CARBON DIOXIDE DRIVEN PH REFERENCE METHOD FOR TRANSFER AND SCALING OF FERMENTATION PROCESSES

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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 pCO₂, 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 both equipment and location independent and that are usually 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 reading. pH in a sample depends on a variety of parameters like CO₂ degassing, temperature, overall respiration of suspended cells and the like, and might differ from the actual bioreactor pH after sampling. Adding to that, offline measurement methods might deliver different results depending on device type, probe age, media properties, daily adjustment procedures, response times, operator effects and so on. Clearly, 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. Direct cross-site comparison of pH values that are desperately needed for efficient process transfer is impossible in required accuracy, relying on sample based pH offline measurement. 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.

In this work, we present a carbon dioxide based alternative method that allows challenging the standard approach, and is able to establish comparable pH values globally by decreasing dependency onto 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. We have shown that pH values that are derived from this chemical relation are superior to standard sample based pH offline reading, and are able to decrease process variability and increase comparability of process performance between runs, scales and sites. Furthermore, troubleshooting efforts as well as process development are a lot more effective, if pH as one key parameter that adds to process variability can be knocked out or at least quantified. Major automation opportunities in scale down model development like fully automated carbon dioxide removal control strategies that massively depend on comparable pH controller behavior were developed. Phase III development of a late stage project delivering bispecific antibodies already was performed exclusively relying on this carbon dioxide based pH reference method. Another project did switch to this method during phase III development to enable more efficient process development. Scale up has been performed successfully up to 400L scale, feasibility studies were performed in 12K scale in three different projects. Results out of this data will be presented, including important considerations for this kind of approach.