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pH Sensor Recalibration Based on Exhaust CO_2 Concentration for Bioprocess Transfer and Scaling

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Abstract

The pH of the culture medium is a critical process parameter in mammalian and microbial bioprocesses. To successfully transfer processes between bioprocess systems and sites – in the course of process development and scale-up, for example – it is essential to ensure that pH readings in the different systems are comparable. Bioprocess engineers use offline pH measurements for the recalibration of online pH sensors. These can be inaccurate, because factors like sensor age, temperature, and CO_2 degassing influence the measurements, making direct cross-site comparisons of pH values difficult. In this application note, bioprocess engineers from Roche Pharma Technical Development describe a method for accurate in-line pH sensor recalibration based on CO_2 concentration in the exhaust, using a DASGIP® GA4 exhaust analyzer. This method allows effectively matching of the starting pH of carbonate-buffered systems in process transfers, scale up and scale down; cross plants, sites, and scales.

Introduction

Bioprocess development usually starts with small working volumes. Once the producer cell line, medium composition, and critical process parameters have been defined and optimized at small scale, scientists scale the process up to larger manufacturing volumes. This usually involves the transfer of the process to another bioprocess system, often located at a different site. It is crucial that critical factors like the growth rate, quality attributes of the product, and product concentration are comparable between processes at different scales and locations. To ensure this, scientists identify critical process parameters and keep them constant in the course of process transfer. The pH of the culture medium strongly influences process performance. Although bioprocess controllers and software allow for online control of pH, monitoring and adjustment after sensor sterilization can have pitfalls. pH measurement requires the calibration of a pH sensor using buffers of known pH to set the offset and slope. In cell culture bioprocessing, pH sensor calibration usually follows this workflow (Figure 1): The pH sensor is calibrated outside the bioreactor and then built into the bioreactor for sterilization. After autoclaving, the bioreactor is filled with sterile medium. Bioprocess engineers now routinely recalibrate the sensor to correct for possible changes caused by the autoclaving. They take a sample of medium from the bioreactor, measure its pH offline with a standard pH meter or blood gas analyzer, and use the offline reading for sensor recalibration. Direct comparability of bioreactor pH sensor readings between systems and sites cannot be ensured, because the offline pH measurements, and therefore the recalibration, are prone to error. There

are many sources of errors. The time between sampling and offline measurement can cause temperature shifts and CO_2 degassing, leading to a pH value of the sample, which does not reflect the value of the medium inside the bioreactor anymore. Furthermore, sensor age, response time, daily adjustment procedures, and other factors may influence offline pH readings. pH offsets of up to 0.3 between sites and plants have been shown to exist, and cannot be detected using the equipment which caused them in the first place. This makes process transfer and troubleshooting difficult.

In this application note, bioprocess engineers from Roche Pharma Technical Development present an alternative recalibration procedure, which is performed in-line without sampling and therefore avoids the possible errors of offline pH measurements. They indirectly determine the pH of cell-free, carbonate-buffered culture medium by measuring the CO_2 concentration in the bioreactor exhaust and use this data for pH sensor recalibration (Figure 1).

