Cultivation of CHO suspension cells and SEAP expression in the HyPerforma Rocker Bioreactor

Summary

This application note describes the cultivation of CHO suspension cells in the wave-mixed Thermo Scientific™ HyPerforma™ Rocker Bioreactor with a maximum working volume of 5 L. Using chemically defined minimal media, cell densities of up to 16.4 x 10^6 cells/mL were achieved. Secreted alkaline phosphatase (SEAP) expression was induced by medium exchange and temperature shift. A maximum SEAP activity of 33.1 U/mL was reached.

Introduction

The HyPerforma Rocker Bioreactor 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 (Figure 1). The scope of the study was to demonstrate the applicability of the HyPerforma Rocker Bioreactor for cultivating CHO XM 111-10, (obtained from Prof. Dr. Martin Fussenegger, Department of Biosystems Science and Engineering of the ETH Zurich) in fed-batch mode. Chemically-defined minimal culture medium was used to express the SEAP model protein. Secretion was induced and supported by medium exchange and a temperature shift.

Thermo Scientific™ HyPerforma™ G3Lab™ Controller was used in conjunction with Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ system to control the HyPerforma Rocker Bioreactor and acquire data (Figure 1). The cells were cultivated in a 10 L Thermo Scientific™ Rocker BioProcess Container (BPC) (maximum working volume of 5 L) equipped with single-use optical pH and dissolved oxygen (DO) sensors.

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

Materials and methods

Overview of procedure setup

- Day –6 and –1: Inoculum production in shaker flasks
- Day –1 and 0: Bioreactor and medium preparation
- Day 0: Inoculation and startup of the HyPerforma Rocker Bioreactor with a seeding density of 0.7 x 10^6 cells/mL in 2 L ChoMaster™ HP-1 growth medium
- Day 2: Sampling, addition of 2.5 L ChoMaster™ HP-5 growth medium, working volume of 4.5 L
- Day 3: Sampling, sedimentation of cells, medium exchange to tetracycline-free ChoMaster HP-5 production medium, working volume of 5 L
- Day 4: Sampling, temperature shift from 37°C to 31°C
- Day 5–9: Sampling, analytics, and harvesting

Media

The cells (CHO XM 111-10, clone 2008) were cultivated in ChoMaster HP-1 and HP-5 chemically defined minimal media (Cell Culture Technologies, CH). 2 g/L Pluronic F-68 and 2.5 mg/L tetracycline (Sigma-Aldrich, CH) were added in order to protect the cells against shear stress and to support cell growth while avoiding SEAP expression in the growth phase (Tet-Off principle). In the production phase an HP-5 production medium containing no tetracycline was used.

Inoculum preparation

The inoculum for the HyPerforma Rocker Bioreactor was produced in Corning™ single-use shaker flasks with working volumes of between 25 and 300 mL. The cells were inoculated with cell densities of 0.5 x 10^6 cells/mL. The shaker flasks were placed in a shaking incubator (Infors HT, CH) at 37°C at a shaking rate of 120 rpm and amplitude of 25 mm.

Three hours prior to the inoculation, 0.47 L of the cell suspension with an average cell density of 2.1 x 10^6 cells/mL was transferred to a sterile beaker. After adding 0.4 L fresh medium, the beaker was placed in the incubator at 37°C without agitation. After cell sedimentation, the supernatant was discarded.

Bioreactor preparation

The Rocker BPC was filled with 1 L HP-1 growth medium and placed on the rocking platform. After the aeration (0.1 slpm) and heating (37°C) were switched on, a two-point calibration was performed for the pH sensor. A sample was taken and its pH was determined using an external pH meter. After injecting CO_2 into the Rocker BPC for 30 minutes at a flow rate of 0.01 slpm, the pH was measured again. The pO_2 sensor was calibrated to 100% saturation.

Inoculum procedure

The inoculation procedure was performed under a laminar flow. A Sartorius™ Flexboy™ media bag with 3 L HP-5 growth medium was connected to the Rocker BPC before inoculation. To achieve the desired cell density of 0.7 x 10^6 cells/mL, 72 mL of the cell suspension with a cell density of 14.1 x 10^6 cells/mL was transferred into the Rocker BPC using a sterile syringe. Afterwards, 928 mL of fresh HP-1 growth medium was added to make an initial working volume of 2.0 L. The Rocker BPC was reconnected to the control unit before all the control loops for DO, pH, and temperature were started. The settings were as defined in Table 1.

Initiation of SEAP production

To initiate SEAP production, the culture medium was exchanged 64 hours after inoculation in order to remove the tetracycline, which suppresses SEAP expression. After adding 0.5 L of fresh HP-5 growth medium from the Flexboy media bag, all controls of the loops were switched off. The Rocker BPC was removed from the rocking platform and placed under the laminar flow. After adding 1 L of fresh HP-5 production medium through a sterile syringe, the Rocker BPC was hung on a tripod to let the cells settle. The Flexboy media bag with HP-5 production medium was connected to the Rocker BPC. After three hours, approximately 5.5 L of the supernatant was removed via the harvest port. As a result, approximately 0.5 L of the cell suspension remained in the bags. Afterwards, approximately 4.5 L of the tetracycline-free HP-5 production medium was added to the Rocker BPC in order to achieve a working volume of 5 L. Finally, the Rocker BPC was placed on the rocking platform and all the controllers were switched on.

