almost exclusively fabricated from glass, are agitated by stirrers because of the wide range of applications and the comprehensive knowledge about fluid flow, power input, mixing, mass transfer, and shear stress. They are used for screening experiments, in the seed train or as scale-down model of larger vessels.

The «SmartGlass» bioreactor, which has a benchtop scale vessel with a maximum working volume of 2.0 L, was recently introduced to the market by the company Finesse Solutions, Inc.. The bioreactor was designed to be suitable for cell culture applications and is agitated by a combination of an axially down-pumping segment blade impeller and a bottom-mounted, radially pumping, modified Rushton turbine. The Finesse G3Lab controller representing an Intel-based micro-controller with the capability and robustness of an industrial automation solution is used for the control of the «SmartGlass» bioreactor. It is operated via the Finesse TruBio µC software developed on a C#/.NET foundation with state-of-the-art graphics, database management, and real-time control algorithms, which further includes pre-configured parameters for controlling of the bioprocess, such as pH, DO, temperature, agitation etc..

The present study focusses on the cultivation of *Sf-9* insect cells producing the secreted alkaline phosphatase (SEAP) with the baculovirus expression vector system. After expanding the cells in batch mode for two days using serum-free medium without components of animal origin, the cells were infected by the baculovirus with defined infection characteristics. The bio-reactor cultivation ran up to eight days achieving maximum viable cell densities of $4.964 \cdot 10^6$ cells·mL^{-1}. The maximum SEAP activity was about 56.4 U·mL^{-1}. By increasing impeller speed and sparging of oxygen, it was possible to maintain the dissolved oxygen level around the set-point of 50 %sat, while preventing excessive shear stress or foam production through aeration.

Zurich University of Applied Sciences, Department Life Sciences and Facility Management, Institute of Biotechnology
Contact CH-8820 Wädenswil, Switzerland
Stephan C. Kaiser Phone: +41 (0) 589 34 5464
Stephan.Kaiser@zhaw.ch Fax: +41 (0) 589 34 5001

Materials and methods

Overview of setting-up procedures

Day -6: Inoculum production with *Sf-9* suspension cells in shake flasks.
Day -1/0: Bioreactor preparation and sterilization
Day 0: Inoculation and starting-up of the glass bioreactor with seeding density of $1 \cdot 10^6$ cells·mL^{-1} in 1.6 L Gibco® Sf-900™ III SFM (Invitrogen); Sampling
Day 1: Sampling
Day 2: Sampling; Removal of about 400 mL cell suspension and addition of the virus and fresh media to achieve final volume of 2.0 L and infected cell density (ICD) of $2 \cdot 10^6$ cells·mL^{-1} at a multiplicity of infection (MOI) of 0.01 pfu·cell^{-1} Increase of impeller speed to maintain comparable specific power inputs.
Days 3 – 8: Sampling, analytics and harvest.

Media

The Gibco® Sf-900™ III SFM (Invitrogen), a serum-free medium without components of animal origin, was used as supplied. According to the manufacturer, it is specifically designed to offer high performance and yield using *Sf-9* cells in both baculovirus and stable expression systems.

Inoculum and bioreactor preparation

The inoculum for the glass bioreactor was produced in single-use shake flasks at maximum working volumes of 150 mL. The cells were inoculated with cell densities of about $1.0 \cdot 10^6$ cells·mL^{-1} and subcultivated at about $3 \cdot 10^6$ cells·mL^{-1}. The flasks were shaken at a shaking rate of 120 rpm and amplitude of 25 mm. Before the seeding, the cell suspension of three shake flasks was pooled in a sterile glass bottle (Duran, 1 L) and the cell density was determined to estimate the required volume of the inoculum.
The pH sensor was calibrated using pH 4.01 and pH 7.0 buffers (Mettler Toledo, Germany). The pH and DO probes were installed in the vessel and 800 mL PBS was filled into the glass vessel for sterilization (30 min, 121 °C).
After sterilization, the bioreactor was aerated and tempered to 27 °C for the calibration of the DO sensor. Under the safety cabinet, a sterile glass bottle with antifoam solution (3 g·L^{-1} Emulsion C, Sigma Aldrich) was connected. The PBS solution was removed from the vessel by use of a vacuum pump and 800 mL pre-warmed culture medium was provided into the bioreactor. Afterwards, the inoculum was transferred via a funnel and culture medium was added to meet the desired initial cell density of $1.0 \cdot 10^6$ cells·mL^{-1} at an initial volume of 1.6 L. About 30 min after the cell transfer, the first sample was taken.

