RESEARCH ARTICLE

Mammalian Cell Cultures

Biotechnology Journal

www.biotechnology-journal.com

On-Line Control of Glucose Concentration in High-Yielding Mammalian Cell Cultures Enabled Through Oxygen Transfer Rate Measurements

*Stephen Goldrick, Kenneth Lee, Christopher Spencer, William Holmes, Marcel Kuiper, Richard Turner, and Suzanne S. Farid**

Glucose control is vital to ensure consistent growth and protein production in mammalian cell cultures. The typical fed-batch glucose control strategy involving bolus glucose additions based on infrequent off-line daily samples results in cells experiencing significant glucose concentration fluctuations that can influence product quality and growth. This study proposes an on-line method to control and manipulate glucose utilizing readily available process measurements. The method generates a correlation between the cumulative oxygen transfer rate and the cumulative glucose consumed. This correlation generates an on-line prediction of glucose that has been successfully incorporated into a control algorithm manipulating the glucose feed-rate. This advanced process control (APC) strategy enables the glucose concentration to be maintained at an adjustable set-point and has been found to significantly reduce the deviation in glucose concentration in comparison to conventional operation. This method has been validated to produce various therapeutic proteins across cell lines with different glucose consumption demands and is successfully demonstrated on micro (15 mL), laboratory (7 L), and pilot (50 L) scale systems. This novel APC strategy is simple to implement and offers the potential to significantly enhance the glucose control strategy for scales spanning micro-scale systems through to full scale industrial bioreactors.

1. Introduction

Mammalian cell cultures are the primary source of therapeutic proteins in the biopharmaceutical industry.[1] A platform for commercial production of these proteins involves a fed-batch protocol where the initial media charge supports growth and production while subsequent feed additions prevent nutrient depletion and sustain protein production.[2,3] Luan et al.[4] initially demonstrated the benefits of conventional fed-batch operation of mammalian cells through enhanced cell viability while maintaining a cell density equal to 2.1×10^6 cells mL^{-1} in addition to increasing the monoclonal antibody (mAb) concentration four-fold to $0.140\,g\,L^{-1}$ compared to similar batch cell cultures. Over the last three decades, significant advances in cell lines,[5] media optimization,[6] bioreactor design,[7,8] and process understanding[9,10] have enabled fed-batch mammalian cultures to reach cell densities greater than 100×10^6 cells mL^{-1} and mAb titers in excess of $8\,g\,L^{-1}$.[11] The ability of a fed-batch protocol to support high cell densities and work across multiple scales (15 mL to 20 000 L) while maintaining low operational complexity demonstrates why the fed-batch protocol is the standard operation for the majority of biopharmaceutical facilities.

In fed-batch mammalian cell cultures, the primary carbon source is glucose. Glucose is typically fed as a bolus addition based on daily off-line concentrations of the nutrient. A drawback of this control strategy is the resultant rapid change to the cellular environment resulting in metabolic deregulation of the glucose uptake rate.[12,13,14] Furthermore, excessive glucose feeding has been shown to increase toxic by-products such as lactate and ammonia.[15–18] whereas insufficient feeding can lead to nutrient depletion. More recently the glucose concentration was shown to influence glycosylation and antibody production.[19] The glucose concentration can be maintained at a lower concentration by increasing the frequency of the off-line samples and subsequent bolus additions, however this strategy results in a higher contamination risk and an

Dr. S. Goldrick, Prof. S. S. Farid
The Advanced Centre of Biochemical Engineering
Department of Biochemical Engineering
University College London
Gower Street, WC1E 6BT, London, United Kingdom
E-mail: s.farid@ucl.ac.uk

Dr. S. Goldrick, Dr. C. Spencer, Dr. W. Holmes, Dr. M. Kuiper, R. Turner
MedImmune
Milstein Building, Granta Park
Cambridge, CB21 6GH, United Kingdom

