Cultivation of Sf9 insect cells and SEAP expression in the HyPerforma Glass Bioreactor

Summary

This application note describes the cultivation of Sf9 suspension cells in the Thermo Scientific™ HyPerforma™ Glass Bioreactor with a maximum working volume of 2.0 L. Using a serum-free medium without components of animal origin, viable cell densities of up to 4.964 x 10^6 cells/mL were achieved. Expression of secreted alkaline phosphatase (SEAP) protein was induced by the baculovirus expression vector system, which provided maximum SEAP activity of 56.4 U/mL.

Introduction

Although single-use disposable bioreactors are increasingly being introduced in 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 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 models for larger vessels.

The HyPerforma Glass Bioreactor, which has a benchtop-scale vessel with a maximum working volume of 2.0 L, 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 HyPerforma Glass Bioreactor can be controlled using the Thermo Scientific™ HyPerforma™ G3Lab™ Controller in conjunction with Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ system (Figure 1).

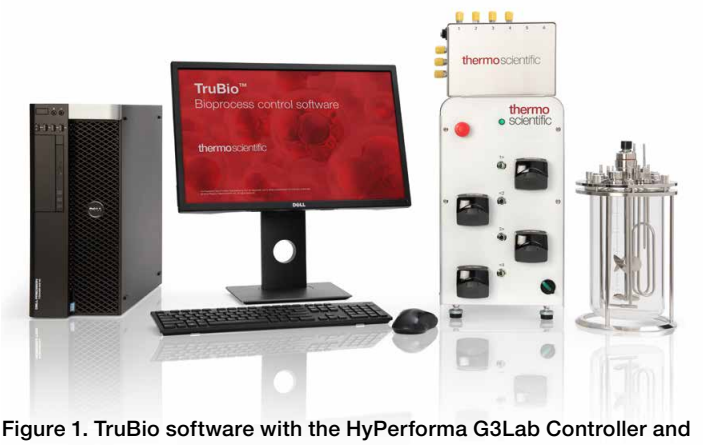

Figure 1. TruBio software with the HyPerforma G3Lab Controller and the HyPerforma Glass Bioreactor.

The present study focuses on the cultivation of Sf9 insect cells producing the 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 bioreactor cultivation ran up to eight days, achieving a maximum viable cell density of 4.964 x 10^6 cells/mL. The maximum SEAP activity was 56.4 U/mL. By increasing impeller speed and sparging of oxygen it was possible to maintain the dissolved oxygen (DO), level around the set point of 50% saturation, while preventing excessive shear stress or foam production due to aeration.

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Materials and methods

Overview of procedure setup

- Day –6: Inoculum production with Sf9 suspension cells in shaker flasks.
- Day –1 and 0: Bioreactor preparation and sterilization.
- Day 0: Inoculation and startup of the bioreactor with a seeding density of 1 x 10^6 cells/mL in 1.6 L Gibco™ Sf900™ III SFM; sampling.
- Day 1: Sampling.
- Day 2: Sampling; removal of approximately 400 mL cell suspension and addition of the virus and fresh medium to achieve final volume of 2.0 L and infected cell density (ICD) of 2 x 10^6 cells/mL at a multiplicity of infection (MOI) of 0.01 PFU/cell. Increase of impeller speed to maintain comparable specific power inputs.
- Day 3: Sampling, analytics, and harvesting.

Media

The Sf900 III SFM, a serum-free medium without components of animal origin, was used as supplied. It is specifically designed to offer high performance and yield with Sf9 cells in both baculovirus and stable expression systems.

Inoculum and bioreactor preparation

The inoculum for the HyPerforma Glass Bioreactor was produced in single-use shaker flasks at maximum working volumes of 150 mL. The cells were inoculated with cell densities of about 1.0 x 10^6 cells/mL and sub-cultivated at about 3.0 x 10^6 cells/mL. The flasks were shaken at a rate of 120 rpm and amplitude of 25 mm. Before seeding, the cell suspension from three shaker 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). The pH and DO probes were installed in the vessel, and 800 mL PBS was added to 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 Emulsion C, Sigma Aldrich) was connected. The PBS solution was removed from the vessel by use of a vacuum pump, and 800 mL of prewarmed culture medium was added to 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 x 10^6 cells/mL at an initial volume of 1.6 L. About 30 minutes after the cell transfer, the first sample was taken.

