Cultivation of Sf9 insect cells and rSEAP expression in the HyPerforma Rocker Bioreactor

Summary

This application note describes the cultivation of Sf9 suspension cells in the Thermo Scientific™ HyPerforma™ Rocker Bioreactor with a maximum working volume of 5.0 L. Using a serum-free medium without components of animal origin, viable cell densities of up to 6.2 x 10^6 cells/mL were achieved. Expression of recombinant secreted alkaline phosphatase (rSEAP) was induced using a baculovirus expression vector system. rSEAP activity up to a maximum of 58.5 U/mL was achieved. The results are in the typical range observed in previous cultivations using wave-mixed bioreactor types.

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

Introduction

The HyPerforma Rocker Bioreactor (Figure 1) is a lab-scale rocker-type bioreactor that is characterized by low shear stresses and is often used for seed train production in biopharmaceutical production processes.

The HyPerforma Rocker 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).

The scope of the study was to demonstrate the applicability of the HyPerforma Rocker Bioreactor for cultivating a *Spodoptera frugiperda* (Sf9) insect cell line in a biphasic production process using serum-free medium and expressing a model protein, rSEAP. The production process was based on a baculovirus expression vector system (BEVS).

Materials and methods

Overview of procedure setup

- Day –6/–1: Inoculum production with Sf9 suspension cells in shaker flasks.
- Day 0: Bioreactor preparation, inoculation with seeding density of 1 x 10^6 cells/mL in 3.0 L of Gibco™ Sf-900™ III SFM.
- Day 1: Sampling, starting of control loop for pH.
- Day 2: Sampling; addition of the virus suspension and fresh medium to achieve a final volume of 5.0 L and infected cell density (ICD) of 2 x 10^6 cells/mL at a multiplicity of infection (MOI) of 0.01 PFU/cell.
- Day 3: Sampling, analytics, and harvesting. Production was stopped 7 days postinfection. This corresponds to a time of harvest (TOH) of 164 hours.

Medium

Gibco™ Sf9 cells were cultivated in Sf-900 III SFM containing Gibco™ Pluronic™ F-68 and L-glutamine. The expression of rSEAP was induced by a baculovirus expression vector system (Sil9.1.1_GFP_SEAP_His MP 9.8 V2, provided by ZHAW IBT Molecular Biology) with a virus titer of 1.5 x 10^9 PFU/cell.

Inoculum preparation

The inoculum for the HyPerforma Rocker Bioreactor was produced in single-use 250 and 500 mL shaker flasks at working volumes of 100 and 200 mL. The cells (passage #33) were inoculated at cell densities of about 0.7–1.0 x 10^6 cells/mL. The flasks were placed in a shaking incubator (Ecotron™, Infors™ HT, CH) at 27°C with a shaking rate of 100 rpm and an amplitude of 25 mm. A cell suspension of 1,200 mL with a cell density of approximately 3.3 x 10^6 cells/mL was produced for inoculation of the HyPerforma Rocker Bioreactor.

Bioreactor preparation

A NaOH solution was produced, put in Duran™ glass bottles, and autoclaved for 20 minutes at 121°C. The HyPerforma Rocker BioProcess Container (BPC) was filled with 1 L of the fresh medium using a sterile syringe connected to the luer lock connector, and placed on the rocking platform. After the aeration (0.25 slpm) and heating (27°C) were switched on, one-point calibration was performed for the pH sensor. A sample was taken and the pH was determined using an external pH meter (Mettler-Toledo). The pO_2 sensor was calibrated to 100% saturation.

Inoculation preparation

The inoculation procedure was performed under a laminar flow, where the Duran glass bottle with the NaOH solution (0.1 M) was connected to the HyPerforma Rocker BPC via a luer lock connector directly before inoculation. In order to achieve the desired cell density of 1 x 10^6 cells/mL, 0.95 L of the cell suspension was added to the bioreactor through a sterile funnel, followed by 1.05 L of fresh medium, to achieve the initial working volume of 3.0 L. Afterwards, the bioreactor was reconnected to the control unit and all the control loops for dissolved oxygen (DO) and temperature were started. The settings were defined as given below. The control loop for pH was started after 24 hours of process time.

Process parameters

Temperature was controlled automatically via an integrated heater in the rocking platform. DO concentration was controlled using a cascade function with rocking rate as the primary factor, and the addition of pure oxygen as the secondary factor. To automatically control the pH during the growth phase, a control loop with the addition of 0.1 M NaOH solution at a maximum pump speed of 50 rpm (corresponding to a flow rate of 16 min/mL) was set up. The pH control was started 24 hours after inoculation.

