Noninvasive Oxygen and pH Sensors

Principles and Applications of Disposable Equipment in Bioprocess Development

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Screening suitable cell lines for protein expression and the development of scale-up processes requires optimal culture conditions. **PreSens** (www.presens.de) has developed a chemical optical measurement technology for noninvasive monitoring of the important culture parameters—oxygen, pH, and CO_2.

A wide variety of disposable vessels with integrated sensors and measurement systems for all relevant culture formats are available. This technology can be used in applications ranging from cell and mutant screening to promoting high-performance cell culture in bioreactors.

Monitoring dissolved oxygen concentration (DO) and pH with conventional measurement systems can involve considerable effort, especially in small-scale cultivation formats. Invasive measurement techniques (e.g., electrodes) are problematic when used in contamination-free production processes.

Using chemical optical sensors, culture parameters can be monitored noninvasively. The sensor spots are mounted inside disposable vessels and measurements are taken through the transparent vessel wall. Therefore, optical sensors are minimizing the number of parts that need to be discarded.

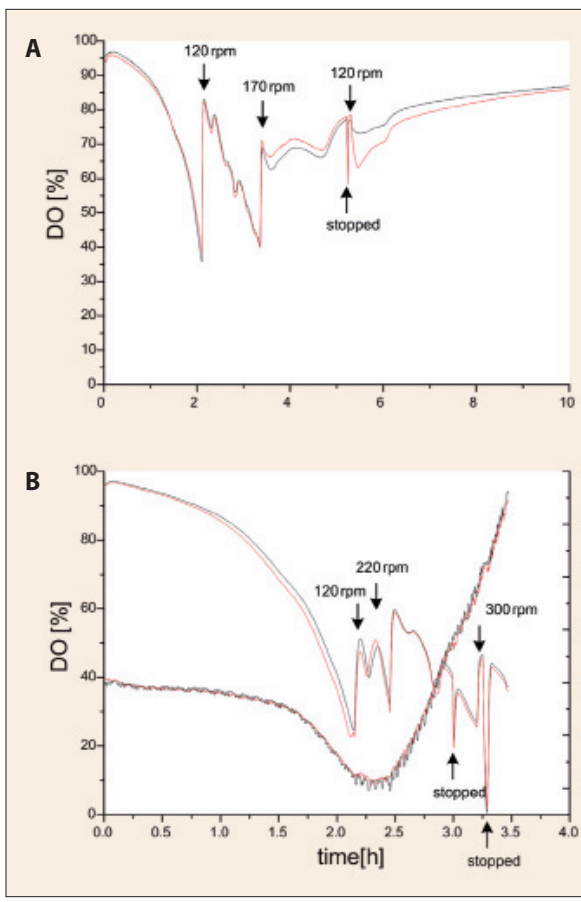

Figure 1. Time course of DO and pH for two batch cultivations of *E. coli* DH5a on LB complex medium in 250 mL baffled glass shake flasks with oxygen sensor (A). 250 mL unbaffled plastic shake flasks equipped with an oxygen and pH sensor. Cultivations were carried out at 37°C and a shaking frequency of 90 rpm in the beginning (B). Changes in shaking rate are indicated by arrows. Duplicates are shown.