Culture conditions	
Culture volume	1.6–2.0 L
Agitation speed	150–180 rpm
pH value	5.82–6.2 (non-regulated)
Temperature	27°C
Aeration rate	0.2 slpm (air, headspace) 0–0.1 slpm (oxygen, sparger)
Seeding density	1.0 x 10^6 cells/mL
Cultivation time	8 days

Sampling and analysis

Samples were taken in place at least twice a day by connecting a sterile 10 mL syringe via a Clave™ adapter. The pH value was determined by a pH meter (Mettler Toledo). In-process control was performed using a Cedex™ HiRes Analyzer (Roche Diagnostics) to determine total and viable cell density, viability, and the cell diameter. The concentrations of substrates and metabolites (e.g., glucose, glutamine, glutamate, lactate, and ammonium) were measured by the BioProfile™ 100 Plus bioanalyzer (Nova Biomedical). For these analyses, the cell suspension was centrifuged at 5,000 x *g* for 2 min, and the supernatant was analyzed. Approximately 2 mL were stored at 4°C until the photometric analysis using a Thermo Scientific™ Multiskan™ microplate photometer to detect the dephosphorylation of para-nitrophenol-phosphate (pNPP) to para-nitrophenol (pNP). The SEAP activity was calculated from the change of absorbance per minute of reaction.

Results

In Figure 2, the profiles of the total cell density and viability and SEAP activity during a cultivation time of 8 days are given. Starting from the initial cell density of 1.01 x 10^6 cells/mL, the cells grew with a mean growth rate of 0.66 per day, corresponding to a doubling time of 25.3 hours. About 48 hours after starting the cultivation, 400 mL of cell suspension was removed from the vessel, and the virus and fresh culture medium were added to achieve the desired ICD of 2 x 10^6 cells/mL at the maximum working volume of 2 L. The growth rate after the medium addition was lower at 0.43 per day, so that the viable cell density increased to 4.8 x 10^6 cells/mL at the end of the exponential growth phase.

48 hours postinfection (after 92 hours of cultivation), the cells started to die because of the virus infection. This resulted in a progressive reduction in 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 x 10^6 cells/mL 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 and 7 mmol/L, respectively. Thus, it can be concluded that the cells were not limited by those substrates. Furthermore, over the complete process, the lactate and ammonium concentrations were below 0.2 g/L and 5.2 mmol/L, respectively.

The SEAP activity started to increase after about two days postinfection (Figure 2B), which aligned with our expectations. Afterwards, the activity increased rapidly and a final SEAP activity of 56.4 ± 0.2 U/mL 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, which is only slightly lower than in the first run. However, the difference is within the confidence range of the SEAP analysis and, therefore, very high reproducibility can be inferred.

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 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, consistency was obtained in the second run, although the profile showed an offset of about 0.3 µm compared to first run.