Culture conditions	
Temperature	27°C
DO concentration	50% saturation
pH	6.2
Rocking rate	18–32 rpm
Rocking angle	6°
Air flow rate (headspace)	0.25 min/L
Maximum O_2 flow rate (headspace)	0.25 min/L

Initiation of rSEAP production

The production of rSEAP was induced by infection of the Sf9 cells with the baculovirus. Virus quantification was performed using three different methods: plaque assay (PA), endpoint dilution assay (EDA), and cell growth cessation assay (CGCA), as described [1]. The effective virus titer VT_{eff} was then calculated from a combination of the outcomes of these assays, as follows:

$$VT_{eff} = \frac{\left(\frac{VT_{PA} + VT_{EDA}}{2}\right) + VT_{CGCA}}{2}$$

where VT_{PA}, VT_{EDA}, and VT_{CGCA} are the virus titers (in PFU/mL) from the individual virus assays. The infection parameters, multiplicity of infection (MOI) of 0.01 PFU/cell and infected cell density (ICD) of 2 x 10^6 cells/mL, were selected based on preliminary studies performed in our laboratory. Two days after inoculation, when the live cell density in the HyPerforma Rocker BPC had reached 3.6 x 10^6 cells/mL, the control loops were switched off and the HyPerforma Rocker BPC was disconnected from the control unit.

The infection procedure was performed under a laminar flowhood. After ensuring a live-cell density of 2 x 10^6 cells/mL in a working volume of 5 L, the Rocker BPC was placed on scales, and 66.7 mL of the virus preparation at a dilution of 1:1,000 was added via a sterile syringe to achieve the desired MOI of 0.01 PFU/cell. The culture volume was topped with 2.35 L of fresh medium to achieve a total volume of 5 L. Afterwards, the HyPerforma Rocker BPC was placed on the rocking platform and all the controllers were switched on. The rocking rate was set to 25 rpm for 1 hour.

Process analysis

Samples of at least 4 mL (6 mL during the production phase) were taken at least once a day from the HyPerforma Rocker BPC via the luer lock sampling port using sterile 10 mL syringes. Cell densities and viability were determined using a NucleoCounter™ NC-100™ Mammalian Cell Counter (ChemoMetec). Dilutions were performed with PBS, after the cell density reached 6 x 10^6 cells/mL. A Cedex™ HiRes cell counting device (Roche Diagnostics) was used to 6 determine cell diameter. Nutrients and metabolites were measured using a BioProfile™ 100 Plus multi-biosensor analysis system (Labor-Systeme Flükiger AG).

The rSEAP activity was measured indirectly via enzymatic transformation of *p*-nitrophenyl phosphate into *p*-nitrophenyl at 405 nm, using a magnesium chloride buffer (magnesium chloride, homoarginine hydrochloride, and diethanolamine adjusted to pH 9.8) and *p*-nitrophenyl phosphate (disodium salt, hexahydrate) as a substrate solution. The quality of the expressed product was not investigated.

Results

During the first 48.5 hours, the cell density in the HyPerforma Rocker BPC increased from 1 x 10^6 cells/mL to 3.9 x 10^6 cells/mL with a specific growth rate of 0.029 per hour, corresponding to a doubling time of 23.9 hours (Figure 2A). After infection with the baculovirus, the cells grew exponentially over the next 2 days and reached a maximum viable cell density of 6.1 x 10^6 cells/mL. The cell viability in the HyPerforma Rocker Bioreactor was 98.9 ± 0.4% up to this point in time. The process was aborted after 163 hours postinfection (213 hours total time), when the cell viability dropped to zero.

A slightly increased cell diameter of 14.9 µm and minor rSEAP activity of about 0.1 U/mL were detected 2 days postinfection (Figure 2B). Afterwards, the rSEAP activity increased rapidly and reached 59 ± 4 U/mL at the end of the process (163 hours postinfection). This is approximately 40 hours later than in previous experiments conducted in our laboratory, despite comparable peak cell densities [2]. The reason could have been the fact that less of the virus was added, which caused fewer cells to be infected by primary infection. Hence, more infection cycles were required until all the cells in the HyPerforma Rocker BPC had been infected. The increase in rSEAP activity was accompanied by an increase in the average cell diameter to a maximum of 16.3 µm after 142 hours postinfection.

