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

Summary

This application note describes the cultivation of CHO suspension cells in the Thermo Scientific™ HyPerforma™ Glass Bioreactor with a maximum working volume of 2.0 L. Using chemically defined minimal media, cell densities of up to 7.44 x 10^6 cells/mL were achieved. Expression of SEAP protein was induced by medium exchange and temperature shift. Maximum SEAP activity of 63 U/mL was reached.

Introduction

The HyPerforma Glass Bioreactor has been designed for mammalian cell culture and microbial fermentation in benchtop-scale applications. With a maximum working volume of 2.0 L, the stirred bioreactor can be controlled using the Thermo Scientific™ HyPerforma™ G3Lab™ Controller in conjunction with the Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ system (Figure 1). Our industry-standard, cGMP-validated software offers state-of-the-art graphics, database management, and real-time control algorithms. It also includes preconfigured settings for controlling parameters such as pH, dissolved oxygen (DO), temperature, and agitation.

The present study focuses on the cultivation of CHO suspension cells in fed-batch mode using chemically defined minimal culture medium. SEAP expression was induced by medium exchange and temperature shift. The bioreactor was agitated by a combination of a modified Rushton turbine and a three-bladed segment impeller, which were found to be suitable for cell culture applications based on previous fluid dynamics investigations.

The bioreactor cultivation ran for 9 days, achieving cell densities of up to 7.44 x 10^6 cells/mL at high cell viabilities of >96% until the end of the stationary growth phase. The maximum SEAP activity was about 63 U/mL. By increasing impeller speed and sparging of oxygen, it was possible to maintain the DO level around the set point of 40% saturation, while preventing excessive shear stress or foam production due to aeration.

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

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Materials and methods

Overview of procedure setup

- Day –6: Inoculum production with CHO suspension cells (CHO XM111-10) in shake flasks.
- Day –1 and 0: Bioreactor and medium preparation.
- Day 0: Inoculation and startup of the glass bioreactor with a seeding density of 0.6 x 10^6 cells/mL in 1.0 L ChoMaster™ HP-1 growth medium (supplemented with 2.0 g/L Pluronic™ F-68 surfactant and 2.5 mg/L tetracycline).
- Day 2: Sampling, addition of 1.0 L ChoMaster™ HP-5 growth medium. Increase of impeller speed to maintain constant specific power inputs.
- Day 3: Sampling, sedimentation of cells, and removal of supernatant for medium exchange to tetracycline-free ChoMaster HP-5 production medium.
- Day 4: Sampling, temperature shift from 37°C to 31°C.
- Day 5–9: Sampling, analytics, and harvesting.

Media

For the seed inoculum production and the start of the cultivation in the glass bioreactor, ChoMaster HP-1 medium supplemented with 2.0 g/L Pluronic F-68 surfactant and 2.5 mg/L tetracycline (Cell Culture Technologies) was used. The feeding was realized with ChoMaster HP-5 growth medium, and the SEAP secretion was induced by medium exchange to tetracycline-free ChoMaster HP-5 production medium.

Inoculum preparation

The inoculum for the glass bioreactor was produced in single-use shake flasks at a maximum working volume of 300 mL. The cells were inoculated at densities of about 0.5 x 10^6 cells/mL and subcultivated at about 3 x 10^6 cells/mL. The flasks were shaken at a rate of 120 rpm and amplitude of 25 mm. Before the seeding, fresh medium was added to the shake flasks, and the cells were allowed to settle. The supernatant was removed after about three hours, and the cells were transferred into the glass vessel.

Bioreactor preparation

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 the glass vessel was filled with 800 mL PBS for sterilization (30 min, 121°C).

The ChoMaster HP-5 growth and production media bags (FlexBoy™ 3 L, Sartorius Stedim Biotech) were connected to the bioreactor via luer lock connectors in a safety cabinet. Two sterile glass bottles for media removal and antifoam solution (5 g/L Antifoam C Emulsion, MiliporeSigma) were connected.

After sterilization, the PBS was replaced by 800 mL ChoMaster HP-1 growth medium and the bioreactor was connected to the control unit. Temperature and agitation control were started for sterility testing about 24 hours prior to inoculation. The seed was poured into the bioreactor via sterile funnel in the sterile cabinet, and ChoMaster HP-1 growth medium was added to meet the desired initial cell density. About 30 minutes after the cell transfer, the first sample was taken.

Culture conditions	
Culture volume	1–2 L
Agitation speed	140–180 rpm
pH value	7.2
pH regulation	CO_2 (< 0.1 slpm)
Temperature	37°C (growth) 31°C (protein production)
Aeration rate	0.1 slpm (air, headspace) 0.05–0.1 slpm (oxygen, sparger)
Seeding density	0.6 x 10^6 cells/mL
Cultivation time	9 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. In-process control was performed using the NucleoCounter™ NC-100 Mammalian Cell Counter (cell density, viability; ChemoMetec) and BioProfile™ 100 Analyzer (substrate and metabolite concentrations; Nova Biomedical). Furthermore, the pH value was determined by a pH meter (Mettler Toledo).

Results

In Figure 2, the profiles of the total cell density and viability, the glucose and lactate concentrations, and SEAP activity during a cultivation time of 9 days are given. Starting from the initial cell density of 0.6 x 10^6 cells/mL, the cells grew with a mean growth rate of 0.898 per day corresponding to a doubling time of 18.6 hours. About 36 hours after starting the cultivation, 1 L fresh growth medium was added. The growth rate after the medium addition was slightly lower at 0.871 per day, so the total cell density prior to the medium exchange was 4.64 x 10^6 cells/mL. The maximum cell density of 7.44 x 10^6 cells/mL was achieved after 161 hours of cultivation. The cell viability remained high (over 96%) until the end of the stationary phase, where all substrates were depleted. Afterwards, it dropped rapidly to zero within one day, when the cultivation was stopped.