Fig. 1: Picture of the novel glass bioreactor together with Finesse TruViu RDPDmini control system

Culture conditions

Culture volume:	1.6- 2.0 L
Agitation speed:	150-180 rpm
pH value:	5.85 – 6.2 (non-regulated)
Temperature:	27 °C
Aeration rate:	0.2 slpm (air, headspace)
	0-0.1 slpm (oxygen, sparger)
Start cell density:	$1.0 \cdot 10^6$ cells mL^{-1}
Cultivation time:	8 days

Sampling and analysis

Samples were taken in place at least once a day by connecting a sterile 10 mL syringe via a clave adapter. The pH value was determined by a pH meter (Mettler Toledo, Switzerland). In-process-control was performed by Cedex HiRes (Roche

Zurich University of Applied Sciences, Department Life Sciences and Facility Management, Institute of Biotechnology
Contact CH-8820 Wädenswil, Switzerland
Stephan C. Kaiser Phone: +41 (0) 589 34 5464
Stephan.Kaiser@zhaw.ch Fax: +41 (0) 589 34 5001

Diagnostics, Germany) determining total and viable cell density, viability as well as the cell diameter. The concentrations of substrates and metabolites (i.e. glucose, glutamine, glutamate, lactate, ammonium) were measured by the multi-bioanalyzer BioProfile 100plus (Nova Biomedical, USA). For these analyses, the cell suspension was centrifuged at 5000 g for 2 min and the supernatant was analyzed. About 2 mL were stored at 4 °C until the analysis of the SEAP activity. This was determined photometrically with a MutliSkan plate reader (Thermo Electron Corporation) detecting the dephosphorylation of para-Nitrophenol-phosphat (pNPP) to para-Nitrophenol (pNP). The SEAP activity was calculated from the change of absorbance per minute of reaction.

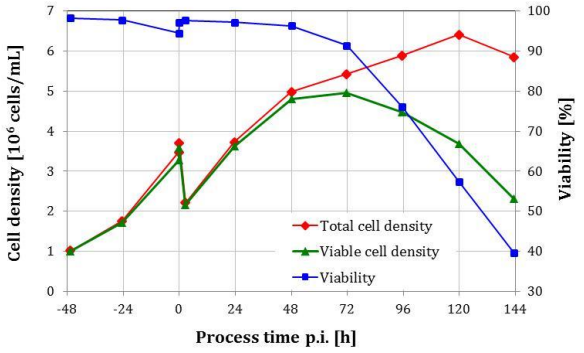
Results

In Fig. 2, the profiles of the total and viable cell density, the viability, and SEAP activity during a cultivation time of 8 days are given. Starting from the initial cell density of $1.01 \cdot 10^6$ cells·mL^{-1}, the cells grew with a mean growth rate of about 0.66 d^{-1} corresponding to a doubling time of 25.3 h. About 48 h after starting the cultivation, 400 mL cell suspension were removed from the vessel, and the virus and fresh culture medium were added to achieve the desired infected cell density (ICD) of $2 \cdot 10^6$ cells·mL^{-1} at the maximum working volume of 2 L. The growth rate after the media addition was lower with 0.425 d^{-1} so that the viable cell density increased up to $4.8 \cdot 10^6$ cells·mL^{-1} until the end of the exponential growth phase.

After 48 h post infection (92 h of cultivation), the cells started to die because of the virus infection. This resulted in a progressive reduction of the cell viability until the end of the process, which was stopped when the cell viability was below 40 %. However, the total cell density further increased and the maximum cell density of $6.41 \cdot 10^6$ cells·mL^{-1} was achieved five days post infection.

The substrate consumption and metabolite production were comparable to our experiences with similar stirred benchtop scale bioreactors (data not shown). During the complete process, the glucose and glutamine concentrations were above 12.5 g·L^{-1} and 7 mmol·L^{-1} respectively. Thus, it can be concluded that the cells were not limited by those substrates. Furthermore, the lactate and ammonium concentrations were, over the complete process, below 0.2 g·L^{-1} and 5.2 mmol·L^{-1} respectively.

a



b

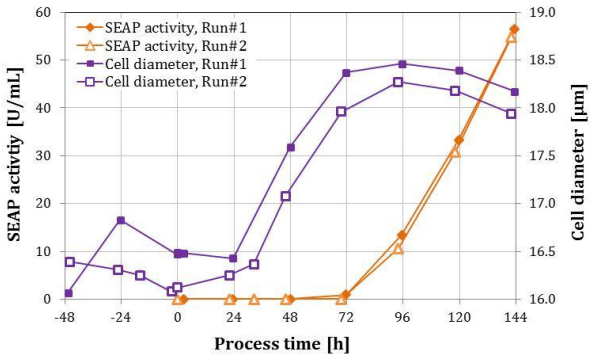


Fig. 2: Offline-data of the *Sf-9* insect cell culture in the «SmartGlass» bioreactor (a) Total and viable cell density and viability, (b) SEAP activity and average cell diameter.