Process parameters

The temperature was controlled automatically via a heater integrated in the rocking platform. 24 hours after the medium exchange, the temperature set point was decreased from 37°C to 31°C. A cascade configuration with the addition of CO_2 was set up to automatically control the pH. The DO concentration was also controlled by a cascade function with a rocking rate ranging from 15 to 20 rpm as the primary factor, and addition of pure oxygen as the secondary factor. After adding 2.5 L medium (HP-5), the lower and upper limits of the rocking rate were increased to 20 and 25 rpm, respectively.

Table 1. Culture conditions

Parameter	Value
Temperature	37/31°C
DO concentration	30% saturation
pH value	7.2
Rocking rate	15–25 rpm
Rocking angle	6°
Air flow rate (headspace)	0.1 L/min
Maximum O_2 flow rate (headspace)	0.25 L/min
CO_2 flow rate (headspace)	0–0.1 L/min

Process analysis

Samples of 4–8 mL were taken twice a day during the growth phase and daily during the production phase using sterile 10 mL syringes from the luer lock sampling port. Cell density and viability were determined using a Cedex™ HiRes cell counting device (Roche Diagnostics, Germany). Nutrients and metabolites were measured with a BioProfile™ 100 Plus multi-biosensor analysis system (Labor-Systeme Flükiger AG, CH).

SEAP activity was measured indirectly via the enzymatic transformation of p-nitrophenyl phosphate into para-nitrophenyl at 405 nm using a magnesium chloride buffer (magnesium chloride, homoarginine hydrochloride, and diethanolamine adjusted to pH 9.8) and p-nitrophenol phosphate di-sodium salt hexahydrate as a substrate solution.

Results

The cells in the Rocker BPC grew at a maximum specific growth rate of 0.043/hr, corresponding to a doubling time of 16.1 hours. During the first 40 hours, the total cell density increased from 0.71 x 10^6 to 4.05 x 10^6 cells/mL (Figure 2A). By adding 2.5 L of HP-5 growth medium (arrow 1), to achieve a total volume of 4.5 L, the cell density was diluted to 1.83 x 10^6 cells/mL. The secretion of SEAP was induced by exchanging the medium with tetracycline-free ChoMaster HP-5 production medium (arrow 2). Since cell sedimentation was incomplete and the medium removal resulted in cell loss, the total cell density after medium exchange was 30% (from 4.7 x 10^6 to 3.3 x 10^6 cells/mL) lower than prior to medium exchange. Only 10% of the decrease can be explained by dilution resulting from the larger working volume in the production phase compared to the growth phase (4.5 L versus 5 L). The maximum total cell density in the Rocker BPC of 16.4 x 10^6 cells/mL was measured after a process time of 168 hours (97 hours in the production phase). The cell viability was 98.0 ± 0.8% by the end of the stationary phase after approximately 190 hours of cultivation time.

The initial glucose concentration was 3.8 g/L, and 3.3 g/L after medium addition (Figure 2B). The glucose concentration decreased and achieved a minimum of 0.7 g/L before medium addition and 1.2 g/L before medium exchange. Therefore, sufficient substrate was available for cell growth during the complete growth phase. The maximum lactate concentration in the culture medium of 2.4 g/L was measured before medium exchange and dropped to 0 g/L after 190 hours. SEAP activity of about 1.1 U/mL was first detected 50 hours after medium exchange (one day after temperature shift) and the maximum activity of about 33.1/mL was determined after 214 hours process time (Figure 2C).

