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High cell density *Escherichia coli* cultivation in a BIOSTAT® A

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turning science into solutions

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Introduction

Escherichia coli is commonly used as a host organism for recombinant protein production. Due to its ease of handling, well known metabolism and genetic characterization, *Escherichia coli* strains like BL21 (DE3) are used for training purposes especially in educational environments. Moreover, many protocols for batch as well as fed-batch cultivations of *Escherichia coli* strains are available.

Fermentation workshops should focus on key learning aspects of controlled cell growth in bioreactors. Hands on operational practice should be made efficient by user friendly automation. Ease-of-use from calibration of probes to system operation is essential for benchtop bioreactors used for educational purposes. The intuitive user interface of the BIOSTAT® A speeds up training and makes it ideal for beginners in fermentation. Digital pH and pO^2 sensors along with the new tablet PC menu facilitate easy and intuitive handling. Digital pH and pO_2 probes help the operators recognize at first glance whether a probe can be used for the next cultivation. Moisture-resistant plug connectors ensure secure data transmission to the BIOSTAT® A at all times – even when the sensor connectors get wet. Its novel aeration module provides continuous and automatic flow control over the full range of each gas used and the chiller unit allows fermentation in any laboratory by providing cooling water to the culture vessel independently of the availability of a water supply line. For precise fed-batch substrate addition, substrate profiles can be easily implemented in the software to control an external speed controlled pump.

The aim of this application note is to show the BIOSTAT® A's ability to perform challenging fed-batch *Escherichia coli* cultivations (specific growth rate of μ_{set} = 0.15 h^{-1}). The peak cell density should be above 60 g/L dry cell weight to underline its suitability as a benchtop bioreactor for educational purposes.

1. Material and Methods

1.1 Strain and medium

The cultivation was performed using strain *Escherichia coli* BL 21(DE3). LB-medium, containing 20 g/L LB-medium powder (Roth), was used for the preparation of the first seed culture. The second seed and main culture utilized a chemically defined medium (Riesenberg et al. 1991) (containing 10 g/L glucose for the second seed culture and 30 g/L glucose for the main culture). The culture medium was directly autoclaved within the UniVessel® 5 L. Glucose was added separately, after autoclaving, as a stock solution. During cultivation a feed solution was supplied to elongate the growth phase with 770 g/L glucose and 19.7 g/L $MgSO_4 \cdot 7H_2O$.

1.2 Cultivation steps

As a first step, aliquots of a Working Cell Bank were distributed on Petrie dishes with LB-Agar and incubated for 24 h at 37°C. The first seed culture was prepared in a 100 mL Erlenmeyer flask filled with 20 mL LB-medium and incubated at 37°C for ≈ 14 h. Utilizing a portion from the first seed culture, the second seed culture was inoculated (initial OD_{600} = 0.1) in a 1 L Erlenmeyer flask filled with 200 mL chemically defined medium and incubated for ≈ 8 h at 37°C. Both seed cultures were incubated in a CERTOMAT® Tplus with a shaking rate of 150 rpm and a 50 mm orbit diameter.

The BIOSTAT® A with UniVessel® 5 L system was prepared for cultivation with the following accessories:

- for temperature control – the chiller unit, cooling finger and heating blanket
- for agitation – 2 × 6 disk blade (rushton turbine)
- for gassing – a ring sparger
- a baffle cage

The vessel was filled with 3.5 L chemically defined medium and the initial cell density was OD_{600} (optical density at 600 nm) = 0.25.

Figure 1: BIOSTAT® A setup for the *Escherichia coli* high cell density fermentation experiment

Once the glucose in the culture medium was completely consumed a highly concentrated feed was supplied with an exponential increasing rate.

The specific growth rate was controlled to μ_{set} = 0.15 h^{-1} during the fed batch phase. Specific growth rates of $\mu > 0.15\ h^{-1}$ lead to unfavorable production of metabolic by-products (Riesenberg et al. 1991).

Figure 2: Calibration of the digital pH and pO_2 probes

Furthermore, cooling issues due to increased heat generation can occur, especially at larger scales | culture volumes. During cultivation the pH was controlled to 6.8 by adding 20% ammonia solution. Dissolved Oxygen levels were automatically controlled to 20% by keeping the stirrer speed constant at 800 rpm (tip speed = 2.7 m/s) and enriching the sparged gas with pure oxygen.

Current status of the probes, calibrations, deviations and process control parameters are directly displayed (see figure 2). The exponential feed profile was also controlled with the tablet feature.

2. Results

As a case study, a high cell density *Escherichia coli* fermentation was performed to assess if a BIOSTAT® A fulfills the requirements of this challenging process. In figure 3 the characteristics of the OD_{600} (proportional to the cell density increase) and the specific growth rate are shown to verify the growth behavior.

During the batch and fed batch phase the cell density increased exponentially. The slope of the proliferation was lower in the fed batch phase due to the controlled µ. At the end of the batch phase an OD_{600} of 35 (Dry cell weight = 13.6 g/L) was measured. This corresponds to a $Y_{X/S}$ of 0.45 $g_{DCW}/g_{glucose}$, indicating an expected substrate consumption and therefore optimal growth conditions (Stanbury et al. 1995). A final cell density of OD_{600} = 191 (DCW = 72 g/L) was achieved.