Dr. K. Lee
MedImmune LLC
Gaithersburg Headquarters, Gaithersburg, MD 20878, USA

increased workload. Therefore, a non-invasive on-line glucose prediction is preferred. With the evolution of the process analytical technology (PAT) initiative[20] there have been significant advancements related to soft-sensor development enabling on-line control of the key process parameters of mammalian cell operation.[21,22] A wide range of spectroscopic devices demonstrate the ability to monitor glucose on-line, including near infrared,[23] mid-infra-red[24] and Raman spectroscopy.[25–27] However, the wide-spread application of spectroscopic PAT devices has been limited due to their high cost and the challenges associated with conversion of spectroscopic data into useful and reliable on-line predictions [28]. Alternative methods to estimate on-line glucose involve evaluation of the oxygen uptake rate through the dynamic method (OUR_{dynamic}) and the development of a stoichiometric relationship between glucose and oxygen consumption rates.[29–32] However, the OUR_{dynamic} method results in significant dissolved oxygen (DO_2) concentration fluctuations ranging from 20 to 70% of saturation throughout the cell culture process.[29,30] Furthermore, this method typically ignores adjustments to agitator speed or shifts in gas-flow rates by assuming a constant oxygen mass transfer coefficient (k_La). Off-gas analyzers have also been demonstrated as an effective tool to monitor and control mammalian cell cultures.[33–35] However these sensors can be expensive and installation can be problematic on micro-scale systems.

The methodology outlined here requires no adjustments to normal operation and utilizes readily available process measurements. Furthermore, the method automatically adjusts the generated correlation to account for metabolic shifts throughout the process and therefore can handle various nutrient uptake rates present in different cell lines. The proposed on-line glucose advanced process control (APC) strategy correlates the consumption rate of glucose to the oxygen transfer rate (OTR) enabling an on-line prediction of glucose throughout the cell culture run. The on-line glucose APC strategy maintains the glucose concentration closer to its target set-point with a significant reduction in glucose concentration fluctuations and was demonstrated across multiple scales. Comparable cell culture profiles and product quality were also observed as compared to conventional fed-batch bolus glucose control. The ability of the on-line glucose APC strategy to visualize and manipulate the glucose concentration in real-time is a step toward enhanced process understanding enabling further capabilities for process optimization. Furthermore, the recent shift toward a "Quality by Design" approach emphasizing product and process understanding through improved control strategies and sound science is demonstrated by this methodology.

2. Experimental Section

2.1. Cell Line and Culture Propagation

Cell culture runs were performed using five different recombinant Chinese hamster ovary (CHO) cell lines expressing high levels of therapeutic proteins. The cell lines were cultured in animal component-free chemically defined CHO media. The cells were maintained in shake flasks at 37 °C under 5% carbon dioxide, shaken at a constant rpm and passaged 2–3 times per week for propagation and scale-up for inoculation.

2.2. Bioreactor Systems

Three different bioreactor systems were used for this study. Micro-scale experiments were performed on the Ambr™-15 system (TAP Biosystems, Greenville, DE) that was operated as described in Ref. [36] with 24 single vessels split into two separate culture stations where each vessel was operated with a 11–15 mL working volume. Laboratory-scale experiments were performed in 7 L bioreactors (Applikon Biotechnology, Schiedam, The Netherlands) with a working volume of approximately 5 L. Large-scale experiments were performed at the 50 L bioreactor scale (Applikon Biotechnology).

2.3. Cell Culture Process

All cell culture runs had an initial seeding density of roughly 1×10^6 cells mL^{-1}. Temperature was maintained at 35.5 °C. The dissolved oxygen set point was 50% of saturation and was maintained through gassing with air flow supplemented with oxygen and manipulation of the agitation rate. The nutrient feeding strategy consisted of six equally spaced additions of a proprietary feed. The culture pH was controlled through the addition of sodium carbonate and sparging CO_2 gas with a pH dead-band equal to 0.1. Antifoam was added as required. Daily off-line samples were analyzed for viable cell concentration (VCC) and viability using the Vi-Cell Automated cell viability analyzer (Beckman Coulter, Brea, CA, USA). Glucose and lactate were analyzed using the Bioprofile flex (Nova Biomedical Corporation, Waltham, MA, USA).

2.4. Titer Analysis

Volumetric antibody titres in cell culture supernatants were quantified by protein A affinity chromatography using a protein A ImmunoDetection sensor cartridge (Applied Biosystems, Warrington, UK) coupled to an Agilent 1200 series HPLC (Agilent, Berkshire, UK). Peak areas relative to a reference standard calibration curve were used to calculate titres. These samples were analyzed for titer on days 4–14 for each cell culture run.

