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Application Note

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High Cell Density *Escherichia Coli* Fed-Batch Cultivation in the New Biostat® B-DCU

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Introduction

The requirements of a bioreactor | fermentor for microbial fermentations are fundamentally different to those for mammalian cell cultivations. Microbial processes require higher gas flow rates, tip speeds and greater cooling capacities in order to support the higher growth rates and greater oxygen demands of microbial cultures. Moreover, highly dynamic microbial processes cause rapid changes of process parameters and place great demands on the performance of the bioreactor controller. Precise control of critical process parameters is needed to achieve optimal product quality and prevent the formation of unwanted byproducts. This application note describes a high demanding *Escherichia coli* fed-batch cultivation and evaluates the performance of the new Biostat® B-DCU in such a process. It focusses on describing the control accuracy of the main process control parameters for pO_2, pH and temperature.

Maintaining a constant pO_2 during the course of a microbial cultivation is a challenging task for a bioreactor system and requires precise control over a large range of gas flow rates. In addition, a microbial bioreactor system for process development and characterization should be capable of being used with a range of different cultivation volumes. The Biostat® B-DCU meets these requirements and features digital mass flow controllers with a gas flow range of 1:200 (e.g. 0.05 – 10 lpm) in combination with the possibility to combine additional mass flow controllers to further extend the flow rate ranges. This enables different cultivation volumes with the same bioreactor controller.

Cultivations of *E. coli* with high growth rates generate a large amount of biological heat. To remove this requires an adequate cooling system that provides effective heat transfer and temperature control close to a set point.

To deliver the optimum product quality scientists must have a thorough understanding of the manufacturing processes. The Biostat® B-DCU features advanced sensors and analytics that form the basis for comprehensive process understanding. Off-gas analysis supports the characterization of cell metabolisms during microbial processes. During these experiments, the Biostat® B-DCU was equipped with the BioPAT® Xgas off-gas analyzer. In addition online glucose, ethanol and methanol determination as well as inline turbidity and capacitance sensors provide even greater process understanding when used in conjunction with the Biostat® B-DCU.

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Material & Methods

The cultivation was performed using strain *E. coli* W3110 thyA36 supO λ- (Ordering number: DSM: 5911, ATCC: 27325). The process was started with a single colony from an LB-agar plate and followed by two preculture steps according to DECHEMA guidelines [1] (Table 1).

Table 1: Preculture steps

Steps	Medium	Growth conditions	Duration
1. LB-Agar Plate	–	–	–
2. 1st Preculture	LB-Medium (20 g/L LB-medium powder Roth, Art. Nr.: X964.2)	Incubation shaker, 37 °C 50 mm orbital diameter at 180 rpm	8 h
3. 2nd Preculture	Biener Medium [2] (10 g/L Glucose)	Incubation shaker, 30 °C 50 mm orbital diameter at 180 rpm	16 h

For the main culture, Biener medium [2] was used – with medium 1 for the main feed during the fed-batch phase as well as the bolus feed medium (medium 2). The mineral salt component was autoclaved directly within the Univessel® Glass 5 L. Glucose and trace elements were added separately as a stock solution after autoclaving. The total batch medium volume was 2.7 L. The fed-batch cultivation was conducted with a growth rate of μ_{Set} = 0.4 h^{-1} using an exponential pump feeding profile according to Riesenberg [3]. The cultivation temperature was 37 °C and the pH was maintained at pH 6.8. The pO_2 cascade is given in Fig. 1, with a pO_2 set point of 35% at maximum gassing rate of 1 vvm. The set-up had previously been shown to generate a k_La of 740 1/h through characterization with the gassing out method [4] (data not shown).

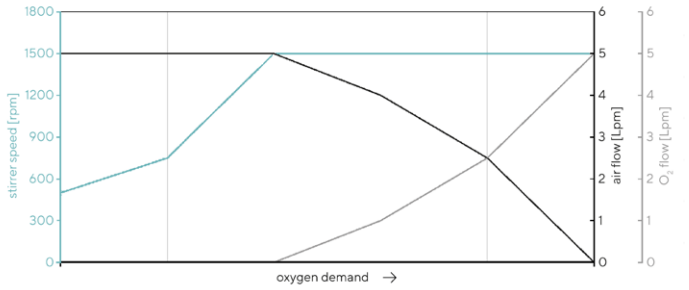

Figure 1: Schematic pO_2 cascade of both cultivations. Starting with a constant air gas flow of 5 lpm, pO_2 was controlled by increasing tip speed to 1500 rpm and supplementing oxygen.

The cultivations were performed within a Univessel® Glass reaction vessel with a maximum working volume of 5 L. The vessel was equipped with two 6-blade disc impellers for effective gas dispersion and good homogenization of the cell broth. A ring sparger with upturned holes was used. Additionally, the vessel was assembled with several ports for feeding, incorporating digital pH as well as digital optical pO_2 sensors, an exhaust cooler and Sartofluor® 150 gas filters.

