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Precise Control of Gas Flows Within the Biostat® B-DCU

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Introduction

To meet the oxygen demand of cells a stable pO_2 control is an essential part of every cultivation. The objective of this application note is to demonstrate the performance of a new generation of mass flow controllers in the Biostat® B-DCU bioreactor system. Several comparative cell cultivations were performed.

The Biostat® B-DCU is a benchtop bioreactor specifically designed for process development and process characterization. It can be operated with Univessel® Glass 1 L, 2 L, 5 L, 10 L and Univessel® SU 2 L bioreactors. The newly developed intelligent mass flow controllers (MFC) have a flow range ratio of 1:200 with regards to the maximum flow rate and communicate digitally with the control system. This enables a wide flow range per gassing line and a smooth opening and closing of the MFC.

The demand for oxygen changes constantly during a cultivation. At the beginning it is beneficial to work with a constant gas flow rate. This leads to a predictable foaming behavior andminimizes particle burden to the exhaust filter. At first, no addition of pure oxygen is needed and only air and nitrogen are sparged to the liquid. With increasing oxygen demand of the culture it is necessary to reduce the nitrogen input to 0 Lpm and add pure oxygen. In this transition phase fluctuations of pO_2 can occur. Therefore, opening and closing characteristics of mass flow controllersat low flow ranges are very important for precise pO_2 control. Therefor, the newly integrated mass flow controllers of the Biostat® B-DCU can control the pO_2 with greater accuracy.

Moreover, by combining a low flow and a high flow mass flow controller for a specific gas (e.g. air), it's possible to realize a flow range of 1 ccm up to 20 liter per minute in a single control system. This allows using the same Biostat® B-DCU system to perform a 1 L cell cultivation or a 10 L microbial fermentation without a physical change of mass flow controllers.

Find out more: www.sartorius.com/en/products/fermentation-bioreactors/benchtop-bioreactors/biostat-b-dcu

1. Material & Methods

A Biostat® B system was compared to a new Biostat® B-DCU system with regards to MFC performance. The MFCs integrated into the Biostat® B have a flow range ratio of 1:50 and feature an analog communication, whereas those integrated into the Biostat® B-DCU have a flow range ratio of 1:200 and a digital communication. Table 1 shows an overview about the MFCs of each gassing line and their flow ranges.

Table 1: Mass flow controller in Biostat® B and B-DCU

Gas	Biostat® B	Biostat® B-DCU
N_2 [Lpm]	0.03 – 1.5	0.005 – 1
Air [Lpm]	0.01 – 0.5	0.005 – 1
O_2 [Lpm]	0.01 – 0.5	0.005 – 1
CO_2 [Lpm]	0.06 – 0.3	0.003 – 0.5

The Univessel® Glass 5 L was equipped with two 3-blade segment impellers for low shear stress and effective mixing of the cell broth. The blade angle was 30° and set to down pumping. A ring sparger with holes facing down and a micro sparger with a porous frit were used during the evaluation. The vessel was operated with several ports for feeds, a classical pH and pO_2 probe, exhaust cooler and gassing filters.

The pH set-point was maintained at pH 7.15 using additive CO^2 sparger gassing. The pO_2 set point was controlled to 60 % saturation with an advanced pO_2 controller comprising several gassing steps and parallel adaption of actuator outputs (see Figure 1).

A mixture of N_2 and air at 0.15 Lpm was used at the start of the culture to achieve a pO_2 of 60 % saturation. The N_2 gassing was reduced from 0.15 to 0 Lpm while air gassing increased from 0 to 0.15 Lpm according to the oxygen demand of the culture. During the next step of the cascade, the flow rate of air is reduced and O_2 gassing increased to 0.15 Lpm. The total gas flow of oxygen is increased up to 0.5 Lpm in the event that the oxygen demand increases further. As a last step of the advanced cascade the agitation rate was increased from 250 to 400 rpm.

