BlueSens
understanding bioprocesses
Application Report

Report from

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Control of dissolved carbon dioxide in driving force limited cell culture processes

Abstract:
A fundamental requirement of bioprocesses is their scalability. Causes of "no scalability" are variances from uncontrolled process parameters. In mammalian cell culture processes pH, dissolved oxygen (dO_2) and partial pressure of carbon dioxide (pCO_2) are reported critical process parameters (CPP). However, in current control concepts those process parameters are not controlled independent from each other. Central challenge is the monitoring of the pCO_2.
Current solutions use pCO_2 probes, but they have the tendency to drift. This paper presents a control strategy, which uses an off-gas analyzer to monitor of the partial pressure of carbon dioxide as control variable.
The application has shown that an independent control of pH, dO_2 and pCO_2 is possible under boundary conditions. Finally, we assessed the validity and found that the transport of carbon dioxide (CO_2) is driving force limited. As part of the presented work, a driving force limitation in weak aerated bioreactors at laboratory scale could be observed for the first time. To date this was known only from large-scale bioreactors.
This allows a process control under defined conditions and consequently increases process robustness. In addition a significant simplification and an increase the robustness of the off gas analysis in low aerated small- and lab- scale cell culture bioreactors can be reached.

Introduction:
Numerous publications investigated influences of various process parameters on the process performance. In this context, scale-dependent process parameters are of particular interest. Present scale-up and scale-down issues often result from insufficient knowledge about potential process-related influences of these parameters [1-4]. However, only a small number of process relevant measurements are collected in production processes.
Reasons for this are that (i) the measurement real-time, (iii) there is no feedback to the process and (iv) no economic benefit. With focus on mammalian cell culture processes particularly the effects of pH, the

dissolved oxygen tension (dO2) and the partial pressure carbon dioxide (pCO2) are well described in literature [4-7]. These three parameters are widespread CPP's. From this follows the need of the monitoring and the control of pH, dO2 and pCO2 in a defined design space.

Additional to temperature and power input, the dissolved oxygen and pH belongs to the most in real-time measured and controlled process variables. In contrast, the dissolved carbon dioxide is commonly not or only at-line measured. The reason for this is that the monitoring of the dissolved carbon dioxide concentration is challenging. A real-time capable method for the in situ measurement of dissolved carbon dioxide already exists [8]. The principle based on a semipermeable membrane, which is pervious for carbon dioxide. The carbon dioxide dissociated in a buffer and changes its pH-value. The measured pH of this buffer correlates to the partial pressure of carbon dioxide. This pH probe is drifting during longer processes. A recalibration of the offsets is possible by proper sample handling, but technologically very complex [8]. Based on this Eisenkrätzer et al. developed a control strategy for a pCO2 control [8]. Due to the complexity, this strategy is not widespread.

The aim of this application note is to present a methodology the control of pCO2 without applying a pCO2-probe in a cell culture processes. The novelty is the usage of a gas analyzer for monitoring the partial pressure of carbon dioxide and uses it for control of pCO2. This allows redundancy to drift-prone in-situ probes. Off gas sensors have a variety of advantages compared to in-situ pCO2-probes.

Off gas sensors (i) enable a stable measurement signal for a long time, (ii) are installed behind the sterile barrier and have therefore no direct contact with the product, (iii)provide additional information (off gasanalysis), (iv) are relatively inexpensive and (v)widely used [9]. However, the main benefitof this measurement type is the overall consideration of the system. In contrast to a local selective measurement by an in situ probe, the off gas analysis provides an averaged pCO2.

Material and Methods:

The listed experiments were performed in a 3.6 L Labfors 5 bioreactor system (Infors, Switzerland). The initial working volume was 2 L.The aeration was realized by four MFCs forair, oxygen, carbon dioxide and nitrogen with an operating range of 0.2-25 [mln / min] (Vögtlin Instruments, Switzerland). As total aeration 30 [mln / min] were defined. The aeration is composed of a constant flow of air (5 [mln / min]) and a total of 25 [mln / min] of the cumulative other gas flows. This corresponds to a flow per minute and liquid volume (vvm) from 0.010-0.015 [Ln / (L · min)]. As in-line measurement devices a pH probe (EASYFERM PLUS K8 200, Hamilton, Switzerland), a pO2 probe (VISIOFERM DO ARC 225, Hamilton, Switzerland), a pCO2 probe (InPro 5000 (i), METTLER TOLEDO, Switzerland) and a temperature probe (PT 100, Infors, Switzerland) were used. The proportions of oxygen, carbon dioxide and water in the off gas were detected on-line by means of an offgas analyzer (BlueInOne Cell, BlueSens, Germany). Lucullus (Soft Cell, Switzerland) was used as Process Information and Management System. As controller, both Step Controller and PI Controller were used.