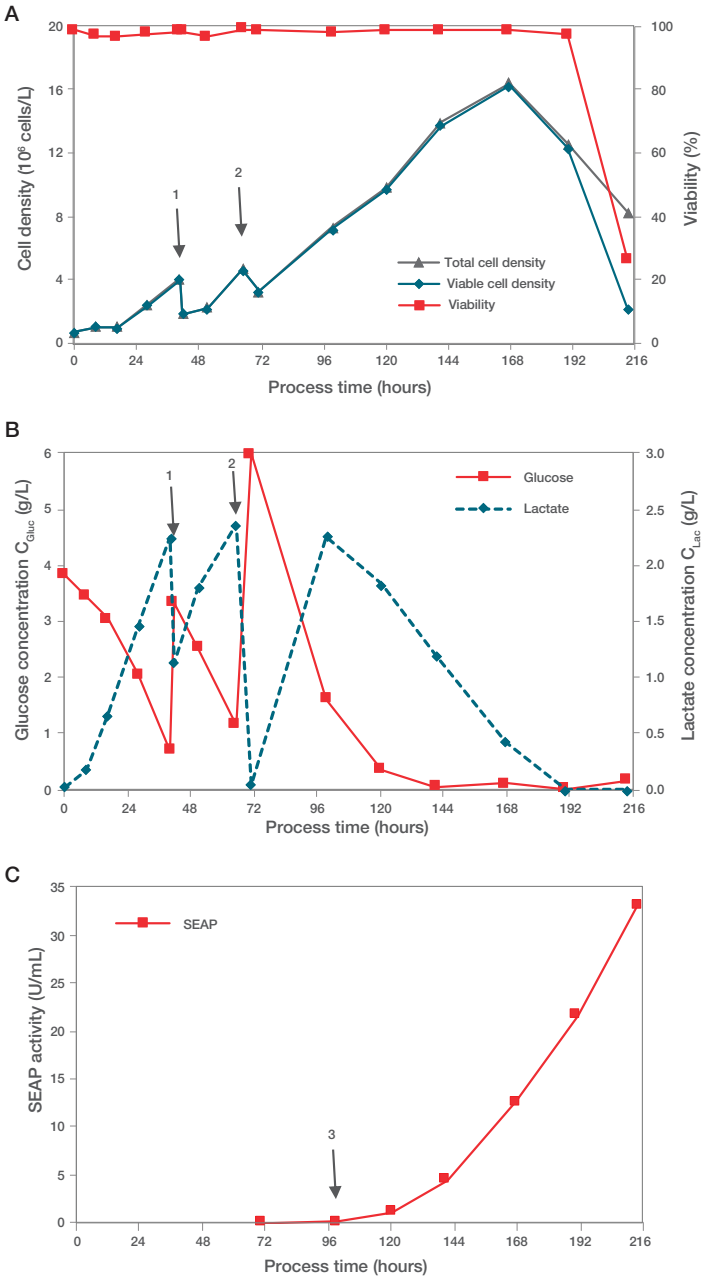

Figure 2. Results showing (A) cell density and viability, (B) concentrations of glucose and lactate, and (C) SEAP activity. The arrows indicate (1) medium addition after 41 hours, (2) medium exchange after 66 hours, (3) temperature shift after 101.4 hours of cultivation.

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During the entire cultivation, the DO level was maintained above the critical value of 30% saturation, ensuring the cells had sufficient oxygen (Figure 3). After medium exchange, the DO concentration decreased to a critical value and pure oxygen was automatically added. After approximately 141 hours process time, the glucose was completely depleted and the addition of pure oxygen was no longer required.

The pH value in the Rocker BPC was regulated by the addition of CO_2. The online pH values measured with the optical pH sensor were nearly identical to the pH values measured with the external pH meter, with a maximum deviation of 0.2 pH units at sampling points (data not shown). This indicated that the pH was correctly regulated and the pH measurements from the optical probes were consistent.

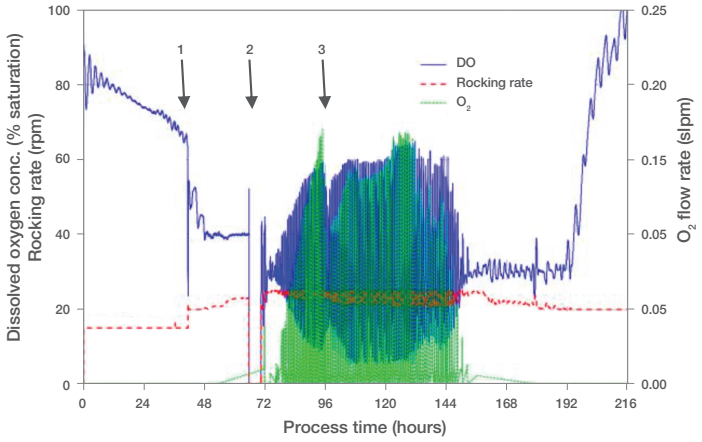

Figure 3. Online data for dissolved oxygen concentration, agitation, and oxygen flow rate in the Rocker BPC.

Conclusions

The novel wave-mixed single-use HyPerforma Rocker Bioreactor was successfully tested for cultivating CHO suspension cells in fed-batch mode and expressing SEAP. The maximum total cell density in the Rocker BPC of 16.4 x 10^6 cells/mL was achieved after 167 hours. The maximum SEAP activity of 33.1 U/mL was measured at the end of the process, after 218 hours. Based on our experience, the results were in the typical range observed in previous cultivations in other wave-mixed bioreactor types (Eibl et al., 2014). The main process parameters of DO, pH value, and temperature were well regulated by the HyPerforma G3Lab Controller operated by TruBio software.

Reference

Eibl R, Löffelholz C, and Eibl D (2014). Disposable bioreactors for inoculum production and protein expression. *Methods Mol Bio* 1104:265-284.

Authors

Nadezda Perepelitsa, GMP Process Engineer, Johnson & Johnson, Basel, Switzerland

Stephan C. Kaiser, Staff Scientist, Research and Development, Thermo Fisher Scientific, Santa Clara, USA

Dieter Eibl, Professor, ZHAW School of Life Sciences and Facility Management, Centre for Biochemical Engineering and Cell Cultivation Technique, Wädenswil, Switzerland

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