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understanding bioprocesses

Application Report

Report from

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Carbon dioxide driven pH reference method for transfer and scaling of fermentation processes

Abstract:
In this work, we present a carbon dioxide based alternative method that allows challenging the standard approach of offline pH measurement, and is able to establish comparable pH values globally independent from the local procedure for sample based pH offline measurement. In cell free culture media, a bioreactor state where carbon dioxide addition equals carbon dioxide removal leads to stable pH and a net carbon dioxide mass transfer between the gas phase and the liquid phase of zero. In this case, carbon dioxide concentration in the gas phase is not any more a function of parameters that influence mass transfer kinetics, and can therefore be considered scale independent.

Introduction:
For the last decades, engineering aspects were in the focus for scale up, scale down as well as transfers of fermentation processes.

For cell culture processes however, comparability of process parameters like pCO_2, lactate concentration, growth rates, base addition and ultimately product concentration and quality attributes between large and small scale was not sufficiently addressed by those parameters alone. On the other hand, parameters exist like pH and dissolved oxygen that are independent from both equipment and location. Usually they are maintained by respective control loops. Especially pH has been proven to be of particular significance for process performance, and therefore for scaling purposes as well.
The current standard approach to monitor and adjust bioreactor probes however relies on sample based pH offline readings. Unfortunately, pH in a sample depends on a variety of parameters. CO_2 degassing, temperature, overall respiration of suspended cells and other parameters might differ from

the actual bioreactor pH after sampling. In addition to that, offline measurement methods might deliver different results depending on probe type and age, media properties, daily adjustment procedures, response times, operator effects etc. It becomes clear that the sum of those offsets cannot be detected or quantified using the very same sample based pH offline measurement that introduces those offsets in the first place. Relying on sample based pH offline measurement, direct cross-site comparison of pH values that are desperately needed for efficient process transfer are impossible in required accuracy. To decrease the risk of process variability and potential quality issues, increase efficiency of troubleshooting, scaling and process transfers, a method that allows detecting otherwise undetectable pH offsets is essential.

Background

The standard industry approach of offline pH measurement is using a variety of devices and methods to measure pH in a sample that has to be removed from the bioreactor prior to measurement. Regularly internal probes after sterilization (Figure 1) are adjusted based on an offline value in fermentation processes. Offsets are thereby unavoidable due to method specifications, variable procedures, carbon dioxide degassing, temperature drops, ongoing cell metabolism and more. Offsets for offline pH measurement may be up to 0.3 between sites and plants, making process transfers difficult, and requiring time and money consuming trouble-shooting efforts.

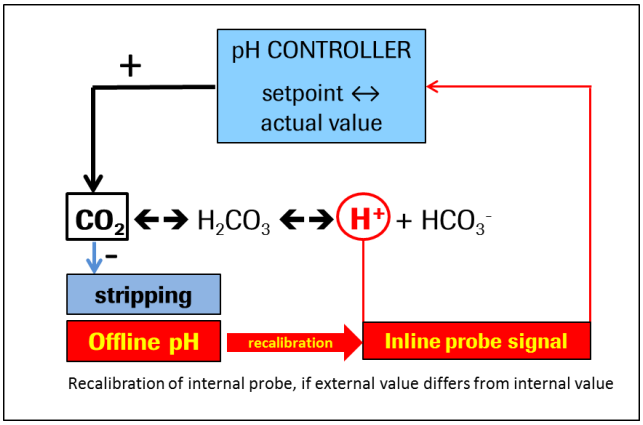

Figure 1: Standard set up for pH control using carbon dioxide gas as acidic pH correction agent

The hereby proposed alternative (Figure 2) using the off gas CO_2 data is able to eliminate all offsets that might be introduced by pH offline measurement and allow adjustment of internal pH probes before inoculation without even sampling. The CO_2 signal is directly referring to the inline probe signal which in return communicates with the pH controller.