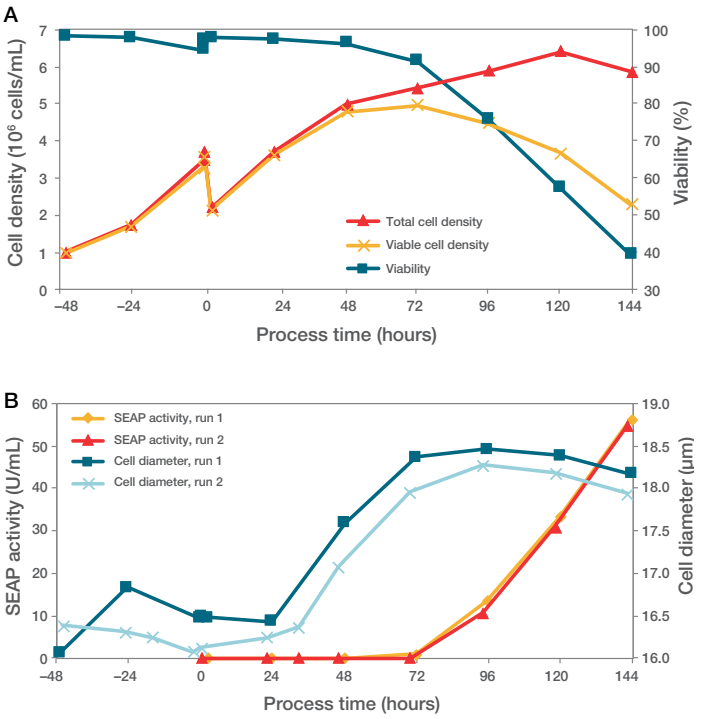

Figure 2. Offline data from the Sf9 insect cell culture in the HyPerforma Glass Bioreactor. (A) Total and viable cell density and viability. **(B)** SEAP activity and average cell diameter.

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During the complete cultivation, the DO level was maintained above critical levels, preventing oxygen limitations of the cells (Figure 3A). The DO was controlled within a range of 40% to 60% saturation 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).

Consistency between offline measured pH values and online data was found over the complete process (Figure 3B). No drift of the pH probes was found and, therefore, no recalibration of the sensor was required. The pH value decreased from 6.24 in the beginning of the cultivation to pH 6.02 before the infection, and to pH 5.87 after about 75 hr postinfection, 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, as measured in the last sample.

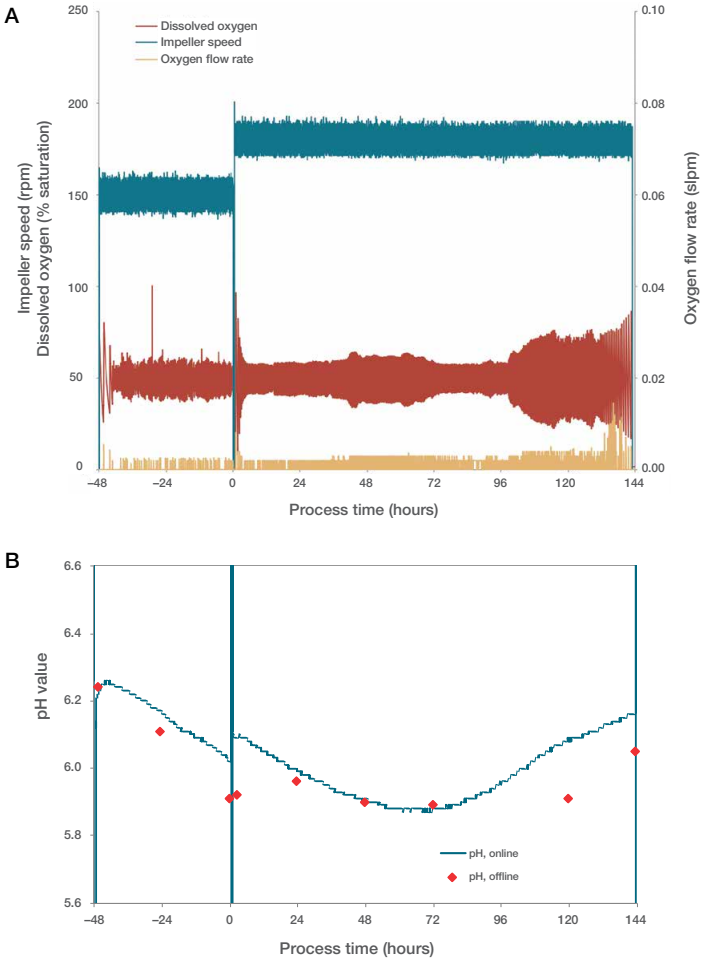

Figure 3. Online data of the Sf9 insect cell culture in the HyPerforma Glass Bioreactor. (A) Comparison of online measured pH values with offline analyses. **(B)** The impeller speed, DO level, and O_2 flow rate used for the DO control. The virus infection was realized at 48 hr of cultivation, which can easily be identified by the peak in the pH profile resulting from the disconnection of the pH probe from the control unit.

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