The bioreactor was controlled with the new Biostat® B-DCU controller. It was equipped with mass flow controllers with a flow range of 0.05 – 10 lpm for air and oxygen. The control system automatically adjusted the ratio of air and oxygen according to the oxygen demand of the culture.

Results & Discussion

The new Biostat® B-DCU was set up as described above and a fed-batch cultivation with a growth rate of μ_{Set} = 0.4 h^{-1} was conducted. The results were compared to historical data from a fed-batch cultivation conducted in a Biostat® benchtop bioreactor with the same growth rate and identical preculture steps. The culture profile of both cultivations is given in figure 2. The cultivations started with an OD_{600} of 1. The duration of both processes was approximately 12 h. After 5.2 h of batch phase the depletion of glucose initiated the fed-batch phase. At an OD_{600} of 150, feed medium 2 was given as a bolus feed in addition of the continuing exponential feed of medium 1. The end of the cultivation was marked when the maximal filling volume was reached and the final OD_{600} was 265 (former cultivation: 260) and a respective cell dry weight of 87 g/L (former cultivation: 84 g/L), indicating a high cell density culture. The cultivations were stopped after approximately 2.3 L of feeds had been added and the maximum volume of the reaction vessel was reached.

The pO_2 value was approximately 35% during the entire cultivation. The fluctuation was significantly lower than compared to the previous cultivation, indicating a sufficient oxygen supply. The pH was well maintained at pH 6.8 by adding base throughout the cultivation. Minor peaks in the pH were caused by the consumption of acetate at the end of the batch and bolus addition of the secondary feed.

The extracellular glucose concentration measurements showed that glucose had been completely metabolized during the batch phase and the cells remained carbon limited during the fed-batch phase. Acetate was formed up to a concentration of 0.4 g/L during the batch phase in both fermentations. After the initiation of the fed-batch phase, acetate was consumed completely and increased only at the end of the process to 4.8 g/L due to the high growth rate and high cell density. The acetate formed was near the growth inhibitory concentration of 5 g/L and even at 1 g/L negative effects could be observed [5]. Consequently, the growth rate was reduced and glucose over feeding was observed during this highly challenging process.

Even at the end of the cultivation at high cell density with a high growth rate of 0.4 h^{-1} and during unlimited growth in batch phase, the temperature was maintained at approximately 37 °C. The new Biostat® B-DCU is clearly able to facilitate high cell density *E. coli* cultivations.

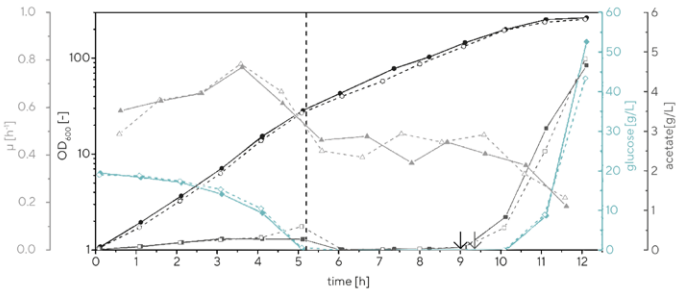

Figure 2: Overview of the cultivation in the Biostat® B-DCU (solid lines) with a Univessel® Glass 5 L compared to a former cultivation (dashed lines) with a Univessel® Glass 5 L. Shown is the optical density at 600 nm (black), growth rate (grey), acetate (light grey) and glucose (yellow) concentration. The start of the feed is indicated by the dashed line and the bolus feed 2 is indicated by the arrow.

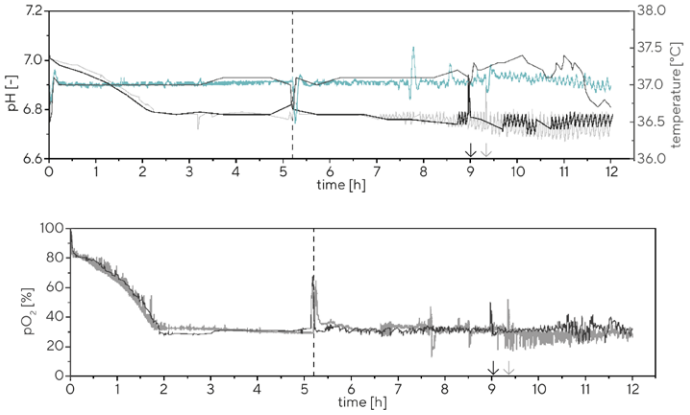

Figure 3: Process control of both cultivations. The pO_2, was controlled by changing the tip speed and supplementing oxygen (fig. 1). Additionally, pH and temperature were also controlled. Biostat® B-DCU data were colored and data from a former cultivation were light-colored, respectively.