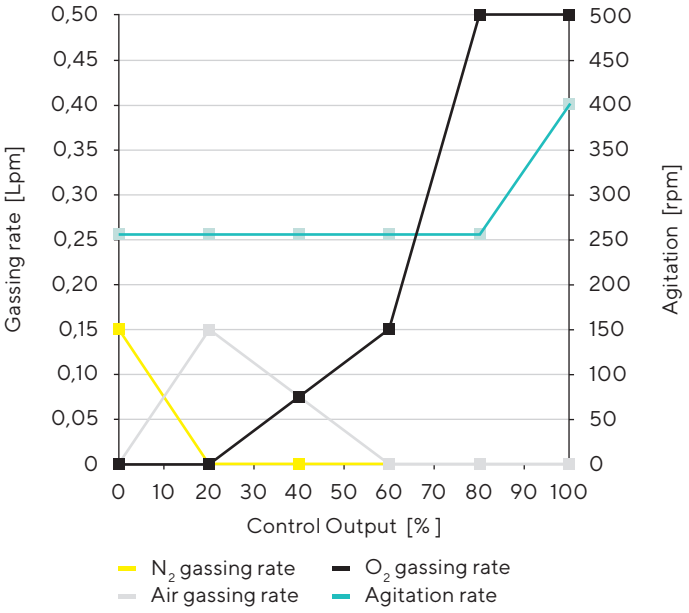

Figure 1: Setup of advanced pO_2 controller

The transitions from N_2 to air at 20 % controller output and air to O_2 at 80 % are especially critical since the MFCs operate at their minimum flow rates during these phases. With standard analog MFCs these transitions often cause high fluctuations in the pO_2 value, due to them being operated at the lowest flow rate, where the noise of the analog signal is causing a fluctuating opening and closing of the MFCs. The new generation of MFCs in the Biostat® B-DCU is now capable of operating accurately at 0.5 % of total gas flow making the pO_2 value more stable during gas transitions.

A CHO fed-batch process was used to evaluate the function of the new mass flow controllers in the Biostat® B-DCU. The 17-day cultivation comprises of a 3-day batch phase and a 14-day fed-batch phase. The culture was inoculated with 0.3 + 10^6 cells/mL and the peak viable cell density (VCD) is typically reached at day 8 with 25 – 30 + 10^6 cells/mL and a viability of 99 %.

The bolus feeding from day 3 comprises feed medium A (FMA), feed medium B (FMB) and a highly concentrated glucose solution (400 g/L). The amount of FMA and FMB added is constant throughout the complete fed-batch phase. Typically, on day seven, additional glucose is needed to maintain a glucose concentration of 3 g/L in the cell broth. The feeding process is automated using balances and pumps connected to the digital control unit (DCU) and a MFCS (multi fermenter control system) recipe. Harvesting is performed during the dying phase. The VCD (viable cell density) should be above 10 + 10^6 cells/mL with a viability of more than 50 %.

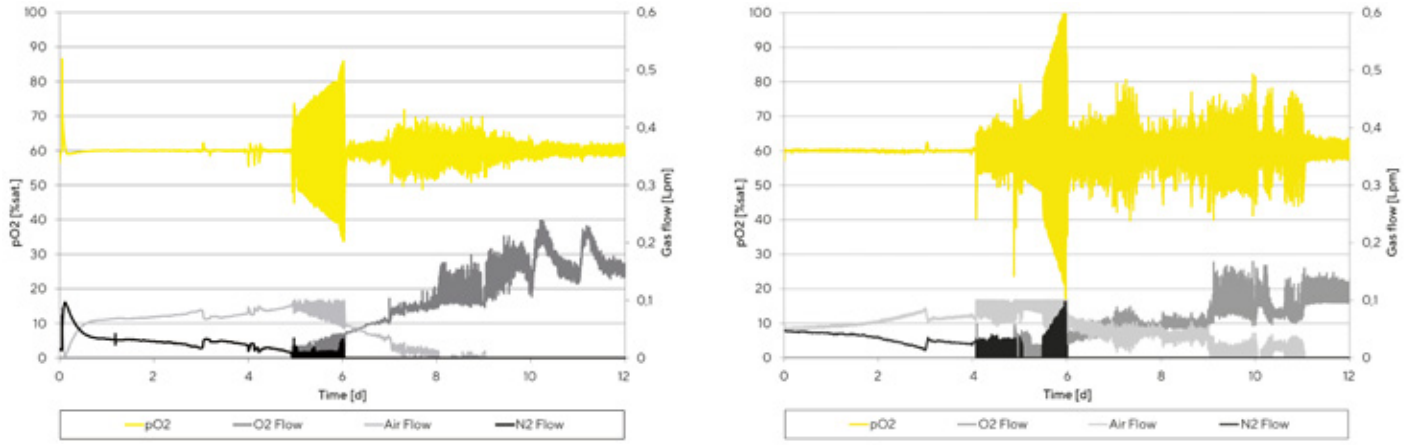

Figure 2: pO_2 and gassing trend – Univessel® Glass 5 L – micro sparger – Biostat® B-DCU (left) Biostat® B (right)

