HAMILTON

Real-time Monitoring of DCO_2 in addition to DO

In-situ PAT applied to Hybridoma Cell Culture

Industry Segment: Biopharma R&D
Application Field: Monitoring of Cell Culture for mAb Production
Hamilton Products: CO_2NTROL & VisiFerm

Utilizing real-time PAT to ensure cell culture productivity

Biopharmaceutical products represent some of the most effective medicines to cure diseases such as cancer, leukemia, diabetes, arthritis (or prevent serious disease through vaccines). Industrial production processes based on cell cultures to produce biotherapeutics are extremely challenging and complex, especially when compared to other processes such as small molecule medicines production. Products like monoclonal antibodies (mAb), for example, are constituted by ~25,000 atoms, in comparison with small drugs such as Aspirin API, 21 atoms. Such biotechnologically produced pharmaceuticals (biopharmaceuticals) can be obtained from production in cell cultures – living organisms – which are complex to control, and with many parameters having the ability to influence each production step. The best way to gain as much control as possible is to apply the principles of the FDA PAT initiative: shift as much quality control as possible from laboratory to production floor. In other words, monitor as many critical process parameters (CPPs) and key performance indicators (KPIs) as possible in-situ/in-line. For example, in the bioreactor steps, in-line monitoring of CPPs such as Dissolved Oxygen (DO) and Dissolved CO_2 (DCO_2) enables gas control strategies which maintain KPIs such as Viable Cell Density (VCD) at levels required to provide the desired productivity. Research in novel control strategies and important parameters to promote high productivity processes is ongoing within the industry.

Fig. 1: In-line Arc Sensors CO_2NTROL (DCO_2) and VisiFerm (DO)

In this application note, the utilization of a DCO_2-probe in addition to a DO-probe at Ostwestfalen-Lippe University of Applied Sciences and Arts (Technische Hochschule Ostwestfalen-Lippe, TH OWL) in Lemgo, Germany, Biotechnology & Bioprocess Engineering, is briefly described using cell cultivation with varying stirring speeds to demonstrate the value added of inline dissolved gas measurement.

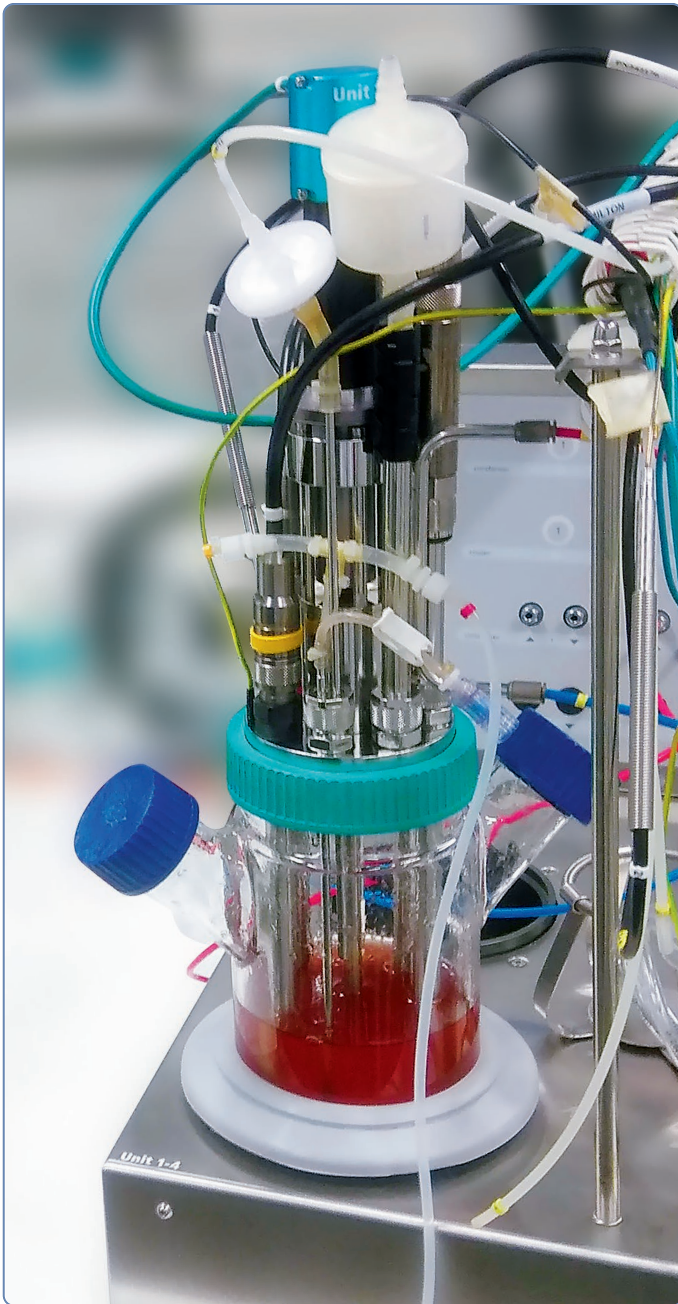

Fig. 2: Cultivation setup with sensors for in-situ measurements of DCO_2 and DO.