2.5. Fed-Batch Bolus Glucose Control

The fed-batch bolus glucose control strategy involved measuring the off-line glucose concentration ($Gluc$) every 24 h. If the glucose concentration was below a pre-defined minimum ($Gluc_{\mathrm{min}}$), a bolus addition of glucose (V_{Gluc}) was added to raise the concentration of glucose in the reactor to a target set-point concentration ($Gluc_{S.P}$). Glucose concentrations above this minimum value resulted in no bolus additions. The fed-batch bolus glucose feeding script is summarized as:

if $Gluc \leq Gluc_{\min}$

$$V_{\text{Gluc}} = \frac{(Gluc_{S.P} - Gluc)V}{C_{\text{Gluc}}}$$

else $V_{\text{Gluc}} = 0$

end

where V represents the volume of the culture and C_{Gluc} the concentration of glucose in the feed solution.

2.6. On-Line Glucose Advanced Process Control Strategy

Figure 1A outlines both the on-line and off-line process measurements in addition to the bioreactor dimensions required for implementation of the on-line glucose advanced process control (APC) strategy. The on-line measurements required by the control strategy for the calculation of the cumulative oxygen transfer rate (*OTR*) are the agitator speed (*RPM*), dissolved oxygen concentration (DO_2), dissolved oxygen concentration at maximum saturation (DO_2^*) and inlet gas flow rate (F_{gas}). The maximum oxygen saturation concentration is defined by the partial pressure of the dissolved oxygen in the inlet gas flow. These measurements enable the calculation of agitator power (P_{ag}) and the superficial gas velocity (V_s) using the bioreactor dimensions defined in **Table 1**A. The k_La coefficients were estimated for each bioreactor scale using the dynamic gassing-out method outlined in Ref. [37] and are defined in Table 1A. The measurements required for the cumulative glucose consumption rate ($Gluc_{\text{con}}$) and the cumulative glucose added ($Gluc_{\text{Add}}$) are the off-line concentration of glucose (*Gluc*) and the volume of glucose added (V_{Gluc}).

The on-line glucose APC algorithm implemented for the 7 and 50 L bioreactor systems is shown in Figure 1B. This control strategy involves the execution of two scripts running at two different frequencies. The outer script runs every n time-points (every 10 s in this work) and continuously calculates the cumulative oxygen transfer rate, *OTR(n)*, and the cumulative glucose added, $Gluc_{\text{Add}}(n)$. The inner script runs every time a new off-line measurement of glucose is added, defined here as every k time-point (approximately every 24 h). The inner script calculates the cumulative glucose consumed, $Gluc_{\text{Con}}(k)$, based on the current and previous glucose concentration and the known amount of glucose added to the bioreactor during this relevant time period. Similarly, the *OTR(k)* is calculated for each of these k time-points. A linear relationship between the $Gluc_{\text{Con}}$ and *OTR* is defined by the equation: $Gluc_{\text{Con}} = m \times OTR + b$. The slope of the line, m, and the intercept, b, are recalculated every k time-point utilising the three most recent values of $Gluc_{\text{Con}}$ and *OTR*. The generated correlation enables an on-line prediction of the cumulative glucose consumed utilizing the *OTR(n)* and the most recent m and b values. To maintain the glucose concentration at its set-point, $Gluc_{S.P}$, the algorithm switches on the glucose feed, F_{Gluc}, if the predicted cumulative glucose consumed is higher than the total glucose added to the vessel after considering the initial glucose concentration. For the Ambr™-15 bioreactor system the script was slightly adjusted to account for the liquid handling system with the outer script running every 30 min to enable time for glucose to be added to each of the 24 vessels after its execution. By defining $Gluc_{S.P}$ as an external variable in the control algorithm, the set-point can be manipulated in real-time throughout the cell culture run.