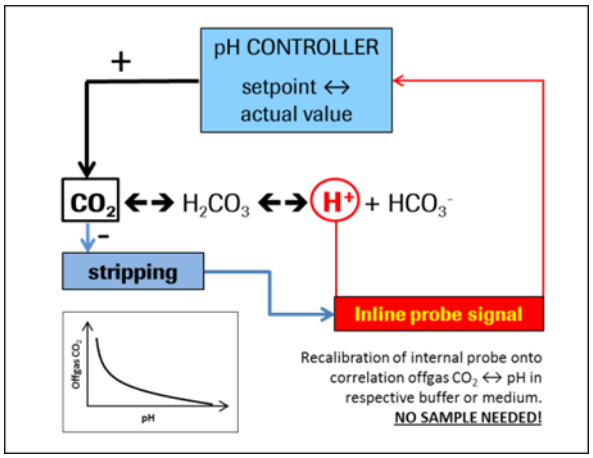

Figure 2: State of the art pH control via off-gas reference method

Principle of approach

To overcome the challenges of offsets created by offline measurement methods one can use the chemical correlation of carbon dioxide in the gas phase and overall proton activity in liquid phase that is described by the pH value. Carbon dioxide concentration in the gas phase has to be identical in identical media as long as temperature and pressure, as well as pH are identical (Henderson-Hasselbalch). This chemical correlation is NOT influenced by parameters that effect mass transfer, because overall net mass transfer is zero in equilibrium (pH, T, p = const.) and is therefore universal (that means e.g. scale independent). Thus, CO_2 levels in e.g. bioreactor off-gas can be used to indirectly monitor, if internal pH is within range or not; without the need to exclusively rely on pH offline measurement that has been proven to be potentially flawed.

Proof of principle

Small scale experiments were carried out in 2L scale using an off-gas analyzer GA4 (Eppendorf, Germany) with embedded BlueSens technology (BlueSens gas sensor GmbH, Germany). Experiments in 2L, 100L and 400L and 10.000 L scale were conducted using both GA4 off-gas analyzers (Eppendorf, Germany) and BlueInOne off-gas analyzers (BlueSens gas sensor GmbH, Germany).

Figure 3 shows pH values and corresponding CO_2 concentrations in the exhaust gas in different working volumes (and therefore different vvm) of a cell culture medium. P/V, kLa and tip speeds varied. Agitation speed applied was up to 1500 rpm. Vessels containing baffles were compared to vessels without baffles and the influence of stirring was investigated by using 2 stirres and 1 stirrer respectively (data not shown here).

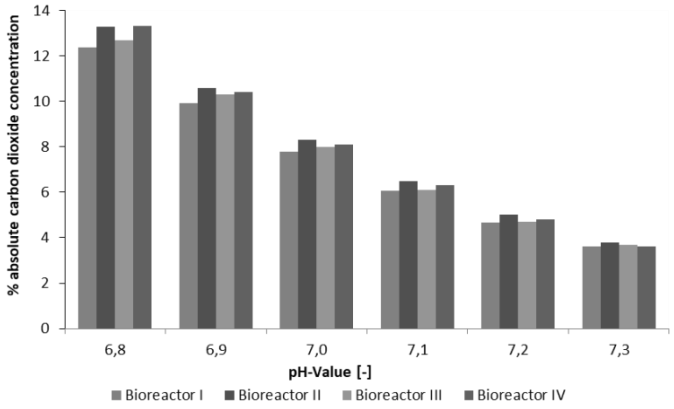

Figure 3: Correlation of off gas CO_2 concentration and pH in different volumes of cell culture media

The correlation of off-gas CO_2 and pH stays identical even at extreme setups like 1500 rpm agitation rate. There was no difference being detected comparing headspace aeration to submerse aeration detected except for the time needed until the equilibrium is reached. To investigate scale independency the experimental process was transferred to bigger scales. Figure 4 compares results for 100L and 400L stainless steel reactors to the approach performed in 2L glass reactors. It shows pH values and corresponding CO_2 concentrations in the exhaust gas after recalibration of internal pH probes and after minimizing pH-controller deviations. Parameters such as P/V, kLa, tip speeds, volumes, vvm and stirrer configuration were not kept constant and thereby varied.