For all calculations MATLAB (Mathworks, R2015a) has been used.

The fermentation was carried out at 37 °C and ambient pressure. The set points for dO_2 were 40 % of the equilibrium concentration under gassing with air. The set point for pCO_2 was 125 mbar equivalents. The pH set point was 6.8.

Results

Monitoring of pCO2:

The monitoring of the concentration of dissolved carbon dioxide (c_{CO2}) is quite challenging. According to Sieblist's discovery the mass transfer of carbon dioxide must be quantified in terms of k_La-value limitation or driving force limitation [10]. To verify this limitation, process events that lead to a change of the k_La-value can be used. Such an event is represented by the addition of antifoam [10]. Figure 1 shows time courses of the dO_2 and the pCO_2. At time point zero a defined amount of antifoam detergent was added. It can be observed that the antifoam addition strongly influences the dO$_2$ but does not affect the pCO$_2$.

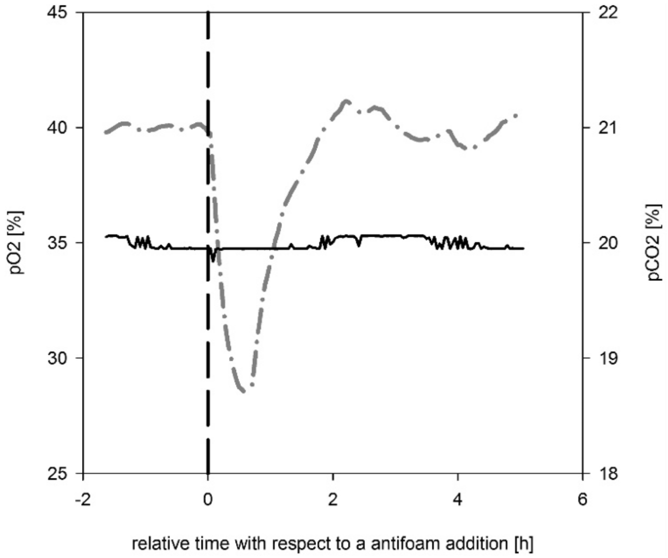

Figure 1: Step response of in situ measured partial pressures of oxygen and carbon dioxide after the addition of antifoam

Assuming the validity of the microscopic film theory this observation can only be explained by Sieblist et al. Thus, it was shown for the first time, that even in small - scale reactors (2 L working volume) states of driving force related transport limitations can occur. The low aeration rate (30 mln/min) in CHO cultivations results in a high mean residence time of the gas phase in the reactor. This is the main cause of the driving force limitation in the investigated system. This leads to two insights: (i) typical problems of large-scale systems can be transferred in small scale systems; (ii) the complex approach of Bonarius et al. and Frahm et al. should not be used to estimate the concentration of dissolved carbon dioxide under given process conditions. Instead the validity of Henry's law can be considered. Thus, the partial pressure of carbon dioxide should be directly proportional to the concentration of dissolved carbon dioxide. Consequently, the partial pressure of carbon dioxide in the off gas under driving force limiting conditions is redundant to in situ measurements. In theory, the off gas signal of pCO2 can be used as control variable for the cCO2.