3. Results

3.1. Correlation Development Between Cumulative Glucose Consumed and Cumulative Oxygen Transfer Rate

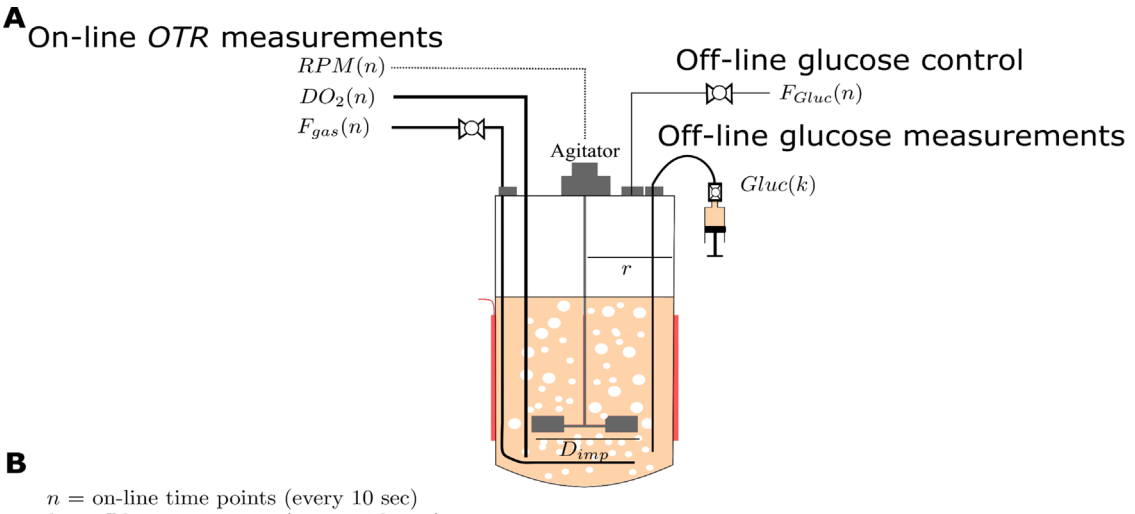
The utilization of the oxygen uptake rate (*OUR*) to predict glucose consumption in mammalian cell culture processes is well established.[29,30,33] However, the dynamic method for *OUR* calculation is cumbersome and requires significant and repeated dissolved oxygen deviations throughout the cell culture. Utilization of the *OTR* to act as a metabolic indicator for mammalian cell cultures is far less publicized. Moreover, the *OUR* and *OTR* are essentially equal during steady-state conditions through analysis of the oxygen mass balance within the bioreactor defined by Eq. (1):

$$\frac{dDO_2}{dt} = \underbrace{k_La(DO_2^* - DO_2)}_{OTR} - OUR \text{ at steady-state}$$

$$\frac{dDO_2}{dt} = 0 \therefore OTR = OUR \quad (1)$$

To demonstrate the ability of the *OTR* to act as a suitable monitoring tool in mammalian cell cultivations, the cumulative sum of the *OTR* was compared against the cumulative sum of the glucose consumed for three highly diverse cell lines (cell lines A, B, and C). The strength of this linear relationship is shown in **Figure 2**A. The on-line *OTR* was calculated continuously using the readily available process measurements defined in Figure 1. Taking the cumulative sum of the calculated *OTR* values filters out some of the rapid fluctuations due to the random deviations in DO_2 and gas flow rates and therefore strengthens the correlation with the cumulative glucose consumed.

Figure 2 A highlights a moderately sigmoidal pattern for each of the cell lines investigated here, particularly for cell lines B and C. These slight nonlinearities represent shifts in the stoichiometric ratio of the glucose to oxygen consumption rates throughout the cell culture run. This is particularly evident during the early and late stages of the run for each of these high-yielding cell lines. The observed changes to this ratio could represent differences in uptake rates as the cells shift from the exponential phase to stationary phase. Furthermore, the metabolic shift from lactate production to lactate consumption could also contribute to this slightly sigmoidal pattern as was demonstrated by Ref. [38] in a mammalian cell culture run. The relationship between the *OTR* compared to the cumulative glucose consumed ($Gluc_{\text{con}}$) was quantified using the correlation coefficient (R^2) displayed in Figure 2B. The strong linear relationship between these *OTR* and $Gluc_{\text{con}}$ demonstrates the ability of this easily calculated and high frequency measurement to be used as a soft-senor for on-line prediction of glucose.



B

n = on-line time points (every 10 sec)
k = off-line time points (every 24 hours)
for n = 1:1:Batch$_{end}$

$$P_{ag}(n) = \mathrm{Po}\rho\left(\frac{RPM(n)}{60}\right)^3 D_{imp}^5 \qquad V_s(n) = \frac{F_{gas}(n)}{\pi r^2} \qquad k_La(n) = \alpha V_s(n)^a \left(\frac{P_{ag}(n)}{V(n)}\right)^b$$

$$OTR(n) = \sum_1^n k_La(n)(DO_2^* - DO_2(n))$$

$$Gluc_{Add}(n) = \sum_1^n \left(\frac{C_{Gluc}(V_{Gluc}(n) - V_{Gluc}(n-1))}{V(n)}\right)$$