In-situ monitoring of DCO_2 and DO

For the real-time monitoring of DCO_2, the Hamilton Solid-State Mid-IR Optical CO_2NTROL Sensor was used; the monitoring of DO was performed applying the optical VisiFerm DO Arc Sensor as shown in Figure 1.

Real-time data facilitates the monitoring of cell-produced CO_2 when the concentration increases or decreases. For the interpretation of the DCO_2-course, other factors affecting the CO_2 concentration, such as changes in pH, aeration or stirring speed, also have to be included. Oxygen consumption over time is also monitored and controlled in order to supervise the culture conditions for the cells in spite of changing process parameters and avoid negative impacts on viable cell density. Continuous in-line data, especially in the case of the additional DCO_2 measurement, allows continuous changes to be recorded and avoid limited data collection as with discrete off-line sampling. Moreover, overall process understanding is improved.

Furthermore, since the respiratory activity of the cells can be observed via the DCO_2- and DO-measurement in combination with further data such as pH and O_2- and CO_2-concentration in the gas phase, this method is suitable to observe the metabolic activity of the cells in a continuous, real-time fashion.

Example bioreactor cultivation for the real-time monitoring of DCO_2 in addition to standard parameters

Mouse-mouse-Hybridoma IV F 19.23 cells were cultivated in a DS1000ODSS DasGip (Eppendorf) bioreactor for working volumes of 400 to 1200 mL. A fed-batch process was started using 400 mL DMEM Ham's F12 medium containing 16.6 mmol/L D-glucose and 4.5 mmol/L L-glutamine. The feed, DMEM Ham's F12 High Glucose medium containing 42 mmol/L D-glucose and 21 mmol/L L-glutamine, started with exponentially increasing feed rate from 94 h after inoculation and ended at 127 h after inoculation, as shown in Fig. 4. The in-situ measurement of DCO_2 and DO have been performed with the sensors mentioned above. Fig. 2 shows a running cultivation in the bioreactor with the two probes included in the setup. The Viable Cell Density (VCD) was checked at least daily by taking samples for off-line microscope counting with Countstar® IC1000 Automated Cell Counter. As a surrogate parameter for substrates, glucose concentration was also measured off-line using a blood glucose meter.

In Fig. 3 and 4, the time courses of different parameters from the cultivation are shown. Dissolved O_2 was measured in % air saturation, the dissolved CO_2 probe was calibrated to 25% in a gas atmosphere containing 25 vol.-% CO_2. In the beginning of the cultivation, bioreactor headspace gassing consisted of air and CO_2 for pH adjustment (compare Fig. 4). The pulsed addition of CO_2 to the gas mixture increased the total gas flow into the headspace, so that the percentage of oxygen in the total gas mixture supplied was then less than 21%. After 50 hours cultivation time, no more CO_2 was included in the gas supply, it now consisted only of air with 21% oxygen until oxygen addition to the gas flow started. From then on, the pH adjustment to 7.0 required NaOH addition only. At about 80 h cultivation time the dissolved O_2 fell below 30% (Fig. 3), so the regulation intervened and mixed pure oxygen into the supply air (Fig. 4).

At 94 h after inoculation, glucose concentration fell below 3 mmol/L (Fig. 3) and the medium feed was started to replenish substrates (Fig. 4). The exponential growth phase of the Hybridoma IV F 19.23 cells continued until 100 h after inoculation. Then growth slowed down, which could be identified by a decreased slope of the DCO_2 curve caused by the decrease of CO_2 production. The slowed growth of the culture was confirmed later by off-line cell counting and validating the earlier observation in the DCO_2 signal. One reason for the apparently slower increase in cell concentration