Control of dissolved carbon dioxide

The carbon dioxide transport rate (CTR) can as well be described by a simple transport term (eq. (1)).

$$CTR = k_{L,CO_2} \cdot a \cdot \left(c^*_{CO_2} - c_{CO_2}\right) \qquad (1)$$

It should be noted that c_{co2} only refers to the concentration of dissolved carbon dioxide. All other carbon species (carbonic acid, carbonate and bicarbonate) are of no importance for this purpose. Considering the film theory k_{L,CO_2} is coupled with k_{L,O_2}. According to Frahm et al. following correlation can be assumed in cell culture media at 37 °C [13] (eq.(2)).

$$k_{L,CO_2} = 0.89 \cdot k_{L,O2} \qquad (2)$$

The CTR can also be influenced by the k_La-value and the driving force. Based on the correlation from the film theory it results that a k_La value dependent control cannot be used in k_La-limited processes. The observation of driving force limitation in small scale systems suggests that a k_La value dependent control is theoretically possible but not ubiquitously applicable. For unknown system conditions (k_La value or driving force limited state) no k_La value dependent control should be used for reasons of robustness. Mandatory for the use of such control application are a constant volume specific power input P and a constant gas superficial velocity $_{sg}$. A fully decoupled control of oxygen and carbon dioxide is accordingly a driving force dependent control of both components. This type of control is only limited by the total pressure in the system (p) and the maximum carbon dioxide transport rate (CTR_{max}) (eq. (3)).

$$CTR_{max} = \frac{1}{V_R} \cdot \frac{p \cdot \dot{V}_{out}}{R \cdot T} \cdot y_{CO2,soll} \qquad (3)$$

In addition to the dissolved carbon dioxide, the other carbon species have to be considered. The relationships of these are described in the literature sufficiently precise [8, 12, 13]. Especially the temperature and pH have an effect on the dissociation equilibrium.This balance needs to be considered depending on the regulating carbon species. Of interest for bioprocesses are mainly carbon dioxide, carbonic acid and carbonate.

$$\frac{dc_{CO2,all}}{dt} = CTR + \sum \frac{dc_{CO2,disturbance}}{dt} + \sum \frac{dc_{HCO_3^-,disturbance}}{dt} + \sum \frac{dc_{CO_3^{-2},disturbance}}{dt}$$

By transport across the interface between liquid phase and gas phase, a direct manipulation of the concentration of dissolved carbon dioxide is possible (eq. (4)). An independently controlled all carbon dioxide species is not feasible due to the pH-dependent equilibrium. It follows that a whole decoupling of pH and all carbon species is physicochemically not possible. However, individual carbon dioxide species can be regulated to a set point. By a corresponding experimental design is an accurate analysis of the effects of each carbon dioxide species hence theoretically possible. A pH shift should be understood as disturbance variable of the concentration of dissolved carbon dioxide. However, this can be counteracted by a shift of the driving force. It should be noted that for the monitoring of concentration of dissolved carbon dioxide a driving force limited process can be exited. In order to quantify this in more detail, a real time simulation can be performed. For that the validity of the film theory needs to be adopted. The necessary calculations are based on Minkevich and Neubert [12]. In kLa value limited process phases using the equations by Minkevic and Neubert is impermissibly. Here, the models of Boranius et al. and Frahm et al. can be applied. In this paper, the influence of pH shifts on the concentration of dissolved carbon is not investigated further.

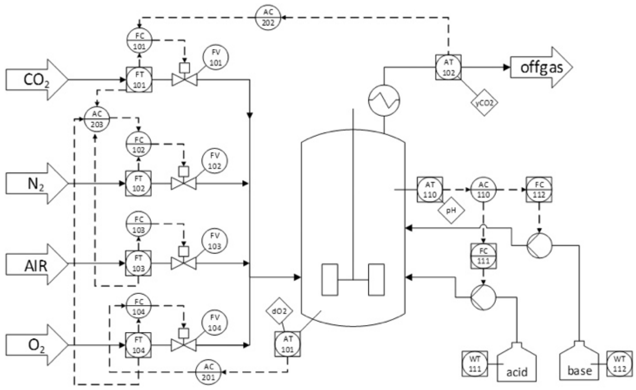

Figure 2: Flow sheet of the process with all the relevant parts for the measurement- and control-systems

The carbon dioxide transport rate can be affected by several manipulable processes parameters in driving force limited processes. Manipulable are the total gas flow and the amount of carbon dioxide in the aeration. The RI - flowsheet for the relevant regulations is shown in figure 2. AC 201 denotes a PI - controller with the in situ measured control variable dissolved oxygen dO2 and as a manipulated variable the oxygen flow rate (FO2). AC 202 is a PI - controller with the on line measured control variable partial pressure of carbon dioxide pCO2 and as a manipulated variable the carbon dioxide flow rate (FCO2). The controller AC 203 regulates the total flow rate (FG). It is designed as a P-T0 element and uses the N2 flow as manipulated variable. The pH control has a dead band of 0.01 and is regulated by a PI controller (AC 110).