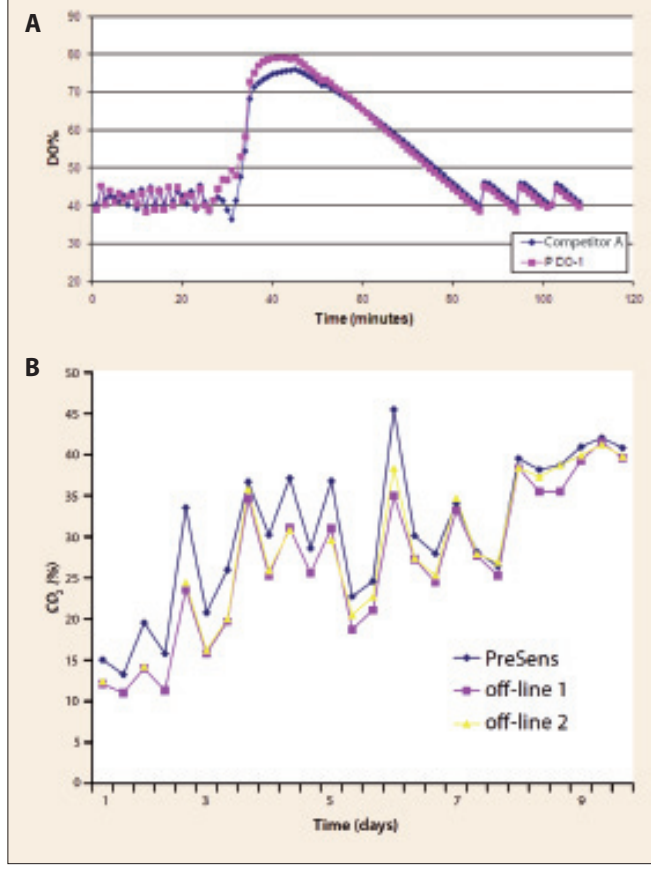

Figure 2. Comparative dissolved oxygen (DO) monitoring in a bioreactor with CHO cells for three weeks—showing effect of medium change on DO concentration. Blue–Competitor A electrochemical DO; red–PreSens optical DO (A). Comparative monitoring of dissolved carbon dioxide (dCO_2) in a CHO-cell bioreactor culture for three weeks–PreSens optical patch sensor vs. offline electrochemical sensors. Note the close trend between real-time and standard electrochemical off-line dCO_2 probes (B).

PreSens chemical optical sensors consist of a thin layer containing an analyte-sensitive (oxygen, pH, or CO_2) dye. These sensor spots, which are excited by light of a certain wavelength, then fluoresce. If the sensor spot encounters a molecule of the respective analyte, the excess energy is transferred in a nonradiative way, decreasing or quenching the fluorescence signal.

This principle of decay time measurement is used to determine the oxygen concentration in the sample. In the case of pH and CO_2 detection, a combination of different fluorescent dyes is used for Dual Lifetime Referencing (DLR), a patented internally referenced measurement principle.

The respective reading systems comprise integrated optical modules applying LEDs, photodiodes, and polymer optical fiber to transfer light to the sensors and read the luminescence response.

Culture Monitoring

Microplates are widely used to increase throughput while decreasing sample volume. The multidishes, available in 24- or 6-well format, contain either an oxygen sensor (OxoDish®) or pH sensor (HydroDish®) at the bottom of each well. Filled with the sample the multidish is put onto the Sensor Dish® Reader (SDR) and the sensors are read out noninvasively. The SDR can be used in incubators and on shakers. (For more information on the SDR, see GEN, March 15, 2011, p. 52).

Shake flask cultures are used across a broad field, including industrial bioprocess

development. PreSens has developed disposable sensors that are mounted inside Erlenmeyer flasks that are used with the Shake Flask Reader (SFR).

The system, which enables simultaneous online monitoring of both oxygen and pH values within up to nine shake flasks, also allows continuous insight into oxygen supply and metabolic activity of a particular culture. The system is mounted on a conventional shaker and is compatible with nearly all shaking systems.

To test the performance quality of the SFR system and reproducibility of retrieved data, *E. coli* DH5a was cultivated in baffled glass and unbaffled plastic shake flasks. The flasks were equipped with an oxygen sensor and, in the case of the plastic flasks, with an additional pH sensor.

Shake flasks with similar k_La values were used for parallel experiments to investigate the reproducibility of measurements. (The k_La is a specific coefficient that quantifies oxygen mass transfer in a given cultivation system.)

All cultivations were carried out in duplicate at 37°C and at an initial shaking frequency of 90 rpm. During cultivation in baffled glass shake flasks an accelerating decline of DO could be observed just 2 h after inoculation with an optical density (OD600 nm) of 0.1 (*Figure 1A*).

In order to avoid oxygen limitation, the shaking rate was increased: from 90 to 120 rpm after 2 hours of cultivation, and again from 120 to 170 rpm shaking rate after 3.5 hours. During the first two hours exponential growth was evident. In the subsequent phase different substrates, available in the used LB medium, were consumed.

Within that period rearrangement of the cellular metabolism could be observed, where DO concentration showed some distinct peaks. After a total of five hours the shaking frequency was reduced to 120 rpm. Due to the gradually decreasing respiratory activity of the culture, DO slowly increased.

In contrast, the profiles of DO and pH of cultures in unbaffled shake flasks (*Figure 1B*) revealed significant differences: the supply of oxygen was clearly insufficient. Unbaffled shake flasks were not so well suited for aerobic cultivation of fast-growing *E. coli*.

The chemical optical measurement technology can also be used for improving process quality in bioreactors. Integrated flow-through cells or optical sensor patches, attached to the transparent bioreactor wall, make it possible to take noninvasive measurements.

Flow-through cells are integrated via Luer-lock connectors at external conduits. The additional monitoring of CO_2, as well as oxygen and pH measurements, in bioreactors allows adjustments to be made. For example, if the CO_2 level is too high, oxygen could be added to the headspace of the sample.

To evaluate the efficiency of the chemical optical technology, tests were carried out comparing the chemical optical sensors with conventional electrochemical systems (*Figures 2A, 2B*). Measurements of DO and CO_2 were taken over a period of three weeks in a CHO cell culture. The optical sensor trended very closely with the standard electrochemical probes. Sensitivity of the optosensoric technology is higher than that of the conventional system.

PreSens' optosensoric technology reviewed in this article can significantly contribute to improving the reproducibility and quality of cell cultivation and be applied in a broad range of scientific research. GEN

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