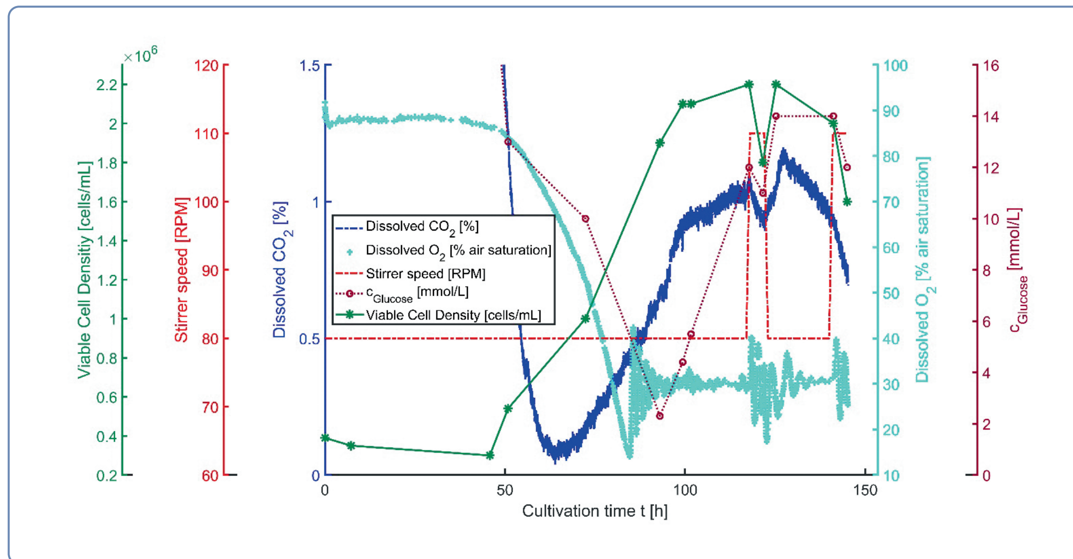

Fig. 3: Inline measured values of DO and DCO_2 along with off-line measuring of VCD and Glucose at different stirrer speeds

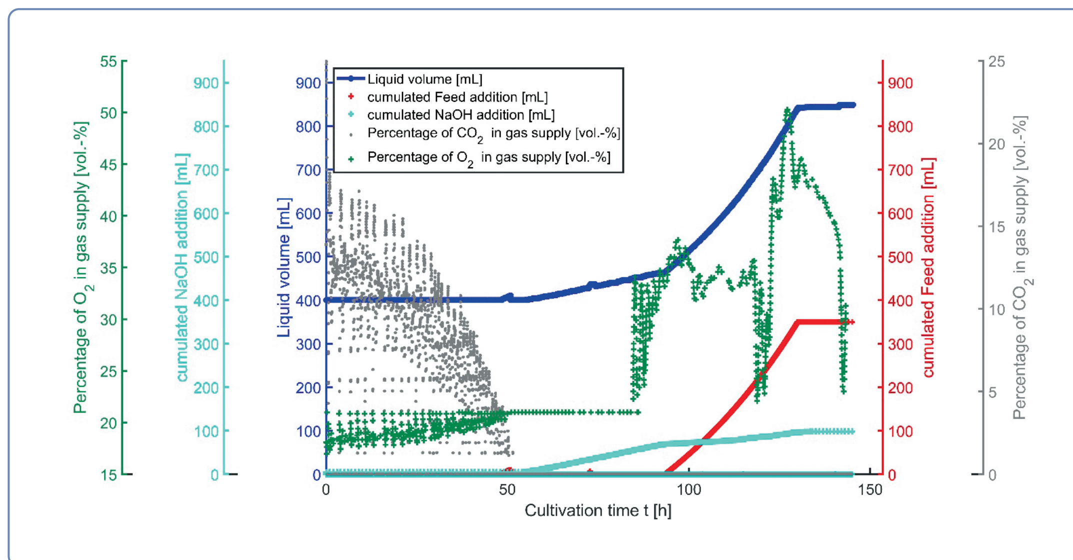

Fig. 4: Bioreactor liquid volume change due to feed and NaOH addition as well as O_2 and CO_2 content in the bioreactor headspace supply

from about 100 h is the dilution effect due to the onset of the feed; it is also possible that a limitation or an inhibition by an unmeasured medium substance occurred.

The agitation speed was increased from 80 RPM to 110 RPM 117 h after inoculation. Of course, possible cell damage due to increased shear stress had to be taken into account. Fig. 3 shows how the CO_2 concentration changed as a result: The DCO_2-curve, which was still increasing slightly up to 117 h, immediately decreased in the dissolved phase as the stirrer speed was increased, with the decrease most likely attributed to increased CO_2 outgassing at the solution surface. Five hours later, the off-line cell counting revealed a sharp drop in VCD, indicating shear damage impacting the cells as the stirrer speed was increased from 80 to 110 RPM. To prevent further cell damage, the stirring speed was reduced again to 80 RPM, 122 h after inoculation and maintained at this point.

Post stirrer speed adjustment, the CO_2 concentration rose much more steeply than before increasing the stirring speed to 110 RPM, suggesting a resurgence of cellular metabolic activity and decreased outgassing at the surface.

Later, off-line measurements showed that the viable cell density indeed had improved temporarily, at least enough to replace the cells that had perished due to the increased stirring speed. After 130 h cultivation time, the death phase began and was observed in both DCO_2 as well as VCD.

The application showed that in-line measurement of dissolved CO_2-concentration with In-line Arc Sensor CO_2NTROL is a suitable means to monitor one more Critical Process Parameter in real time. As this study showed, real-time control of DCO_2 in addition with DO, pH, and aeration or stirring speed enabled supervision of culture conditions despite variable process parameters, resulting in rapid correction of parameters causing negative impacts on viable cell density.

Benefits of real-time DCO_2 monitoring

- Real-time in-situ monitoring of DCO_2 in addition to DO enables acting in time to keep the cell viability as expected
- Enhanced process understanding

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