Cultivation of *CHO* Suspension Cells and SEAP Expression in the Wave-mixed Single-use Finesse SmartRocker Bioreactor

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Summary

This application note describes the cultivation of *CHO* suspension cells in the wave-mixed single-use Finesse SmartRocker bioreactor with a maximum working volume of 5 L. Using a chemically defined minimal medium, it was possible to produce up to $16.4 \cdot 10^6$ cells·mL^{-1}. SEAP expression was induced by medium exchange and a temperature shift. Maximum SEAP activity of up 33.1 U·mL^{-1} was achieved. The results are comparable to cultivations in other 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 Chinese Hamster Ovary (CHO XM111-10, obtained from Prof. Dr. Martin Fussenegger, Swiss Federal Institute of Technology in Zurich) suspension cells in fed-batch mode. We used a chemically defined minimal culture medium and expressed secreted alkaline phosphatase (SEAP), which represents a model protein. Secretion was induced and supported by medium exchange and a temperature shift.

A Finesse G3Lab Universal Bioreactor Controller with a 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.
Days -1/0: Bioreactor and medium preparation.
Day 0: Inoculation of the SmartRocker bioreactor with a seeding density of $0.7 \cdot 10^6$ cells·mL^{-1} 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.
Days 5/9: Sampling, analytics and harvest.

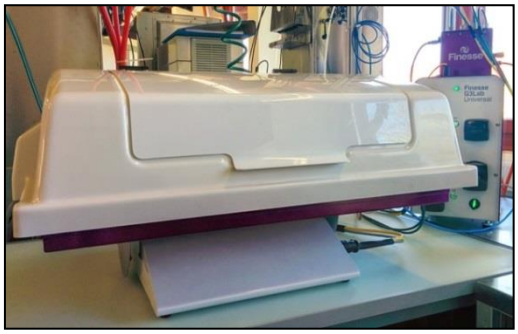

Fig. 1: The novel wave-mixed single-use SmartRocker bioreactor with Finesse G3Lab Universal Bioreactor Controller.

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Materials and Methods

Medium

The cells (CHO XM-111-10, clone 2008) were cultivated in Cho Master HP-1 and HP-5 chemically defined minimal media (Cell Culture technology, CH). 2 g·L^{-1} Pluronic F-68 and 2.5 mg·L^{-1} 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 a HP-5 production medium containing no tetracycline was used.

Inoculum Preparation

The inoculum for the SmartRocker was produced in single-use shake flasks (Corning, USA) with working volumes of between 25 and 300 mL. The cells were inoculated with cell densities of 0.5·10^6 cells·mL^{-1}. The shake 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 2.1·10^6 cells·mL^{-1} 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 SmartBag 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, 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 SmartBag for 30 minutes at a flow rate of 0.01 slpm, the pH was measured again. The pO_2 sensor was calibrated to 100 %sat.

Inoculation Procedure

The inoculation procedure was performed under a laminar flow. A Flexboy® media bag (Sartorius Stedim Biotech, Germany) with 3 L HP-5 growth medium was connected to the SmartBag before inoculation. To achieve the desired cell density of 0.7·10^6 cells·mL^{-1}, 72 mL of the cell suspension with a cell density of 14.1·10^6 cells·mL^{-1} was transferred into the SmartBag 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 SmartBag was reconnected to the control unit before all the control loops for DO, pH and temperature were started. The settings were defined as given below.

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 control the loops were switched off. The SmartBag 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 SmartBag was hung on a tripod to let the cells settle. The Flexboy® media bag with HP-5 production medium was connected to the SmartBag. 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 the tetracycline-free HP-5 production medium was added to the SmartBag in order to achieve a working volume of 5 L. Finally, the SmartBag 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 to 31 °C. A cascade configuration with the addition of CO_2 was set up to automatically control the pH. The dissolved oxygen 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, the lower and upper limits of the rocking rate were increased to 20 and 25 rpm respectively.

Parameter	Value
Temperature	37/31 °C
Dissolved oxygen concentration	30 %sat
pH value	7.2
Rocking rate	15 – 25 rpm
Rocking angle	6 °
Air flow rate (headspace)	0.1 L·min^{-1}
Max. O_2 flow rate (headspace)	0.25 L·min^{-1}
CO_2 flow rate (headspace)	0 – 0.1 L·min^{-1}

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Process Analysis

Samples of at least 4 mL were taken twice a day during the growth phase and daily during the production phase using sterile 10 mL syringes from the LuerLock 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 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.

Results

The cells in the SmartBag grew at a maximum specific growth rate of 0.043 h^{-1}, corresponding to a doubling time of 16.1 hours. During the first 40 hours, the total cell density increased from $0.71 \cdot 10^6$ to $4.05 \cdot 10^6$ cells·mL^{-1} (Fig. 2a). By adding 2.5 L of HP-5 growth medium (1st arrow), to achieve a total volume of 4.5 L, the cell density was diluted to $1.83 \cdot 10^6$ cells·mL^{-1}. The secretion of SEAP was induced by exchanging the medium with tetracycline-free ChoMaster® HP-5 production medium (2nd arrow). Since cell sedimentation was incomplete and the medium removal resulted in cell loss, the total cell density after medium exchange was 30% (from $4.7 \cdot 10^6$ to $3.3 \cdot 10^6$ cells·mL^{-1}) 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 (4.5 L instead of 5 L). The maximum total cell density in the SmartBag of $16.4 \cdot 10^6$ cells·mL^{-1} was measured after a process time of 168 hours (97 hours production phase). The cell viability was 98.0±0.8% by the end of the stationary phase after approximately 190 hours cultivation time.

