Cultivation of *Sf-9* Insect Cells and rSEAP Expression in the Wave-Mixed Single-Use Finesse SmartRocker Bioreactor

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Summary

This application note describes the cultivation of *Sf-9* suspension cells in the wave-mixed single-use Finesse SmartRocker bioreactor with a maximum working volume of 5 L. Using serum-free, it was possible to produce up to $6.2 \cdot 10^6$ cells·mL^{-1}. The rSEAP expression was induced by the baculovirus expression vector system. Maximum rSEAP activity of up to 58.5 U·mL^{-1} was achieved. The results are in the typical range observed in previous cultivations using wave-mixed bioreactor types.

Introduction

The novel single-use SmartRocker bioreactor from Finesse Solutions, Inc. (see Fig. 1) is a lab-scale rocker-type bioreactor that is characterized by low shear stress rates and is often used for seed train production in biopharmaceutical production processes. The scope of the study was to demonstrate the applicability of the SmartRocker bioreactor for cultivating a *Spodoptera frugiperda* (*Sf-9)* insect cell line in a bi-phasic production process using serum-free medium and expressing a model protein: recombinant secreted alkaline phosphatase (rSEAP). The production process was based on the baculovirus expression vector system (BEVS).

A Finesse G3Lab Universal Bioreactor Controller with DeltaV control platform from Emerson process Management was used to control the SmartRocker and provide data acquisition. The system was controlled by Finesse TruBio DV Control Software. The cells were cultivated in a 10 L SmartBag (maximum working volume of 5 L) equipped with single-use optical pH and dissolved O_2 Finesse TruFluor sensors.

Overview of Setting-up Procedures

Days -6/-1: Inoculum production in shake flasks.

Day 0: Bioreactor preparation, inoculation with seeding density of $1 \cdot 10^6$ cells·mL^{-1} in 3 L Gibco® Sf-900™ III SFM medium.

Day 1: Sampling, starting of control loop for pH.

Day 2: Sampling, addition of virus solution and fresh medium to achieve a final volume of 5 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}.

Days 3/7: Sampling, analytics and harvest. Production was stopped seven days post infection. This corresponds to a time of harvest (TOH) of 164 hours.

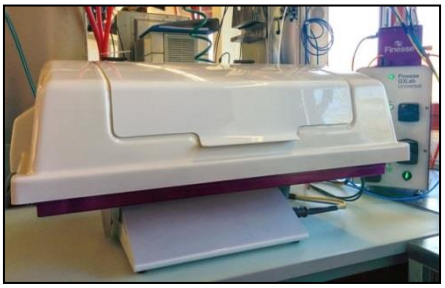

Fig. 1: The novel wave-mixed single-use SmartRocker bioreactor with Finesse G3Lab Universal Bioreactor Controller.

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Materials and Methods

Medium

The cells (non-engineered insect cell line *Sf*-9, Invitrogen, USA) were cultivated in Gibco® Sf-900™ III SFM serum-free medium containing Pluronic® F-68 and L-glutamine (Gibco, Invitrogen, CH). The expression of rSEAP was induced by the baculovirus expression vector system (Sil9.1.1_GFP_SEAP_His MP 9.8 V_2 provided by ZHAW IBT molecular biology) with a virus titer of $1.5 \cdot 10^9$ pfu·mL^{-1}.

Inoculum Preparation

The inoculum for the SmartBag was produced in polycarbonate single-use 250 and 500 mL shake flasks (Corning, USA) at working volumes of 100 and 200 mL, respectively. The cells (passage #33) were seeded with cell densities of 0.7-1.0·10^6 cells·mL^{-1}. The shake flasks were placed in a shaking incubator (Ecotron, Infors HT, CH) at 27 °C, a shaking rate of 100 rpm and an amplitude of 25 mm. 1200 mL cell of suspension with a cell density of approximately $3.3 \cdot 10^6$ cells·mL^{-1} was produced for inoculation of the SmartBag bioreactor.

Bioreactor Preparation

A NaOH solution (0.1M, Sigma-Aldrich, CH) was produced, put in Duran glass bottles and autoclaved for 20 minutes at 121 °C. The SmartBag was filled with 1 L of the fresh medium using a sterile syringe connected to the LuerLock 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, CH). The pO_2 sensor was calibrated to 100% saturation.