The fed-batch cultivation of *E.coli* in Biostat® B-DCU was conducted with a fully integrated BioPAT® Xgas device, which monitored the oxygen and carbon dioxide concentration in the exhaust gas (Fig.4). Compared to a "standard" BioPAT® Xgas the measuring range for carbon dioxide was modified and increased from 10 to 20 %.

Based on these values the oxygen uptake rate and carbon dioxide evolution rate was calculated to characterize the metabolism of the cells. During this highly challenging fed-batch process the measuring range of oxygen and carbon dioxide in the off-gas was exceeded after 10.5 h and the measurement was stopped. Thus, the measuring range for the BioPAT® Xgas will be increased in future to 75 % for oxygen and 25 % for carbon dioxide, respectively.

At around 10.5 h of cultivation the measured CER was 500 mmol L^{-1} h^{-1} and the OUR was 1200 mmol L^{-1} h^{-1}. These are typical values for an *E. coli* high cell density culture at a growth rate of 0.4 h^{-1} [3]. This data allows process control steps like induction profiles at peak OUR or feeding profiles to be defined. In addition, values can be calculated that allow greater process characterization. For example, the RQ-value describing carbon source uptake efficiency could be determined.

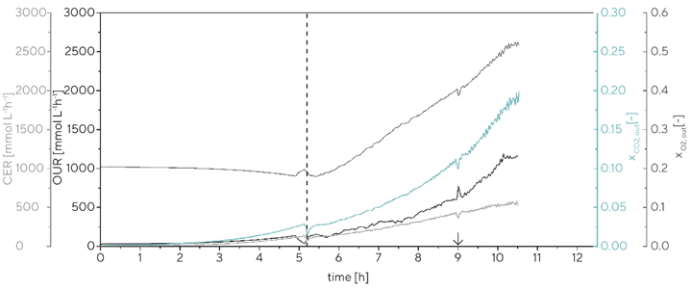

Figure 4: Exhaust gas measurement of the cultivation in the Biostat® B-DCU with a Univessel® Glass 5 L for oxygen (light grey) and carbon dioxide (yellow) as well calculated oxygen uptake rate (dark grey) and carbon dioxide evolution rate (grey). The start of the feed is indicated by the dashed line and the bolus feed 2 is indicated by the arrow. Exhaust gas measurement was stopped when the measuring range of O_2 (50%) and CO_2 (20%) was exceeded after 10.5 h.

Summary and Outlook

A high cell density *E. coli* fed-batch cultivation with high growth rates was successfully performed in a Biostat® B-DCU bioreactor system. The maximum tip speed was 5 m/s and a gassing rate of 1 vvm was selected to avoid foaming and to prevent clogging of the exhaust filter. The culture achieved a final OD_{600} of 265 (equal to a cell dry weight of 87 g/L) after 12 hours.

It is also possible to apply higher gas flow rates to reach higher k_La values of e.g. 1000 1/h at 2 vvm gassing [data not shown] for even higher cell densities with the new Biostat® B-DCU. Bench scale bioreactor systems are capable of performing challenging processes, however, the design space in which they are operated is often constrained to match the technical limitation of large-scale bioreactors to ensure effective scale-up. We were able to demonstrate that the Biostat® B-DCU provides precise control of the critical process parameters even during a highly challenging *E. coli* fed-batch cultivation with a growth rate of $\mu \approx 0.4$ h^{-1} even though the growth rate of industrial *E. coli* processes rarely exceed a $\mu_{Set} > 0.2$ h^{-1}.

Digital mass flow controllers are a significant enabling, underlying technology within the system. The experimental data we have presented shows that fluctuations of the pO_2 were greatly reduced when compared to the control provided by analog mass flow controllers.

The measured value was close to the set point of 35% during the carbon source limited fed-batch phase of the cultivation. Even when the addition of pure oxygen started and the mass flow controller was working at the lower end of its flow range the pO_2 remained almost constant. Moreover, the possibility to combine mass flow controllers with different flow ranges in a single controller facilitates perfect conditions for working volumes from 250 mL – 10 L and makes the bioreactor controller truly universal.

The integration of advanced sensors and analytics allows indepth process monitoring and supports straightforward implementation of advanced process control strategies. In an earlier application note we have discussed the possibilities and advantages of an automated glucose level control with a Biostat® B-DCU [6]. In this application note we focused on integrated exhaust-gas measurement, which allowed continuous monitoring of the cell's metabolism.

In future, the potential to optimize the feeding scheme and process control recipe could be investigated. Turbidity or capacitance measurement could be used to monitor the cell density in real-time and to automatically trigger the induction to initiate the production phase.

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