The SEAP activity started to increase after about two days post infection (see Fig. 2b), which is agreement to our expectations. Afterwards, the activity increased rapidly and a final SEAP activity of 56.4±0.2 U·mL^{-1} was detected at the end of the cultivation. This profile was perfectly reproduced in a second run accomplished with identical infection characteristics. Here, the final SEAP concentration was 54.9±0.74 U·mL^{-1}, which means only slightly lower than in the first run. However, the difference is within the confidence range of the SEAP analysis and, therefore, very good reproducibility can be stated.

The increase of the SEAP activity correlates well with the increase of the average cell diameter, which is an indication of the successful infection. Due to the virus infection and multiplication, the cells were

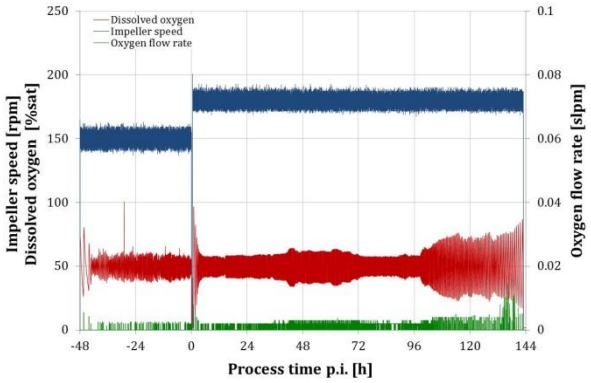
Zurich University of Applied Sciences, Department Life Sciences and Facility Management, Institute of Biotechnology
Contact CH-8820 Wädenswil, Switzerland
Stephan C. Kaiser Phone: +41 (0) 589 34 5464
Stephan.Kaiser@zhaw.ch Fax: +41 (0) 589 34 5001

enlarged about one day after the virus infection, so that the average cell diameter increased from about 16.5 µm to 18.5 µm. Again good agreements were obtained in the second run, although the profile showed an offset of about 0.3 µm compared to first run.

During the complete cultivation, the DO level was maintained above critical levels preventing oxygen limitations of the cells (see Fig. 3). The dissolved oxygen was controlled in a range of 40 %sat to 60 %sat over the majority of the process. Some higher fluctuations only occurred at the beginning of the cultivation and after the virus infection, which may be explained by the limited dynamic range of the mass flow controllers for oxygen and bubble attachment to the membrane of the DO probe. However, foam formation was effectively prevented due to the low gassing rates of below 0.02 slpm (corresponding to 0.01 vvm).

Very good agreement between off-line measured pH values and online data was found over the complete process (see Fig. 3b). No drift of the pH probes was found and, therefore, no re-calibration of the sensor was required. The pH decreased from the initial pH 6.18 to pH 6.02 before the infection and to pH 5.87 after about 75 h p.i., which can be explained by acid and CO_2 formation during the cell growth. During the death phase the pH value increased again to pH 6.14 measured in the last sample.

a



b

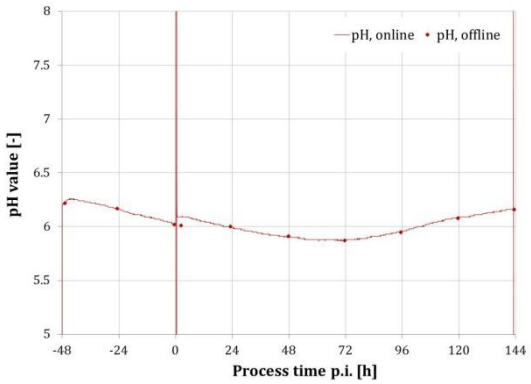


Fig. 3: Online data of the *Sf-9* insect cell culture in the «SmartGlass» bioreactor. (a) The impeller speed, dissolved oxygen level and O_2 flow rate used for the DO control, (b) Comparison of online measured pH values with offline analyses. The virus infection was realized at 48 h of cultivation, which can easily be identified by the peak in the pH profile resulting from the disconnection of the pH probe from the control unit.

Zurich University of Applied Sciences, Department Life Sciences and Facility Management, Institute of Biotechnology
Contact CH-8820 Wädenswil, Switzerland
Stephan C. Kaiser Phone: +41 (0) 589 34 5464
Stephan.Kaiser@zhaw.ch Fax: +41 (0) 589 34 5001