Whitepaper

Endress+Hauser
People for Process Automation

Measuring consistency from laboratory to process

Whitepaper on digital sensors in the biotechnology industry

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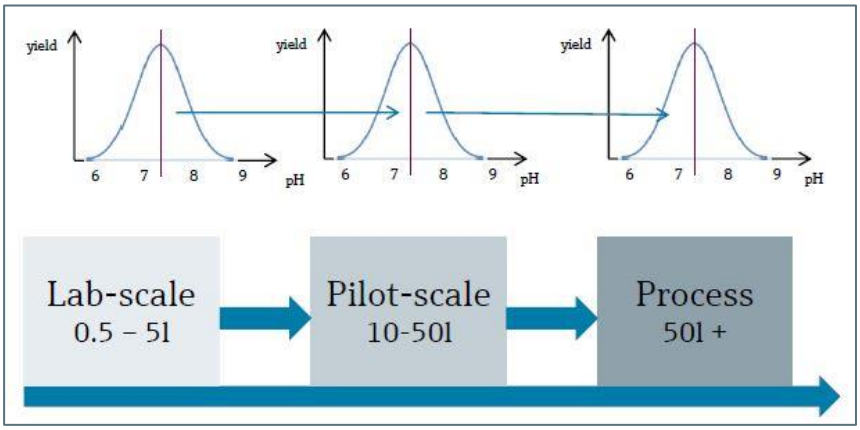
Abstract
In the biotechnology industry analytical sensors are commonly standardized in terms of brand and type during process development. This helps to maintain data consistency when the process is later scaled-up. Despite this, companies can still run into considerable problems caused by unreliable sensor signals and disparities concerning the signal algorithm and sensor handling. Digital sensors offer a solution to guarantee data consistency and a way to easy, uniform sensor management.

The importance of measuring consistency from lab to process

It is vital creating the right conditions in the bioreactor during trials and in the up-scaled process to allow microorganisms or cells to thrive. Correct environment conditions will ensure that the yield is maximized in a stable and predictable manner. Two of the most critical parameters during a fermentation process are pH and oxygen, and both need to be controlled carefully. pH and dissolved oxygen values out of specification lead directly to a loss of yield. For some specific cells, typically Mammalian cells from humans and hamsters, the pH value is highly critical and needs to be controlled in a range better then ± 0.1 to 0.2 pH units to obtain the expected yield. The oxygen concentration gets critical for the batch if it is too low, e.g. less than 20-25%, as there is not enough oxygen for respiration. On the other hand, too much oxygen risks the yield as some bacteria tend to grow in size more than to increase the production of the wanted molecules. Beside that it is also a waste of expensive sterile oxygen.

When a process is scaled up from the initial laboratory fermentation to pilot and to full-scaled, it is stringent to keep all conditions unchanged as well as it is preferred to keep the identical sensors down to brand and type. This is to ensure that no measuring discrepancies occur when the process is up-scaled that could risk a decrease of process yield. Measuring behavior and performance between different sensor brands can occur due to several reasons such as different compensation algorithms, different material performance or different sensor design. Despite standardization of the sensors, discrepancies are anyhow common. They usually can be related to the analytical sensors themselves or to the electrical signals from the sensors. In the following, we will explain different reasons behind errors related to the electrical signals and show how those errors can be eliminated by using digital sensors.



Challenges concerning consistency of pH measurements

One of the largest challenges, especially for pH sensors, occurs already with the bioreactors in the laboratory. During the autoclavation both the glass fermenter and the sensors are exposed to high temperature in combination with steam. If humidity remains on the sensor contacts this will later result in unreliable and unstable measured values.

It is a well-known fact that the high impedance mV-signal from a pH sensor is very sensitive to any humidity or oxides on the metallic cable contacts. Signal drops will result in unpredictable measuring errors and depending on the environment, they can occur randomly. The biggest challenge is if they only appear occasionally, as this makes them hard to detect.