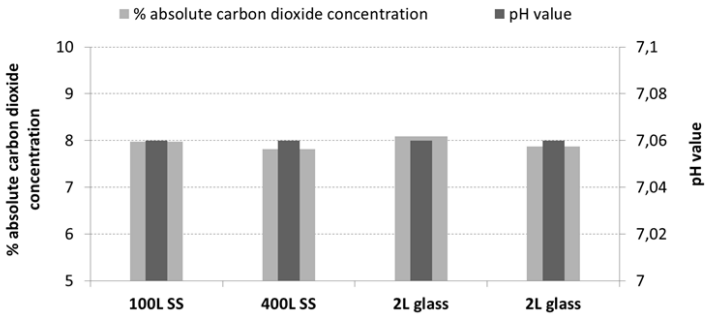

Figure 4: pH values and corresponding carbon dioxide concentration in bioreactor off gas

It could be clearly shown that that the correlation off-gas CO_2 and pH stays identical and that this correlation is independent from scale or any of the other parameters. Provided that the setup is otherwise kept identical Lactate levels can be employed to signal in pH.
Identical pH set points using both methods, offline pH measurement with glass electrode and off-gas based pH reference method, may lead to different internal pH after recalibration of internal probes by dismissing all offsets that are introduced by sampling, and offline measurement. Lactate variability within the set of off-gas based pH recalibration was compared to standard pH offline measurement. Lactate can be considered a very good signaler for pH in extracellular medium. The higher the pH is the more lactate is generally secreted by a CHO cell via lactate-proton symport. pH derived variability therefore can be detected by comparison of lactate levels under otherwise comparable conditions.

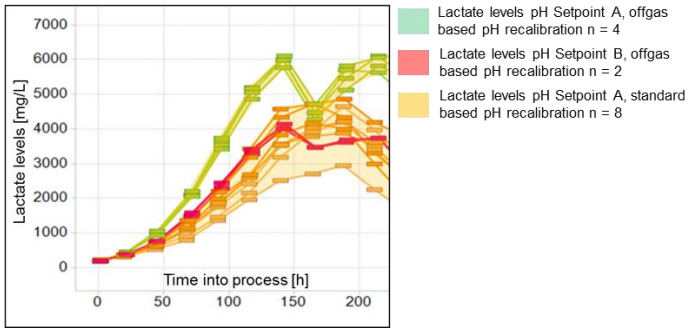

Figure 5: Lactate levels differ if pH is monitored by sample based pH offline measurement under otherwise comparable conditions (orange). If off gas based pH reference method is used to evaluate pH and readjust bioreactor probes, lactate levels become virtually identical with one pH set point (green and red).

Figure 5 shows Lactate levels as pH signaler in identical process setups up to 200 h process time. Within these 200 h only the pH recalibration procedure of internal probes shows significant deviations. Red and green bars indicate for the off-gas based pH reference method whereas orange bars indicate the standard offline pH measurement. Experiments were conducted at 2L scale in the framework of a portfolio project development in non-GMP environment in Penzberg.

Conclusion

Here we presented a simple and non-invasive method to apply CO_2 off gas 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; cross plants, sites and scales.
Troubleshooting can be designed more efficiently. Using the off gas related pH measurement method in future runs pH comparability can be detected and established. The methods results in a definite statement which can be excluded as a source of potential deviations. As a last consequence an increased comparability can be achieved by minimizing variability within production campaigns. Variability in development has been significantly decreased which leads to a more efficient process development. In summary this method allows to reduce the risk of undesired process behavior by potentially flawed sample based pH offline readings. Furthermore, the off gas based pH reference method resembles a simple and cost-effective, reproducible and robust way to detect otherwise undetectable but relevant pH offsets in carbonate buffered systems.