$Gluc(k)$ = Input new off-line glucose measurement
if $Gluc(k)! = Gluc(k)$
$k = k + 1$

$$Gluc_{Con}(k) = Gluc(0) + Gluc_{Add}(n) - Gluc(k) \qquad OTR(k) = OTR(n)$$

$$m(k) = \frac{3(\sum_{j=1}^{j=3}(OTR(j)Gluc_{Con}(j)) - \sum_{j=1}^{j=3} OTR(j) \sum_{j=1}^{j=3} Gluc_{Con}(j)}{3\sum_{j=1}^{j=3} OTR(j)^2 - \left(\sum_{j=1}^{j=3} OTR(j)\right)^2}$$

$$b(k) = \frac{\sum_{j=1}^{j=3} Gluc_{Con}(j) - m(k)\left(\sum_{j=1}^{j=3} OTR(j)\right)}{3}$$

end

$$\text{Predicted } Gluc_{Con}(n) = m(k)OTR(n) + b(k)$$

if Predicted $Gluc_{Con}(n) + Gluc_{S.P} > Gluc_{Add}(n) + Gluc(1)$

$$F_{Gluc} = 10\,(\mathrm{L\ h^{-1}})$$

else

$$F_{Gluc} = 0\,(\mathrm{L\ h^{-1}})$$

end
end

Figure 1. A) Schematic outlining bioreactor dimensions and on- and off-line variables required to implement the on-line glucose APC strategy. B) Outline of on-line glucose APC algorithm manipulating the glucose feed rate based on predicted glucose consumed ($Gluc_{Con}$) derived from the cumulative oxygen transfer rate (OTR).

3.2. Comparison of On-Line Glucose APC Strategy to Fed-Batch Bolus Glucose Control

The on-line glucose APC algorithm developed in this work has been compared to the standard fed-batch bolus glucose feeding strategy for a wide range of cell lines and scales. **Figure 3** summarizes the performance of the algorithm for three separate cell lines carried out at the 7 L scale. The typical controller action of the fed-batch bolus feeding control strategy for cell line A is shown in Figure 3A. The fed-batch bolus feeding strategy is described in section 2.5 and involves measuring the glucose concentration approximately every 24 h. The glucose trigger rate for the bolus addition was above 5 g L^{-1} ($Gluc_{\min}$) resulting in no glucose additions during the batch phase for cell culture run 1, 3, and 5 as demonstrated in Figure 3C, E, and G. To ensure glucose limitation does not occur, the bolus addition is calculated to raise the glucose concentration to a target of 8 g L^{-1} ($Gluc_{S.P}$). The simple and accurate nature of this method is demonstrated for

Table 1. (A) Summary of bioreactor dimensions and oxygen mass transfer coefficients required for implementation of the on-line glucose APC strategy. (B) Comparison of the mean and standard deviation of glucose concentration controlled using a fed-batch feeding strategy (indicated as "Bolus") and on-line glucose advanced process control (APC) strategy (indicated as "APC").

A)

Bioreactor parameter	Micro (15 mL)	Lab (7 L)	Large (50 L)
Bioreactor radius: r (m)	0.0115	0.06	0.175
Impeller dimeter: D_{imp}(m)	0.0114	0.055	0.12
Power number: Po (−)	2.1	1.7	2.2
k_La coefficients: (−)	1.74, 0.30, 0.16	73.5, 0.43, 0.24	81, 0.35, 0.6

B)

Cell Culture ref	Cell line	Scale	Glucose control strategy	Mean glucose conc ($g\,L^{-1}$)	Standard deviation ($g\,L^{-1}$)
Cell culture run1	Cell line A	7-L	Bolus	4.70	1.85
Cell culture run1	Cell line A	7-L	APC	2.47	0.57
Cell culture run 3	Cell line B	7-L	Bolus	5.19	1.59
Cell culture run 4	Cell line B	7-L	APC	2.62	1.01
Cell culture run 5	Cell line C	7-L	Bolus	5.71	1.53
Cell culture run 6	Cell line C	7-L	APC	2.31	0.88
Cell culture run 7–9	Cell line D	7-L	Bolus	4.01	1.71
Cell culture run 10–12	Cell line D	7-L	APC	2.35	1.31
Cell culture run 13–18	Cell line E	7-L	Bolus	4.80	1.41
Cell culture run 19–20	Cell line E	7-L	APC	3.39	1.42
Cell culture run 21	Cell line A	50-L	Bolus	5.56	2.76
Cell culture run 22	Cell line A	50-L	APC	2.91	1.13
Cell culture run 23–34	Cell line A	15-mL	Bolus	5.67	2.32
Cell culture run 35–46	Cell line A	15-mL	APC	3.76	1.73