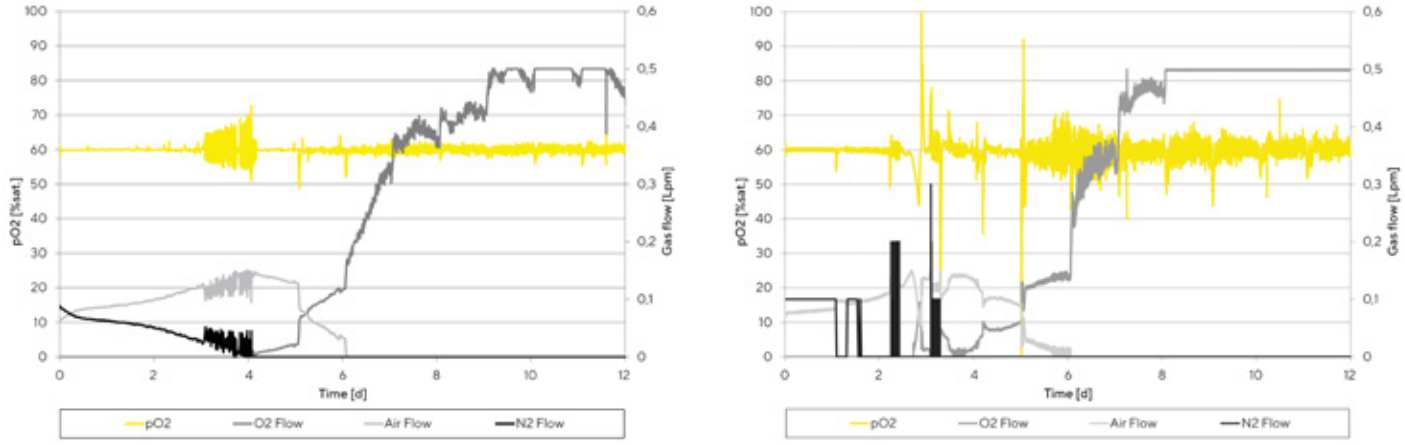

Figure 3: pO_2 and gassing trend – Univessel® Glass 5 L – ring sparger – Biostat® B-DCU (left) Biostat® B (right)

2. Results & Discussion

In Figure 2 and 3 the fluctuations of the pO_2 value in different experiment setups are shown (orange trends), as well as the flow rates of the individual MFCs (grey trends).

Figure 2 shows data of new generation MFCs (Biostat® B-DCU) and previous generation MFCs (Biostat® B), while gassing was performed solely via a micro sparger. In comparison to figure 2, data in figure 3 was gained with a ring sparger instead. Data from both figures shows that the gas transition from N_2 to air to O_2 is resulting in pO_2 fluctuations. However, with the new generation MFCs a significant reduction in pO_2 fluctuation can be seen. All cultivations used the same PID parameter setup. The precise opening and closing behavior of the new generation MFCs can also be seen in the reduced fluctuations of the gas flow rates during the transition phases.

Moreover, the data shows that gassing via a ring sparger allows for a more stable pO_2 control than a micro sparger, since the oxygen transfer is slower with ring sparger. Although, further PID parameter optimization might reduce pO_2 fluctuation significantly.

3. Summary

CHO fed-batch cultivations in the new Biostat® B-DCU were successful. The experiments have shown that the new generation MFCs of the Biostat® B-DCU have a higher accuracy compared to the MFCs integrated into the Biostat® B. Control of a stable pO_2, also at small flow ranges, is not a challenge anymore. This opens up new opportunities for process optimization and subsequently to reach higher titers and better product quality.

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