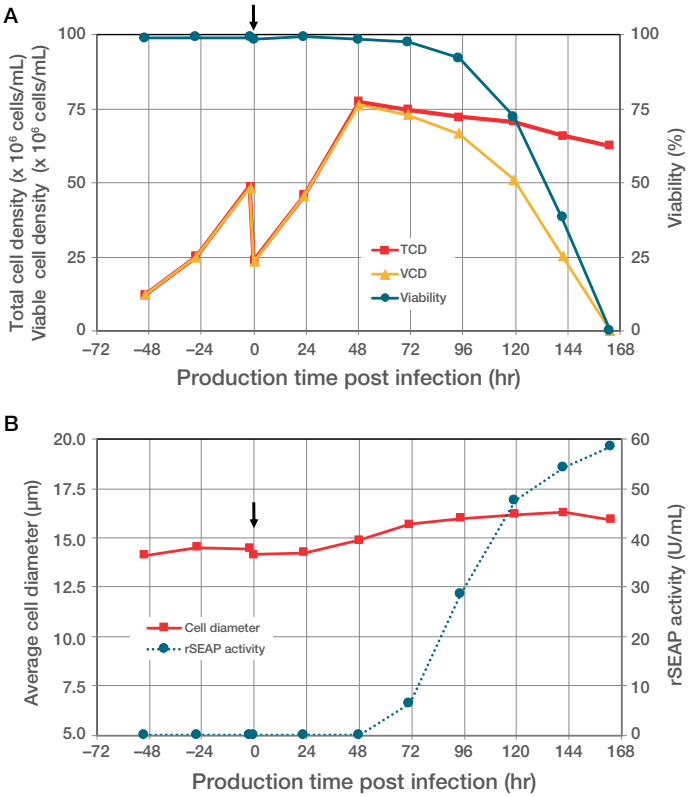

Figure 2. Analysis of samples over the culture period.
(A) Cell densities and viability. **(B)** Cell diameter and rSEAP activity. The arrow indicates the time of virus addition to initiate the protein expression.

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The DO concentration decreased continuously from 86% saturation after inoculation to 59% saturation before virus infection, indicating cell growth. After virus infection (indicated by the arrow in Figure 3), the rocking rate was set to a constant value of 25 rpm for 1 hour in order to ensure sufficient mixing to disperse the virus. After restarting the control loop for oxygen, the DO concentration immediately dropped to 33% saturation and the rocking rate automatically increased in order to maintain the DO concentration at the defined set point of 50% saturation. The rocking rate reached 29 rpm 57 hours postinfection, before the cells entered the stationary phase. The DO concentration in the HyPerforma Rocker BPC remained at 50.02 ± 0.14% saturation until 172 hours postinfection. No addition of pure oxygen was required.

The control loop for pH was started after 24 hours of process time and was run for 26 hours. Overall, 27 mL of 0.1 M NaOH was added. During this time, the pH in the HyPerforma Rocker BPC was 6.20 ± 0.01, indicating correct pH regulation in the HyPerforma Rocker Bioreactor. The pH values agreed well with offline measurements, with deviations below 0.2 pH units.

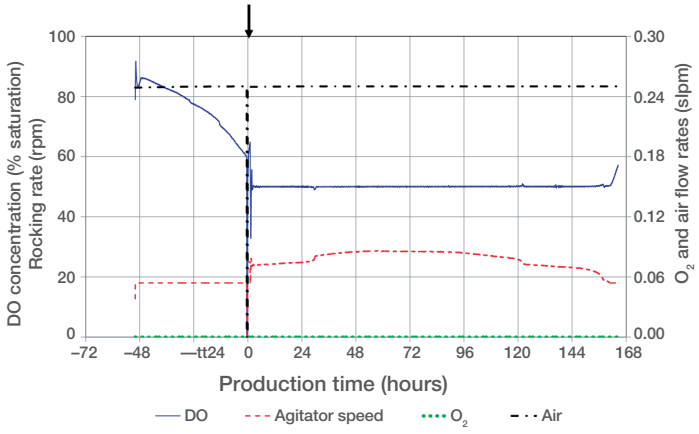

Figure 3. Online data for DO concentration, agitation, and oxygen flow rate in the HyPerforma Rocker BPC.

Conclusions

The HyPerforma Rocker Bioreactor was successfully tested in a biphasic Sf9 cell–based growth and rSEAP production process. The maximum viable cell density in the HyPerforma Rocker BPC of 6.1 x 10^6 cells/mL was measured after 48.3 hours postinfection. The maximum rSEAP activity of 59 ± 4 U/mL in the HyPerforma Rocker BPC was measured 163 hours postinfection (213 hours of process time). The results for cell growth, peak cell density, and maximum rSEAP activity are in the typical ranges observed in previous cultivation using other wave-mixed bioreactor types.

References

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2. Imseng N, Steiger N, Frasson D et al. (2014) Single-use wave-mixed versus stirred bioreactors for insect-cell/BEVS-based protein expression at benchtop scale. Engineering in Life Sciences 14(3):264–271.

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