Cultivation of CHO suspension cells and SEAP expression in the Finesse glass bioreactor

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Summary

This application note describes the cultivation of *CHO* suspension cells in the Finesse glass vessel bioreactor having a maximum working volume of 2.0 L. Using chemically defined minimal media cell densities of up to $7.44 \cdot 10^6$ cells·mL^{-1} were achieved. The SEAP expression was induced by medium exchange and temperature shift. Maximum SEAP activities of up 63 U·mL^{-1} were reached.

Introduction

A novel stirred glass bioreactor suitable for cell culture applications at benchtop scale was designed by Finesse Solutions, Inc.. The bioreactor with a maximum working volume of 2.0 L is controlled by the Finesse TruViu RDPDmini controller representing an Intel-based micro-controller with the capability and robustness of an industrial automation solution. It is operated via the Finesse TruBio PC software developed on a C#/.NET foundation with state-of-the-art graphics, database management, and real-time control algorithms, which further includes pre-configured parameters for controlling of the bioprocess such as pH, DO, temperature, agitation etc.

The present study focusses on the cultivation of CHO suspension cells in fed-batch mode using chemically defined minimal culture media and SEAP expression, which is 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 run up to 9 days achieving maximum cell densities of $7.44 \cdot 10^6$ cells·mL^{-1} at high cell viabilities of above 96 % until the end of the stationary growth phase. The maximum SEAP activity was about 63 U·mL^{-1}. By increasing impeller speed and sparging of oxygen, it was possible to maintain the dissolved oxygen level around the set-point of 40 %sat, while preventing excessive shear stress or foam production through aeration.

Fig. 1: Picture of the novel glass bioreactor together with Finesse TruViu RDPDmini control system

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Materials and methods

Overview of setting-up procedures

Day -6: Iinoculum production with *CHO* suspension cells (CHO XM111-10) in shake flasks.
Day -1/0: Bioreactor and medium preparation.
Day 0: Inoculation and starting-up of the glass bioreactor with seeding density of 0.6 x 10^6 cells mL^{-1} in 1.0 L ChoMaster® HP-1 growth medium (supplemented with 2.0 $g \cdot L^{-1}$ Pluronic F-68 and 2.5 $mg \cdot L^{-1}$ 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.
Days 5 – 9: Sampling, analytics and harvest.

Media

For the seed inoculum production and the start of the cultivation in the glass bioreactor ChoMaster® HP-1 medium supplemented with 2.0 $g \cdot L^{-1}$ Pluronic F-68 and 2.5 $mg \cdot L^{-1}$ tetracycline (Cell Culture Technologies, Switzerland) 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 flaks at a maximum working volume of 300 mL. The cells were inoculated with cell densities of about $0.5 \cdot 10^6$ cells$\cdot mL^{-1}$ and subcultivated at about $3 \cdot 10^6$ cells$\cdot mL^{-1}$. The flasks were shaken at a shaking 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, Germany). The pH and DO probes were installed in the vessel and 800 mL PBS was filled into the glass vessel for sterilization (30 min, 121 °C).
The ChoMaster® HP-5 growth and production media bags (FlexBoy® 3L, Sartorius Stedim Biotech) were connected to the bioreactor via LuerLock connectors in the safety cabinet. Two sterile glass bottles for media removal and antifoam solution (5 $g \cdot L^{-1}$ Emulsion C, Sigma Aldrich) were connected.
After sterilization, the PBS was replaced by 800 mL ChoMaster® HP-1 and the bioreactor was connected to the control unit. Temperature and agitation control were started for sterile testing about 24 hours prior to inoculation.
The seed was filled into the bioreactor in the sterile cabinet and ChoMaster® HP-1 was added to meet the desired initial cell density. About 30 min 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)
Start cell density:	$0.6 \cdot 10^6$ cells mL^{-1}
Cultivation time:	9 days

Sampling and analysis

Samples are taken in place at least twice a day by connecting a sterile 10 mL syringe via a clave adapter. In-Process-Control was performed by NucleoCounter NC-100 (cell density, viability; chemometec), BioProfile 100 (substrate and metabolite concentrations; Nova Biomedical). Furthermore, the pH value was determined by a pH meter (Mettler Toledo)

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Results

In Fig. 2, the profiles of the total cell density and viability, the glucose and lactate concentrations and SEAP activity during a cultivation time of 13 days are given. Starting from the initial cell density of $0.6 \cdot 10^6$ cells·mL^{-1}, the cells grew with a mean growth rate of 0.893 d^{-1} corresponding to a doubling time of 18.6 h. About 36 h after starting the cultivation, 1 L fresh growth medium were added. The growth rate after the media addition was slightly lower with 0.871 d^{-1} so that the total cell density prior to the medium exchange was $4.64 \cdot 10^6$ cells·mL^{-1}.The maximum cell density of $7.44 \cdot 10^6$ cells·mL^{-1} was achieved after 161 h 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 glucose were consumed for the production of 10^6 cells·mL^{-1} and glucose was depleted after 192 h of cultivation. At the end of the exponential growth phase, the cells started to consume the lactate, whereas the maximum lactate concentration after the media exchange was 2.15 g·L^{-1} (see Fig. 2b).