Inoculation Procedure

The inoculation procedure was performed under a laminar flow, where the Duran glass bottle with the NaOH solution (0.1M) was connected to the SmartBag via a LuerLock connector directly before inoculation. In order to achieve the desired cell density of $1 \cdot 10^6$ cells·mL^{-1}, 0.95 L of the cell suspension was added to the bioreactor through a sterile funnel. 1.05 L fresh medium was added 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 DO and temperature were started. The settings were defined as given below. The control loop for pH was started after 24 hours process time.

Process Parameters

Temperature was controlled automatically via an integrated heater in the rocking platform. Dissolved oxygen 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 0.1M NaOH solution at a maximum pump speed of 50 rpm (corresponding to a flow rate of 16 mL·min^{-1}) was set up. The pH control was started 24 hours after inoculation.

Parameter	Value
Temperature	27 °C
Dissolved oxygen concentration	50 %sat
pH value	6.2
Rocking rate	18 – 32 rpm
Rocking angle	6 °
Air flow rate (headspace)	0.25 L·min^{-1}
Max. O_2 flow rate (headspace)	0.25 L·min^{-1}

Initiation of rSEAP Production

The production of rSEAP was induced by infection of the *Sf-9* 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 in Roldao et al., 2009. The effective virus titer VT_{eff} was then calculated from a combination of the outcomes of these assays, as follows:

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

where VT_{PA}, VT_{EDA} and VT_{CGCA} are the virus titers (in pfu mL^{-1}) from the individual virus assays.

The infection parameters, multiplicity of infection (MOI) of 0.01 pfu·cell^{-1} and infected cell density (ICD) of $2 \cdot 10^6$ cells·mL^{-1} were selected based on preliminary studies performed in our laboratory. Two days after inoculation, when the live cell density in the SmartBag had reached a value of $3.6 \cdot 10^6$ cells·mL^{-1}, the control loops were switched off and the SmartBag was disconnected from the control unit.

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The infection procedure was performed under a laminar flow. After ensuring a living cell density of $2 \cdot 10^6$ cells·mL^{-1} at a working volume of 5 L, the SmartBag was placed on scales and 66.7 mL of the virus solution with a dilution of 1:1000 was added via a sterile syringe to achieve the desired MOI of 0.01 pfu·cell^{-1}. The culture volume was topped up with 2.35 L fresh medium to achieve a total volume of 5 L. Afterwards, the SmartBag was placed on the rocking platform and all the controllers were switched on. The rocking rate was set to 25 rpm for one hour.

Process Analysis

Samples of at least 4 mL (6 mL during the production phase) were taken at least once a day from the SmartBag via the LuerLock sampling port using sterile 10 mL syringes. Cell densities and viability were determined using a NucleoCounter® NC-100™ (ChemoMetec, Denmark). Dilutions were performed with PBS, after the cell density reached a value of $6 \cdot 10^6$ cells·mL^{-1}. A Cedex HiRes cell counting device (Roche Diagnostics, Germany) was used to determine cell diameters. The nutrients and metabolites were measured using a Bioprofile 100 Plus multi biosensor analysis system (Labor-Systeme Flükiger AG, CH).

The rSEAP activity was measured indirectly via enzymatic transformation of para-nitrophenylphosphate into para-nitrophenyl at 405 nm, using a magnesium chloride buffer (magnesium chloride, homoarginine hydrochloride and diethanolamine adjusted to pH 9.8) and p-nitrophenolphosphate di-sodium 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 SmartBag increased from $1 \cdot 10^6$ cells·mL^{-1} to $3.9 \cdot 10^6$ cells·mL^{-1} with a specific growth rate of 0.029 h^{-1}, corresponding to a doubling time of 23.9 hours (Fig. 2a). After infection with the baculovirus, the cells grew over the next two days and reached a maximum viable cell density of $6.1 \cdot 10^6$ cells·mL^{-1} after 98 hours process time. The cell viability in the SmartBag was 98.9±0.4% up to this point in time. The process was aborted after 213 hours (163 hours post infection), 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^{-1} were detected after two days post infection (Fig. 2b). Afterwards, the rSEAP activity increased rapidly and reached 59±4 U·mL^{-1} at the end of the process (163 hours post infection). This is approximately 40 hours later than in previous experiments conducted in our laboratory, despite comparable peak cell densities (Imseng et al., 2014). 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 SmartBag 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 191.5 hours process time.