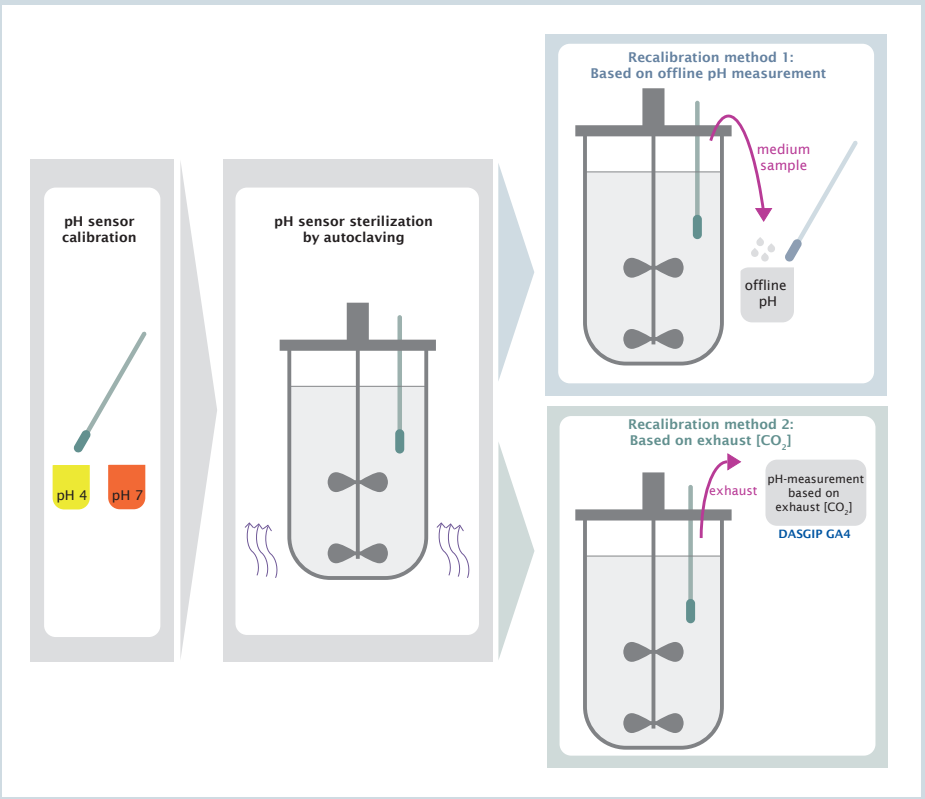

Fig. 1: Workflow for pH sensor calibration and recalibration. The pH-sensor is calibrated outside the bioreactor using buffers at pH 4 and pH 7 (left). The sensor is then built into the bioreactor and sterilized by autoclaving (middle). Afterwards the sensor is recalibrated, to correct for changes caused by autoclaving (right). Routinely, recalibration is based on offline pH readings (right, top). However this method is prone to errors. Alternatively, the pH sensor can be recalibrated based on the CO_2 concentration in the exhaust (right, bottom). This method is described in this document.

Material and Methods

Measuring principle

According to the Henderson-Hasselbalch equation, CO_2 concentration in the gas phase must be identical in multiple systems, if the media, temperature, pressure, and pH are identical. At equilibrium, the net CO_2 mass transfer between the gas phase and the liquid phase is zero. In this state, the CO_2 concentration in the gas phase is not a function of parameters that influence mass transfer kinetics, and can therefore be considered as scale-independent.

Thus, one can indirectly infer to the pH of the culture medium from the CO_2 concentration in the exhaust and use this information for pH sensor recalibration.

Exhaust analyzing

In all experiments, the CO_2 concentration in the exhaust was quantified with a DASGIP GA4 exhaust analyzer (Eppendorf, Germany, Figure 2). The device uses robust dual-beam infrared CO_2 sensor technology by BlueSens® (BlueSens gas sensor GmbH, Germany). Four carbon dioxide analyzer channels allow the precise exhaust analysis of four bioreactors in parallel with a single DASGIP GA4. Integrated sensors allow for an automated compensation of pressure, humidity, and temperature effects.

Fig. 2: DASGIP GA4 exhaust analyzer

Bioprocess system

Experiments at small scale were carried out using a glass bioreactor with a maximum working volume of 2 L. Further experiments were carried out in bioreactors with working volumes of 100 L and 400 L. For the 100 L and 400 L bioreactors it was ensured by installing a bypass, that the gas flow to the DASGIP GA4 did not exceed 250 sL/h.

Correlation of exhaust CO_2 concentration and medium pH

2 L glass bioreactors were filled with cell-free culture medium. The working volumes, agitation speed, gassing (percentage of CO_2, VVM) were varied as described in the figure legends in the results section. The CO_2 concentration in the exhaust was measured. To be able to correlate the CO_2 concentration in the exhaust with the pH of the medium, the latter was measured online using the bioreactors' pH sensor. The pH of the cell-free culture medium was quantified using the internal pH sensors, which were lowered into the bioreactors under non-sterile conditions via the lid. This approach allows verification of bioreactor sensor signals without sampling and offline measurement, although it cannot be applied under sterile conditions.