The substrate consumption and metabolite production were comparable to our experiences with similar stirred benchtop-scale bioreactors. About 0.9 g of glucose was consumed for the production of 10^6 cells/mL, and glucose was depleted after 192 hours of cultivation. At the end of the exponential growth phase, the cells started to consume the lactate, and the maximum lactate concentration after the medium exchange was 2.15 g/L (Figure 2B).

The SEAP activity increased rapidly after the medium exchange (Figure 2C), and the temperature shift (37°C to 31°C) led to an increase of the enzymatic activity. The maximum SEAP activity of 62.7 U/mL was detected after about 210 hours of cultivation.

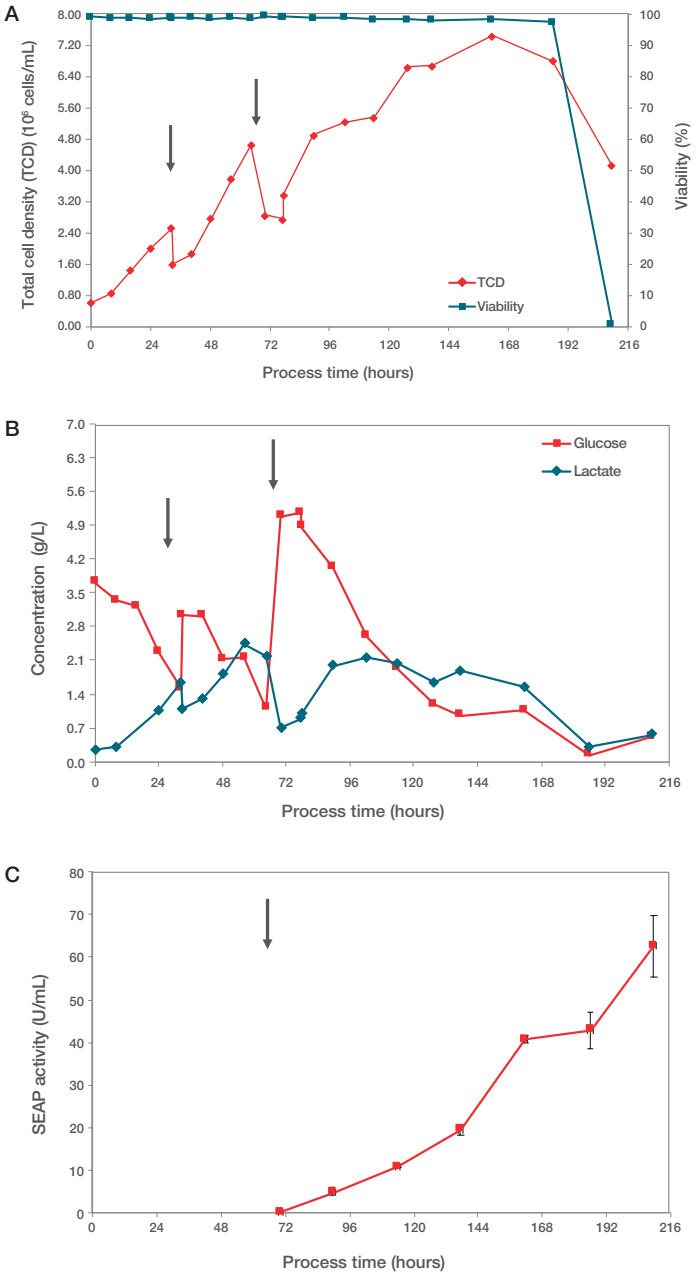

Figure 2. ChemoMetec) and BioProfile™ 100 Analyzer (A) total cell density and viability, (B) concentrations of glucose and lactate, and (C) SEAP activity. The arrows indicate the fresh medium addition after 32 hours of cultivation and the medium exchange to tetracycline-free production medium after 66 hours of cultivation.

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During the complete cultivation, the DO level was maintained above critical levels, preventing oxygen limitations of the cells, except the three hours prior to the medium exchange (Figure 3). Some higher fluctuations occurred at the beginning of the cultivation, after the medium exchange, and during the stationary growth phase, which may be explained by the limited dynamic range of the mass flow controllers for oxygen and attachment of bubbles to the DO probes. However, foam formation was effectively prevented due to the low gassing rates.

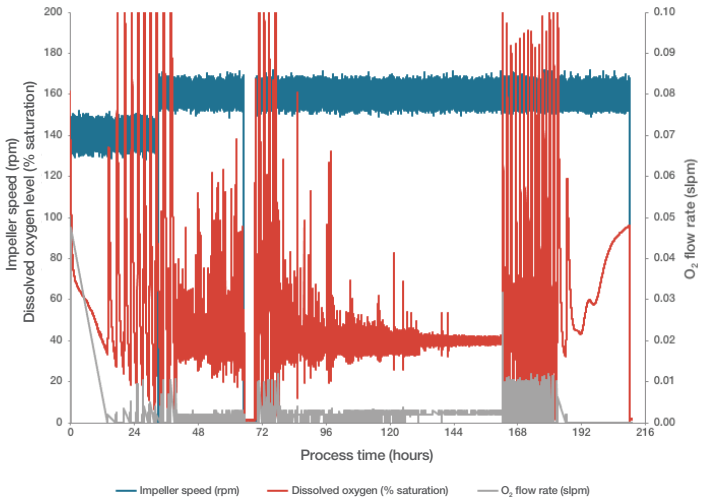

Figure 3. Online data for the impeller speed, dissolved oxygen level, and O_2 flow rate used for the DO control.

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