High cell density *Escherichia coli* cultivation in a BIOSTAT® A
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 MgSO₄·7H₂O.

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 OD₆₀₀ = 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 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.
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 $\mu_{set} = 0.15 \, \text{h}^{-1}$ during the fed batch phase. Specific growth rates of $\mu > 0.15 \, \text{h}^{-1}$ lead to unfavorable production of metabolic by-products (Riesenberg et al. 1991).

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 $= 2.7 \, \text{m/s}$) 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 $\mu$. At the end of the batch phase an OD$_{600}$ of 35 (Dry cell weight $= 13.6 \, \text{g/L}$) was measured. This corresponds to a $Y_{X/S}$ of 0.45 $\text{gDCW/gglucose}$, indicating an expected substrate consumption and therefore optimal growth conditions (Stanbury et al. 1995). A final cell density of OD$_{600} = 191$ (DCW $= 72 \, \text{g/L}$) was achieved.
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 pO₂ decreased exponentially caused by increasing oxygen uptake rate until the set point of 20% was reached. After t = 7.5 h a significant pO₂ 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 pO₂ 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.

In figure 5 the process parameters for the dissolved oxygen control are shown. After the pO₂ 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 O₂-ratio was slower and reached a maximal gassing rate of 4 Lpm (ratio 80% of the total gassing).

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, pO₂ 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.