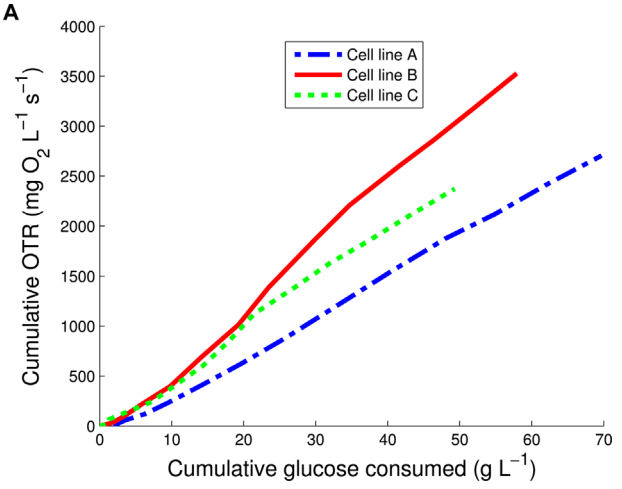
each cell line in Figure 3C, E, and G where the target glucose set-point is reached after each bolus addition. The on-line glucose concentration shown in Figure 3 was estimated by a mass balance incorporating the cumulative glucose consumed using the off-line glucose concentration measurements and the known quantity of glucose added to the reactor.

Figure 3B represents the typical controller action for the glucose flow rate implementing the on-line glucose APC strategy and was demonstrated here for cell culture run 2. The algorithm implements an "if-statement" that continuously switches the glucose pump on or off. The pump is switched on if the predicted cumulative on-line glucose concentration generated through the *OTR* correlation is higher than the cumulative glucose added to the vessel after taking the initial glucose concentration and the set-point ($Gluc_{S,P}$) into consideration. The on-line glucose APC strategy implemented for three separate cell lines is shown for cell culture runs 2, 4, and 6 in Figure 3D, F, and H. The average glucose concentration for the bolus controlled cell culture runs 1, 3, and 5 is approximately $5.2\,g\,L^{-1}$ with a standard deviation equal to $1.66\,g\,L^{-1}$. Enhanced control was observed for the cell culture runs 2, 4, and 6 implementing the on-line glucose APC strategy where the average glucose concentration was equal to $2.52\,g\,L^{-1}$ with a standard deviation equal to $0.71\,g\,L^{-1}$. The target glucose set concentration for all cell culture runs was equal to $2\,g\,L^{-1}$. Furthermore, the on-line glucose APC strategy significantly reduced the high fluctuation in the glucose concentration observed after each daily bolus addition.

3.3. Comparison of Growth Profiles for On-Line Glucose APC Strategy to Fed-Batch Bolus Glucose Control

Figure 4 compares the off-line variables of the conventional fed-batch bolus glucose control and the on-line glucose APC strategy controlled runs during the cultivation of cell lines A, B and C (cell culture runs 1–6). Each of the cell lines had highly varied viable cell densities and different monoclonal antibody production rates resulting in different glucose consumption rates. These highly diverse cell lines therefore represent a significant control challenge requiring an adaptive glucose feeding strategy. The ability of the on-line glucose APC strategy to dynamically adjust the glucose uptake demands of these diverse cell lines is demonstrated in Figure 3 and highlights the robustness of the proposed algorithm. Furthermore, the on-line glucose APC strategy demonstrates excellent comparability to the fed-batch cultures for growth, viability, lactate, and titer as shown in Figure 4A–D, respectively. The very high peak cell density reached (4.5×10^7 cells mL^{-1}) for cell line A during the production phase represents a significant control challenge based on the high glucose consumption demand. Figure 3C highlights the rapid consumption rate of glucose where the glucose concentration is almost depleted between each sample particularly during the production phase of this cell line. Typically to alleviate this issue, biopharmaceutical companies manually raise the target set-point for the bolus additions for the stationary phase to ensure glucose limitation does not

A



B

Batch reference	Cell line	OTR:R²
Cell culture run 1	Cell line A	0.9984
Cell culture run 3	Cell line B	0.9982
Cell culture run 5	Cell line C	0.9978
Average		**0.9981**

