Application Note

INGOLD
Leading Process Analytics

Simplified Oxygen Control in Bioreactors with Optical Technology

Successful mammalian cell fermentation requires careful control of the physicochemical properties of the medium, such as dissolved oxygen (DO). The long batch durations necessitate the use of low maintenance sensors with minimal drift. DO sensors based on optical technology are ideal for this purpose.

Background

Process analytics during fermentation serves to maintain consistent living conditions for the suspended cells or microorganism. This includes monitoring and control of the physicochemical environment such as the level of pH, dissolved oxygen (DO), and dissolved carbon dioxide. Neglecting control of these parameters could potentially impact final product quality. In-line measurement can be employed to maintain the culture in its optimal state.

Cell cultures require oxygen for the production of energy from organic carbon sources. Given oxygen's poor solubility in water, the control of oxygen (air) flow must be carefully regulated to ensure it does not become a rate-limiting factor in the process. In contrast, a hyperoxygenated bioreactor air supply can irreversibly impact culture performance and is also a waste of the energy used for running the air compressor.

Mammalian cells are large, slow growing and very shear-sensitive in comparison with microbial fermentation. Product concentration (titer) is usually very low, and toxic metabolites such as ammonium and lactate are produced during growth. The bioreactor for mammalian cells needs well-controlled homogeneous environmental conditions (correct temperature, pH, DO, and redox potential). Due to the slow growth

METTLER TOLEDO

rate, batch duration can be up to three weeks; therefore, in-line sensors must be very stable throughout the whole run.