The SEAP activity increased rapidly after the medium exchange (see Fig. 2c), whereas 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^{-1} was detected after about 210 h of cultivation.

During the complete cultivation, the DO level was maintained above critical levels preventing oxygen limitations of the cells, except of the three hours prior to the medium exchange (see Fig. 3). Some higher fluctuations occur 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 bubble attachment at the DO probes. However, foam formation was effectively prevented due to the low gassing rates.

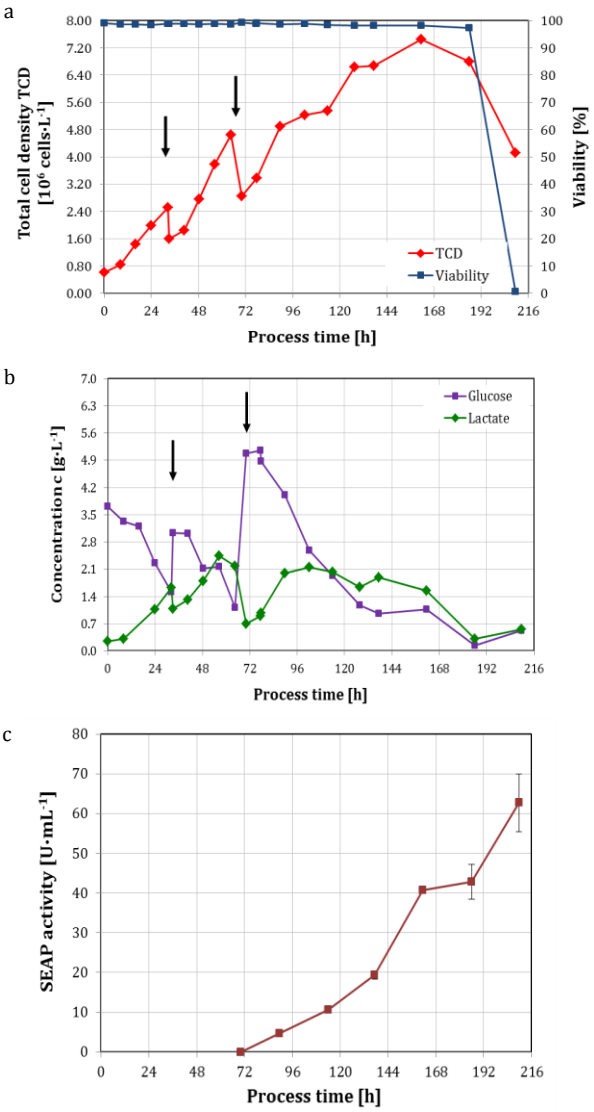

Fig. 2: (a) Total cell density and viability, (b) Concentrations of glucose and lactate, (c) SEAP activity. The arrows indicate the fresh media addition after 32 h of cultivation and the medium exchange to tetracycline-free production medium after 66 h of cultivation respectively.

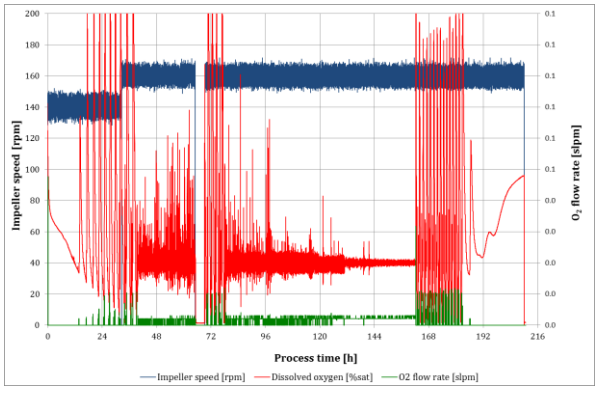

Fig. 3: Online data of the impeller speed, dissolved oxygen level and O_2 flow rate used for the DO control.

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