The correlation of exhaust CO_2 and medium pH are described by a calibration curve that was then used in further experiments for in-line recalibration of the pH sensor.

Verification of scale-independence of the pH sensor recalibration procedure

To verify the method for in-line pH sensor recalibration, the process was scaled up. The experimental procedure described above was applied in bioreactors with working volumes of 100 L and 400 L.

Parameters like power number per volume (P/V), k_La, tip speed, gassing, and stirrer configuration were not kept constant.

The CO_2 concentration in the exhaust was measured with different DASGIP GA4 gas analyzers.

The pH of the cell-free culture medium was measured using the internal pH sensors, which were inserted under non-sterile conditions, as described above.

Verification of the recalibration method by measuring lactate levels

It is well known that secretion of lactate by CHO cells via a lactate-proton symporter increases with increasing pH of the culture medium. Therefore, the lactate concentration in the medium can serve as a reliable indirect signal of the medium pH.

CHO cell bioprocesses were performed in glass bioreactors with a working volume of 2 L. The cells were cultivated for up to 200 hours. Two different pH setpoints were used. Up to eight replicates were analyzed per experimental condition. Before inoculation, the pH sensors were either recalibrated based on the CO_2 concentration in

the exhaust or using a standard procedure based on offline pH readings. All other process parameters were the same for all runs until 200 h into the batch. After that, the feed strategy was modified.

Results

Figure 3 shows the correlation of the exhaust CO_2 concentration and the pH of the cell-free culture medium. The correlation between the parameters was compared for four bioreactors, which were operated under different experimental conditions. Parameters like the agitation speed, P/V, k_La, and the presence or absence of baffles varied, but did not influence the correlation.

Next, the correlation between exhaust CO_2 concentration and pH was compared at different scales. Again, factors including agitation speed, P/V, and gas flow differed at the different scales. Figure 4 shows that the correlation between the two parameters was scale-independent.

In summary, after pH sensor recalibration based on independent readings without sampling and offline measurement, the correlation of exhaust CO_2 and pH has been proven independent from the scale and other parameters, including agitation speed, P/V, and gas flow (Figure 3, 4).

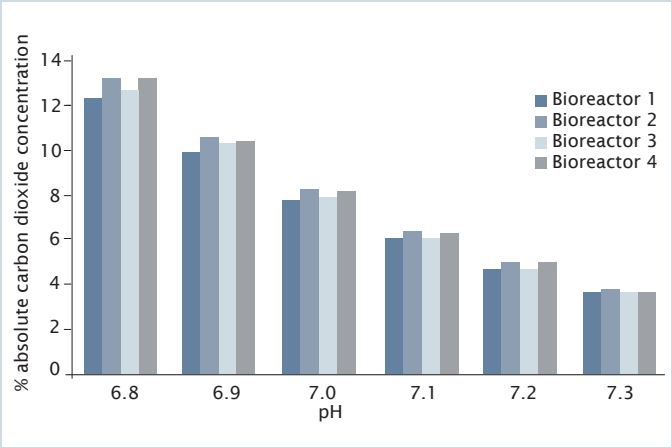

Fig. 3: Measurements were carried out in four glass bioreactors with a maximum working volume of 2 L. The CO_2 concentration in the exhaust was measured at different medium pH values. The four bioreactors were operated under different experimental conditions, with factors like the working volume, agitation speed, P/V, and k_La being different.

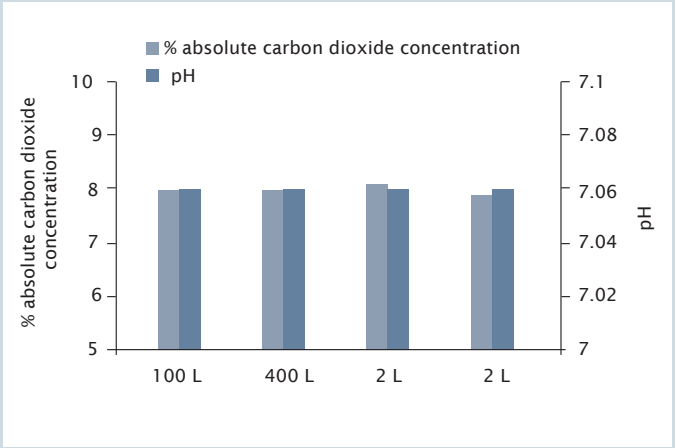

Fig. 4: pH and exhaust CO_2 concentration were measured in a bioreactor with a working volume of 100 L, a bioreactor with a working volume of 400 L, and two 2 L bioreactors.