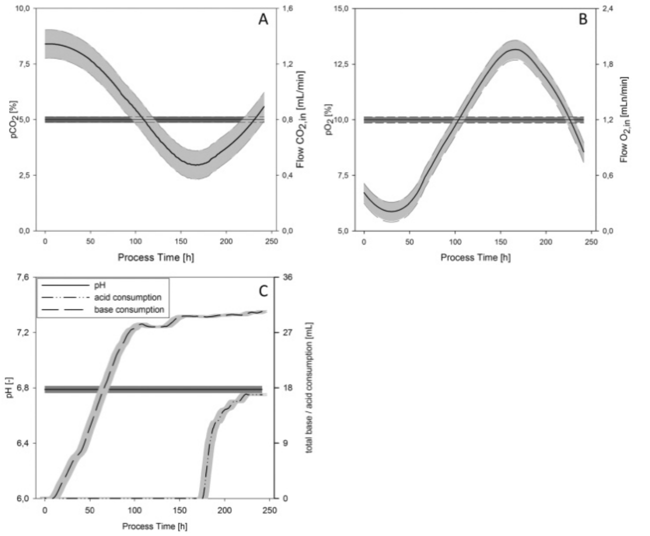

Figure 3: Profiles of control and manipulated variables for the p_{CO2} control (A), the d_{O2} control (B) and the pH control (C). In addition to the smoothed profiles the 95% confidence interval of each parameter is shown.

Application in mammalian cell culture process

In figure 3 the time courses of the controlled and manipulated variables for the dissolved oxygen and the partial pressure of carbon dioxide are shown. The confidence interval for the pCO2 - and for dO2 - control amounted to ± 0.13 [%]. The manipulated variables showed a confidence interval of ± 0.10 [mln / min]. This could be improved by further optimization of the control parameters. However, this is not necessary in view of the observed control deviation. Due to the control strategy, the observed variances in carbon dioxide production and oxygen consumption are set to the mass flow controller signals. Thus process kinetics can be extracted directly from the manipulated variables. Here, the carbon dioxide flow correlates indirectly with the carbon dioxide production rate and the oxygen flow directly to the oxygen consumption rate. These signals may consequently be used as input variables for simple soft sensors to estimate the Viable Cell Density (VCD). In addition to dissolved oxygen and the partial pressure of carbon dioxide, the pH value was controlled. In Figure 3 C the time courses of the controlled and manipulated variables for this control are shown. The 95% confidence interval for the pH - control amounted to ± 0.025 [-].The confidence interval of the illustrated acid- / base consumption was ± 0.12 [g] and is therefore within the range of the resolution limit of ± 0.1 [g] of the used scales. Even in the signals of the manipulated variables for the pH control, the process kinetics can be derived.

Conclusion:

Aim of this application note was to presents a control strategy, which uses an off-gas analyzer to monitor of the partial pressure of carbon dioxide as control variable and regulate the pCO2.

In order to achieve this aim, four subtasks were defined, leading to the following statements: (i) an investigation on the mass transfer of CO2 in weak aerated lab-scale bioreactors which show a driving force limitation in a small scale bioreactor for the first time. Herewith the presented observations represent an essential prerequisite for scale-up and scale-down of cell culture processes with respect to the carbon dioxide mass transfer. Furthermore, this finding has influence on the description of CO2 transport rates and leads to a significant simplification in the corresponding systems. This should be investigated in further studies. (ii) In driving force limited systems the pCO2, measured by off gas analyzers, can be used as control variable. In this case the CO2 inflow can be used as manipulated variable. (iii) As experimental result, we could show that the control was stable over a whole batch process. Furthermore, the process variance was shifted to the manipulated variables. These are more suitable to characterize the process kinetics for example as inputs in soft-sensors, which are useful in the context of PAT.

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