An ideal pH sensor has a zero point at pH 7.00. In other words, in a pH 7.00 solution, an ideal pH sensor provides a 0mV signal. In a pH 8.00 solution the same pH sensor will provide a -59.16 mV signal (at 25°C). Under perfect conditions this signal is measured without interference and converted into the pH value by the transmitter. But when corrosion, humidity or oxides are present on the sensor on the sensor and cable contacts, part of, or in the worst case, all of the 59.16 mV will disappear, and the signal gets closer to 0mV (pH 7.00). The signal from the pH sensor would indicate a lower value than there is in reality and the controller in the fermenter would continue to add reagent to increase the pH. The result in this case would be an overdosing of reagents which results in a pH value out of specification and likely a wasted batch.

Comparability of measurements in the laboratory and in the process

During all the development steps it is common to control and even adjust the on-line pH value in the fermentation by grab-sample analysis, where a relatively small sample is analyzed with a laboratory pH sensor. This is the second challenge regarding consistency of the pH-measurement. It is common that measuring discrepancies occur also between measurements in the laboratory and in the process. There may be several reasons for this. But even high-end pH sensors tend to show discrepancies in measurement if the measurements are carried out with sensors of different brands or types.

Typical reasons for this can be:
- Diffusion potentials in the pH sensor due to different reference systems
- Nonlinearity at high/low pH-values because of different membrane glass
- Different temperature behavior dependent on the isothermal point
- Different compensation algorithms in the pH-transmitter

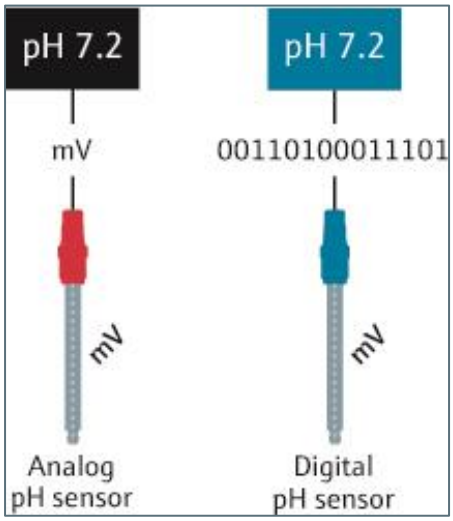
Challenges concerning consistency of dissolved oxygen concentration measurements
There are two types of measuring technologies available for dissolved oxygen measurement: the traditional amperometric and the optical florescence technology. Amperometric oxygen sensors provide a very small nA signal proportional to the oxygen concentration. Commonly a freshly maintained sensor provides 0nA at 0 mg/l (%) and 60 to 70 nA at the saturation point (100%). This small nA current measuring signal requires a sophisticated controller to detect variation in the process.
In contrast the optical measuring principle is based on fluorescence quenching, where oxygen sensitive molecules are integrated into an optically active fluorescence layer. By applying energy, in general light with a specific wavelength on this layer, a response in form of fluorescent light is received that is inversely proportional to the oxygen concentration in the solution. The decay time and intensity of the response signals are inversely proportional to the oxygen content in the solution.
The optical sensor technology has several advantages compared to the traditional amperometric method:

- No fragile membrane and no electrolyte
- No polarization time required
- Very easy maintenance and handling

The challenge with optical and amperometric oxygen sensors is mainly the interference of air bubbles at the O_2-sensitive membrane when the sensor is top-down mounted. A dissolved oxygen sensor should measure the concentration of oxygen that is dissolved in the solution and that can be employed by the bacteria and the cells. It should not be sensitive to the oxygen of the air bubbles in the solution. The oxygen concentration in the bubbles is completely different to what is dissolved. When an oxygen sensor is installed in a laboratory fermenter it is mainly installed top-down. This installation is always a risk as bubbles tend to stick on the O_2-sensitive membrane. The influence can be minimized with electronic filters and damping of the sensor signal. However, this will slow down the sensor response. In a pilot and larger fermenter, the oxygen sensors are installed from the side slightly angled from a horizontal line. In this position the influence of air bubbles is neglectable. The next challenge arises when values from those two applications are compared. The best solution on the market so far is to use an oxygen sensor with a convex sensor tip. It minimizes the risk that bubbles get stuck and also enables top-down installation.