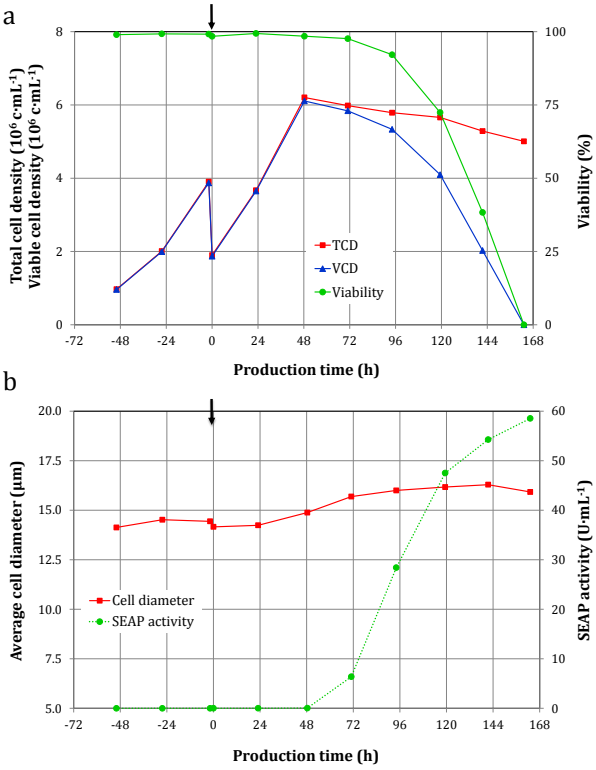

Fig. 2: a) Cell densities and viability, b) cell diameter and rSEAP activity. The arrow indicates the time of virus infection, after 50 hours process time.

The dissolved oxygen concentration decreased continuously from 86 %sat after inoculation to 59 %sat before virus infection, indicating cell growth. After virus infection (indicated by the arrow in Fig. 3), the rocking rate was set to a constant value

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of 25 rpm for one 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 %sat and the rocking rate automatically increased in order to maintain the dissolved oxygen concentration at the defined set-point of 50 %sat. The rocking rate reached 29 rpm at 107 hours process time, before the cells entered the stationary phase. The DO concentration in the SmartBag remained at 50.02±0.14 %sat until 212 hours process time. No addition of pure oxygen was required.

The control loop for pH was started after 24 hours process time and was run for 26 hours. Overall, 27 mL base was added (0.1M NaOH solution). During this time, the pH value in the SmartBag was 6.201±0.008, indicating correct pH regulation in the SmartRocker. The pH values agreed well with offline measurements, with deviations below 0.2 pH units.

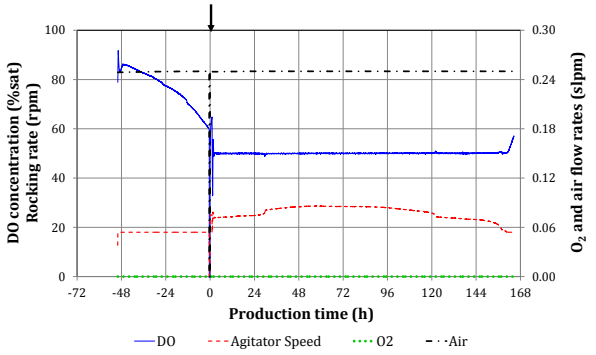

Fig. 3: Online data for dissolved oxygen concentration, agitation and oxygen flow rate in the SmartBag.

Conclusions

The novel wave-mixed single-use SmartRocker bioreactor from Finesse Solutions, Inc. was successfully tested in a bi-phasic *Sf-9* cell based growth and rSEAP production process. The maximum viable cell density in the SmartBag of $6.1 \cdot 10^6$ cells·mL^{-1} was measured after 48.3 hours post infection. The maximum rSEAP activity of 59±4 U·mL^{-1} in the SmartBag was measured after 163 hours post infection (213 hours process time). The results for cell growth, peak cell density and maximum rSEAP activity are in the typical range observed in previous cultivations using other wave-mixed bioreactor types.

References

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