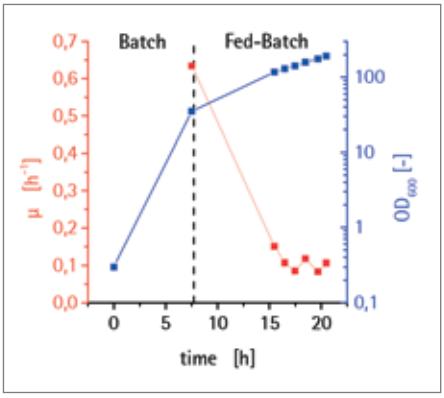

Figure 3: BIOSTAT® A *Escherichia coli* fermentation growth behavior. The OD_{600} and µ characteristics are shown.

Figure 4 shows the Dissolved Oxygen, temperature and pH measured value profiles. These may be used to evaluate if the culture conditions were optimal.

Within the first 6 hours the pO_2 decreased exponentially caused by increasing oxygen uptake rate until the set point of 20% was reached. After t = 7.5 h a significant pO_2 increase was observed, indicating the complete consumption of the initial glucose and the end of the batch phase.

During the remainder of the fermentation the pO_2 was adequately controlled to the set point. Hence, it can be concluded that aerobic conditions were present for the whole cultivation. Temperature control was successfully performed indicating a sufficient cooling capacity of the cooling finger and Chiller Unit. In addition, pH control remained reliable for the entire process.

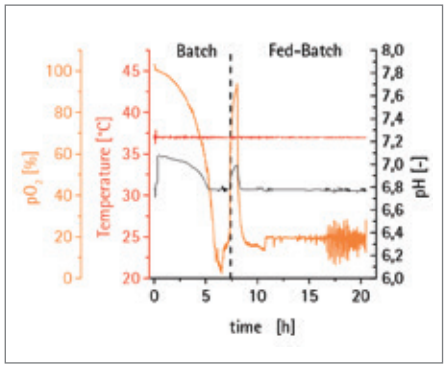

Figure 4: Cultivation conditions showing the characteristics of the pO_2, temperature and pH.

In figure 5 the process parameters for the dissolved oxygen control are shown. After the pO_2 setpoint was reached, pure oxygen was supplied up to 1 Lpm during the batch phase.

Due to the slower growth in the fed batch phase the increase of the O_2-ratio was slower and reached a maximal gassing rate of 4 Lpm (ratio 80% of the total gassing).

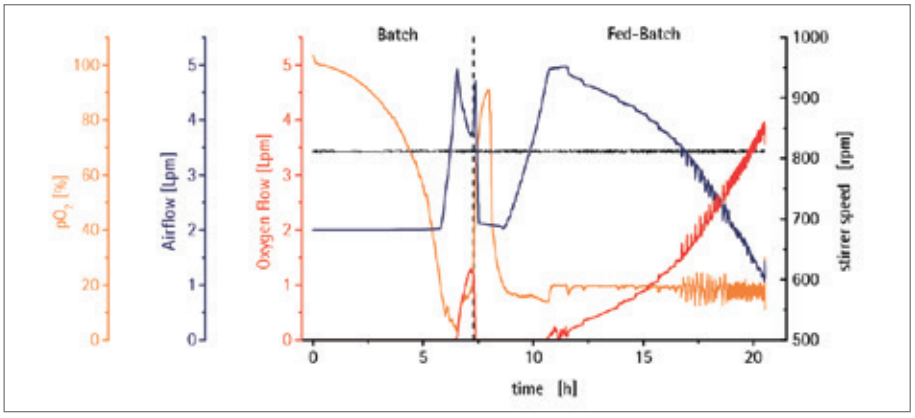

Figure 5: Oxygen partial pressure characteristics and control parameters. The pO_2, stirrer speed, gas flow rate and oxygen gas flow rate are shown.

In figure 6 the characteristics of antifoam addition are shown. Overall 10 mL was supplied (which was within the expected typical range).

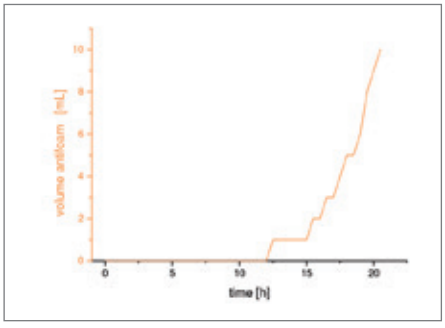

Figure 6: Added amount of antifoam (Antifoam 204, Sigma) solution during the *Escherichia Coli* cultivation

3. Conclusion

The feasibility to perform high cell density batch and fed-batch cultivations of *Escherichia coli* BL21 strain with the BIOSTAT® A was shown within this application note. Cell densities well above 60 g/L dry cell weight could be achieved and all critical process parameters like pH, pO_2 and temperature could be automatically and successfully controlled. Due to continuous gas flow no manual adjustment of the gas flow rates was necessary and anti-foam addition was low.

Remote monitoring and control of the cultivation, even from outside the lab, was possible due to the usage of a tablet for operation of the BIOSTAT® A. Moreover, the ease of which the operators handled the BIOSTAT® A proved its perfect suitability for educational purposes.

References

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