The lactate concentration in the medium was used as an indirect signal of the medium pH as described in the Method section. In the runs in which the pH sensors were recalibrated based on offline measurements the lactate concentrations varied (Figure 4, blue). In the runs in which the pH sensors were recalibrated based on the CO_2 concentration in the exhaust the lactate concentrations in the replicates remained virtually identical (Figure 4, green and red). This indicates that sensors recalibrated based on the exhaust CO_2 concentration deliver more consistent measurements than sensors recalibrated using standard procedures based on offline measurements.

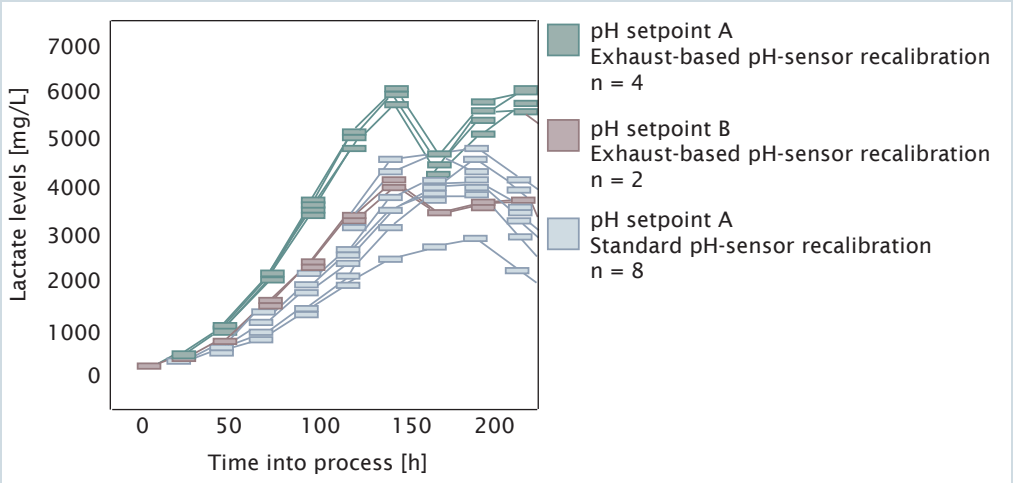

Fig. 4: CHO cell culture processes were performed. The pH sensors were recalibrated either based on the CO_2 concentration in the exhaust (exhaust-based) or based on offline readings (standard).

Conclusion

Scientists from Roche Pharma Technical Development, Penzberg, Germany, present a simple and non-invasive method to apply CO_2 exhaust levels to indirectly monitor and control pH in cell free systems. By applying the method presented it is possible to effectively match start-pH in process transfers, scale up, and scale down, across plants, sites, and scales.

By using the exhaust-related pH measurement method, pH comparability can be established and pH excluded as a source of potential deviation. Troubleshooting can also be conducted more efficiently. Variability in development can also be significantly decreased, which would lead to more efficient process development. In summary, the exhaust-based pH reference method provides a simple, cost-effective, reproducible, and robust way to detect otherwise undetectable but relevant pH offsets in carbonate buffered systems.

Ordering information

Description	Order no.
DASGIP® GA4 Stand-Alone Exhaust Analyzing Module, for 4 vessels, including relative humidity measurement and accessories, O_2 1 – 50 %, CO_2 0 – 25 %	76DMGA4
DASGIP® GA4 Stand-Alone Exhaust Analyzing Module, for 4 vessels, including relative humidity measurement and accessories, O_2 0 – 100 %, CO_2 0 – 25 % (GA4E)	76DMGA4E

Two alternative electrochemical O_2 sensors allow to best serve individual customer's needs (1 – 50 % O_2 or 0 – 100 % O_2)

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