The initial glucose concentration was 3.8 g·L^{-1}, and after medium addition 3.3 g·L^{-1} (Fig. 2b). The glucose concentration decreased and achieved a minimum of 0.7 g·L^{-1} before medium addition and 1.2 g·L^{-1} 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^{-1} was measured before medium exchange and dropped to zero after 190 hours. SEAP activity of about 1.1 U·mL^{-1} was first detected 50 hours after medium exchange (one day after temperature shift) and the maximum activity of about 33.1 U·mL^{-1} was determined after 214 hours process time (Fig. 2c).

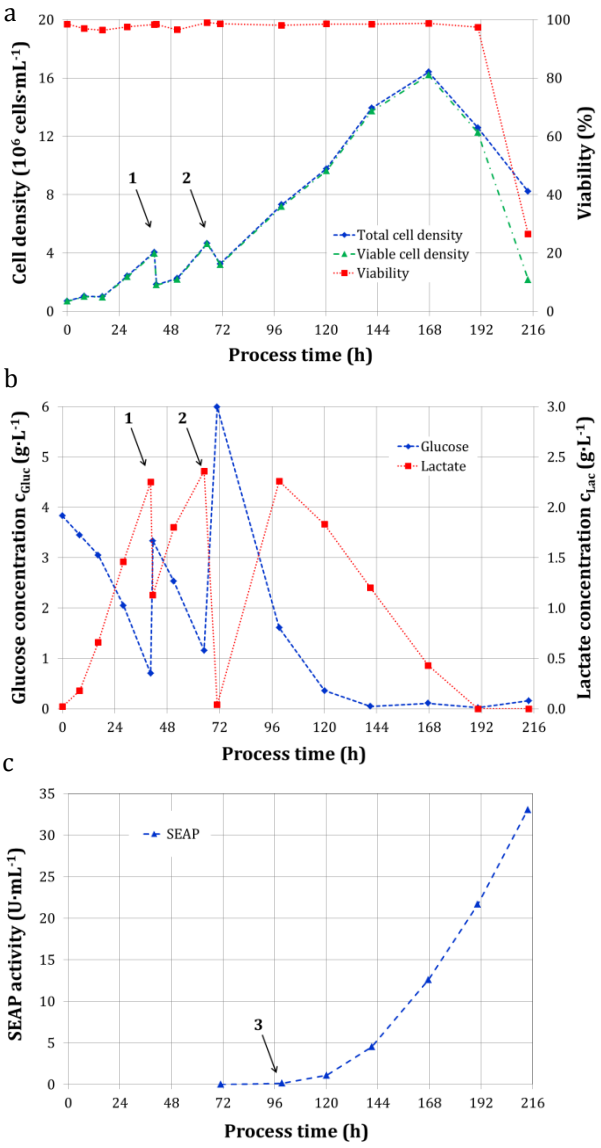

Fig. 2: a) Cell densities and viability, b) concentrations of glucose and lactate, c) SEAP activity. The arrows indicate (1) medium addition, after 41 h, (2) medium exchange, after 66 h, (3) temperature shift, after 101.4 h of cultivation respectively.

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During the entire cultivation, the DO level was maintained above the critical value of 30 %sat, ensuring the cells had sufficient oxygen (Fig. 3). After medium exchange, the dissolved oxygen 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 SmartBag was regulated by the addition of CO_2. The online pH values measured with the optical TruFluor 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.

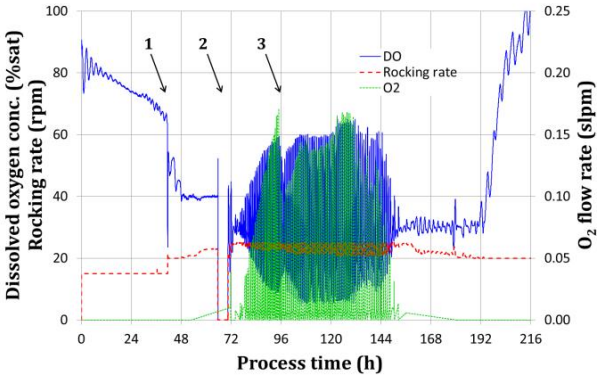

Fig. 3: Online data for dissolved oxygen concentration, agitation and oxygen flow rate in the SmartBag.

Conclusions

The novel wave-mixed single-use SmartRocker bioreactor from Finesse Solutions, Inc. was successfully tested for cultivating *CHO* suspension cells in fed-batch mode and expressing SEAP. The maximum total cell density in the SmartBag of $16.4 \cdot 10^6$ cells·mL^{-1} was achieved after 167 hours. The maximum SEAP activity of 33.1 U·mL^{-1} 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: dissolved oxygen, pH value and temperature were well regulated by the Finesse G3Lab Universal Bioreactor Controller operated by DeltaV control software.

References

Eibl, R., Löffelholz, C., & Eibl, D. (2014). Disposable bioreactors for inoculum production and protein expression. In *Animal Cell Biotechnology* (pp. 265-284). Humana Press.

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