Advantages of digital sensors

Digital sensors can solve the challenges of pH measurement. A digital sensor is, as the name implies, digital. The actual sensing part of the sensor is analog and identical to a conventional analog sensor. The difference is that digital sensors include an additional component in form of a microprocessor that processes measuring signals. Generally, several signals need to be processed and considered in parallel.



The advantage of digital analytical sensors is that they provide 100% signal integrity, improving the reliability of the measurement value. Compared to a measurement loop with analog sensor technology, there is no risk of signal loss between the sensor and the displayed measurement value. Moreover, humidity and oxides on contact surfaces do not cause any issues for the measurement. Either you receive a correct measurement or no measurement at all. This is a great step forward for all fermenter applications in the laboratory, as any remaining humidity on the contact surfaces after the autoclavation will no longer cause distorted or unstable values.
Measuring consistency means maintaining the same sensor brand and type, as well as keeping the calculation algorithms behind the measurement values unchanged when a process is scaled up from the laboratory to pilot and full process capacity. Standardization of digital signal processing is much easier between different transmitters when using digital information as opposed to analog signals.

Sensor adjustment and sensor handling

A second great benefit of digital sensors is that sensor handling and sensor adjustment can be standardized between the laboratory and the process. The adjustment of an analog pH sensor can be challenging at the measuring point as buffer and cleaning solutions need to be brought

from point to point and additional documentation needs to be done. Digital sensors carry their own adjustment data, which means that they can be cleaned, calibrated and adjusted offline in a stable environment and later installed in process or in laboratory applications.

Sensor adjustment in the laboratory provides several benefits. Beside the time saving aspect, also the measuring reliability can be improved. The high concentration of protein molecules in the fermentation can easily contribute to clogging of the pH sensors reference diaphragm. This will in the end shorten the sensor lifetime and contribute to measuring errors if it is not cleaned properly. For reliable measurements batch to batch the sensor needs to be carefully maintained with an acid in combination with a pepsin solution. This maintenance can be done more easily in the laboratory compared to the measuring point. The direct result of sensor maintenance in the laboratory is better performance, higher measuring accuracy and in many cases a prolonged lifetime.
By using a digital sensor technology that in parallel provides the possibility to use a software for sensor maintenance and management, all handling can largely be standardized and simplified. Digital sensor technology also minimizes the risk of discrepancies between the grab sample and the online measurement.
By using the identical digital sensor and signal technology in parallel with appropriate sensor handling, any risk for incorrect values is minimized.

Memosens sensors for reliable, stable measured values
Digital Memosens sensors with inductive coupling are completely resistant to moisture and can even be connected under water. This guarantees maximum accuracy. Memosens digitizes the measured value within the sensor and provides non-contact interference-free transfer to the transmitter/controller. With the CYM17 Memosens Analog Converter, digital Memosens sensors can now be used in biotechnology laboratories too. The sensors can simply be installed in existing fermenters and connected to the converter. Different adapter cables are available for easy and fast conversion. The output signal of the CYM17 converter corresponds to that of a conventional analog pH or oxygen sensor.

The inductive Memosens coupling

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High-performance sensors for biotechnology – in the laboratory and in the process

Memosens CPS171D pH sensor
Thanks to its robust design and long-term stability, the sensor ensures extremely accurate, reproducible and reliable measurement results—even after autoclaving (up to 140°C/284°F).

Memosens COS81D
optical oxygen sensor
The accuracy and long-term stability of its measurements, as well as its permanent self-monitoring, ensure maximum reliability of the measured values.

Memosens analog converter CYM17
enables the use of digital Memosens sensors with the fermenters.

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