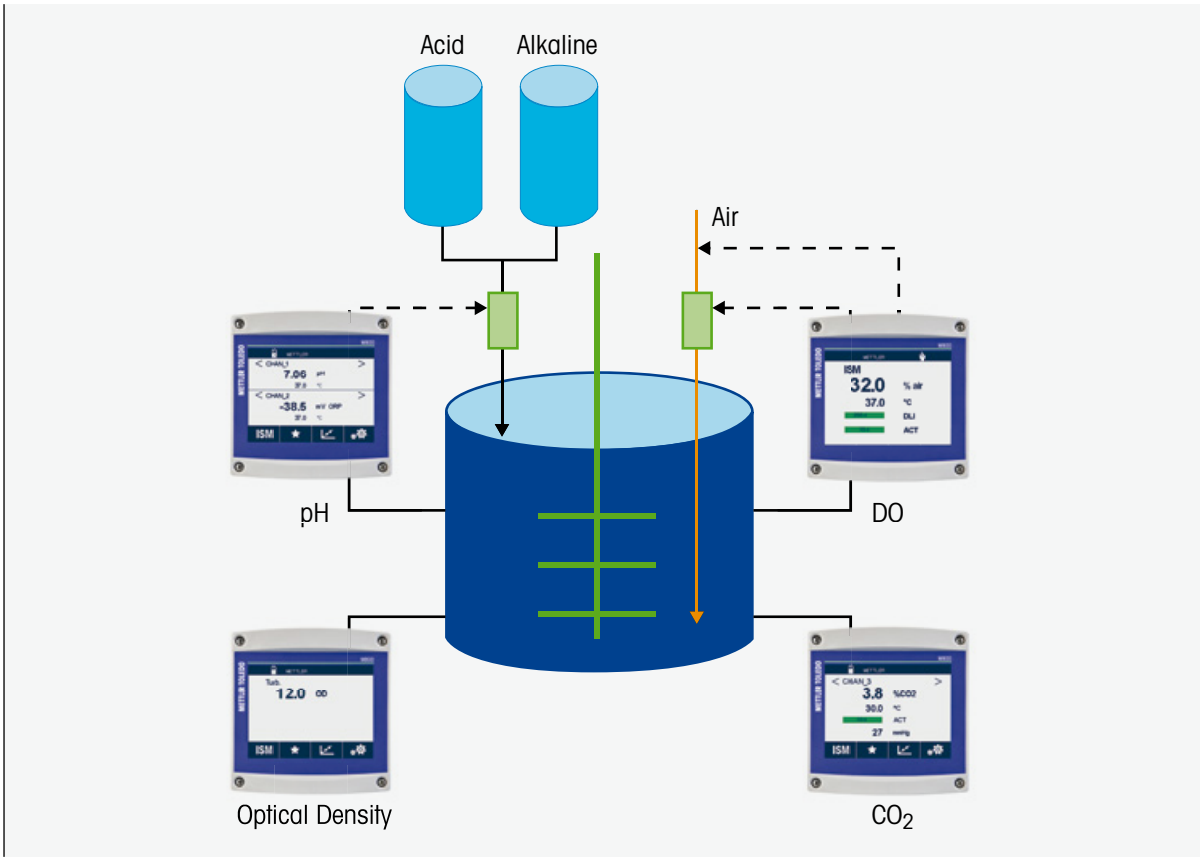
Optical technology DO sensor

In respect of DO measurement, sensors that utilize optical measurement technology offer significant advantages over amperometric technology, as shown in table 1.

METTLER TOLEDO's InPro® 6860i is an optical oxygen sensor designed for the requirements of the pharmaceutical industry. At the heart of the sensors is an oxygen-sensitive layer containing immobilized marker molecules. These molecules absorb light from an LED and are able to release this energy as light of a different wavelength (fluorescence). The time delay between light absorption and emittance is dependent on the partial pressure of oxygen present in the medium. Instead of membrane body, inner body, and electrolyte found in amperometric sensors, only one component, the OptoCap™ (which contains the oxygen-sensitive layer), has to be replaced from time to time as a consumable.

Straightforward calibration of optical DO sensors

It is quite common to perform a sensor calibration after a sterilization cycle in order to achieve a reproducible starting value, e.g. 100 % air saturation or any other desired value. With amperometric sensors, the slope is adjusted during calibration. With optical systems, slope adjustment may result in a corruption of the real calibration data of the sensor because the desired value does not necessarily represent the true oxygen saturation value. For the real oxygen value, process pressure and salinity measurements need to be performed. For end users switching from amperometric to optical sensors, this change in procedure can be confusing and they may prefer to use their existing SOP. With the process calibration option, "scaling", the InPro 6860i can be adjusted to the desired value without changing the process pressure values. This procedure is now very similar to what is done with amperometric sensors with only one difference; instead of a slope correction, a process calibration needs to be performed. (If "scaling" has been chosen, the calibration curve of the sensor will be untouched, but the output signal of the sensor will be scaled.)



Typical setup for bioreactor control

Amperometric technology	Optical technology	Benefits of optical technology
Medium drift rate	Very low drift rate and shorter response time	Highly suited to long batch runs
Frequent membrane and electrolyte exchange. Risk of electrolyte leakage.	Electrolyte-free	Low maintenance (OptoCap exchange after 6 – 7 months). No risk of electrolyte leakage.
6 hours pre-polarization prior to calibration and measurement	No polarization needed	Ready for measuring when connected to a transmitter, even after autoclaving. Works out of the box. High availability.

Table 1: Amperometric vs. optical technology

Lifetime of OptoCap

To reduce the stress on the OptoCap and maximize its lifetime, the sampling rate can be reduced. Using the InPro 6860i sensor, changes in the sampling rate between 1 and 20 seconds do not affect the response time because the system does not perform an averaging of measurements (the recommended sampling rate for biotechnology application is 10 to 30 seconds). During sterilization and CIP, oxygen measurement is not needed. Throughout these processes the measurement is switched off, resulting in a further extension of OptoCap lifetime.

Conclusion

Maintaining ideal conditions during mammalian cell fermentation requires control of a number of parameters, including dissolved oxygen. The response time and drift behavior of METTLER TOLEDO's InPro 6860i oxygen sensors is significantly better than that of amperometric sensors. As batch times for mammalian cell and algae cultures are long, the low drift and low maintenance of the sensors are major benefits.

For more information, visit:
www.mt.com/pro-pharma

InPro 6860i O_2 sensor
- Optical technology
- Slim design allows easy mounting in benchtop bioreactors
- Versatile output – nA or 4 – 20 mA or digital ISM and MODBUS

M800 transmitter
- Multi-parameter and multi-channel
- Color touchscreen simplifies operation

METTLER TOLEDO Group
Process Analytics
Local contact: www.mt.com/pro-MOs

www.mt.com/pro
For more information

PA4081en B
MarCom Urdorf, CH