Figure 2. A) Correlation of cumulative glucose consumed ($Gluc_{Con}$) with the cumulative oxygen transfer rate (*OTR*) for three different cell lines. Cell line A is represented by the blue dashed dot line, cell line B by the red solid line and cell line C by the green dotted line. Color images are available for the on-line version. B) Table highlighting the correlation coefficient (R^2) for *OTR* compared to the cumulative glucose consumed for cell culture runs 1, 3, and 5.

occur as demonstrated in Ref. [19]. The adaptive nature of the on-line glucose APC strategy ensures that glucose levels are maintained near their set-point and require no manual adjustments throughout the cell culture run. Furthermore, this control strategy demonstrates consistent therapeutic protein production for highly varied cell lines including high yielding cell culture runs enabling titres in excess of $6\,g\,L^{-1}$ as demonstrated in Figure 4D.

3.4. Consideration of Lactate Metabolism

The on-line glucose APC strategy assumes that the *OTR* requirements of the cell culture indicate the required glucose demand for cell proliferation and protein production. The energy required by these cellular activities is produced by the TCA cycle driven by the metabolism of glucose to pyruvate by glycolysis. The proposed APC strategy only considers the aerobic metabolism of glucose to energy and does not take into account the anaerobic conversion of glucose to lactate. To investigate whether the inclusion of lactate in the proposed APC strategy would improve the control of glucose, the algorithm was adjusted to take into account the off-line measurements of lactate that were recorded every 24 h similar to the off-line glucose measurements. The modified algorithm is similar to the APC strategy outlined in Figure 1 however the cumulative glucose consumed is defined as:

$$Gluc_{Con}(k) = Gluc(0) - Gluc_{Add}(n) - Gluc(k) - Lact(k) \quad (2)$$

where (k) and (n) refer to off-line samples (every 24 h) and on-line samples (every 10 s), respectively. The off-line lactate concentration is represented as $Lact(k)$ and the cumulative glucose consumed is defined as $Gluc_{Add}$. The inclusion of lactate in the control algorithm resulted in tighter control of glucose by reducing the standard deviation of the glucose concentration compared to the on-line glucose APC algorithm without lactate. Zhou and Hu[29] also included the lactate in their stoichiometric relationship with the *OUR* measurements and demonstrated high correlations between the cumulative glucose consumed and the cumulative $OUR_{dynamic}$. Although the inclusion of lactate marginally improved the APC strategy in this work, the additional off-line measurement further complicates the algorithm and is not necessary based on the significantly enhanced glucose control demonstrated utilising solely the off-line glucose measurement. Furthermore, the inclusion of the lactate in the control algorithm requires the additional validation of the off-line lactate measurement in comparison to the standard operation implementing a fed-batch bolus glucose feeding regime.[39]

3.5. Validation of On-Line Glucose APC Strategy Across Multiple Scales and Multiple Cell Lines

To demonstrate the robustness and adaptability of the proposed on-line glucose APC strategy, the performance of the algorithm was compared against conventional fed-batch bolus glucose control for five different cell lines operated at three scales. The three scales chosen to validate the APC algorithm were a micro-scale using the advanced micro-bioreactor system (Ambr™-15) with a volume of 15 mL, a standard laboratory scale with a 7 L volume, and a 50 L pilot scale system. To implement the APC strategy on the different scales, the bioreactor dimensions and the k_La coefficients had to be adjusted for each system as defined in Table 1A. To challenge the algorithm at pilot scale, Cell line A (high glucose consumption demand) was chosen to validate the method at the 50 L scale. A comparison of the performance of the on-line glucose APC strategy and fed-batch bolus glucose control is summarized in Table 1B. The algorithm demonstrated a significant reduction in the mean glucose concentration and glucose deviations across all cell culture runs for all scales investigated. Furthermore, across all scales, the bioreactor growth profiles were highly comparable to those using bolus feeding.

3.6. Product Quality Comparisons Between Fed-Batch Bolus Glucose Control and On-Line Glucose APC

Glycosylation is a critical protein quality attribute that can modulate the efficacy of a commercial therapeutic.[40] Maintaining consistent glycosylation profiles between cell culture runs is therefore necessary to ensure constant therapeutic efficacy for commercial products. To ensure the glycosylation patterns of the proteins produced were comparable for each glucose control method, the N-linked glycosylation patterns of

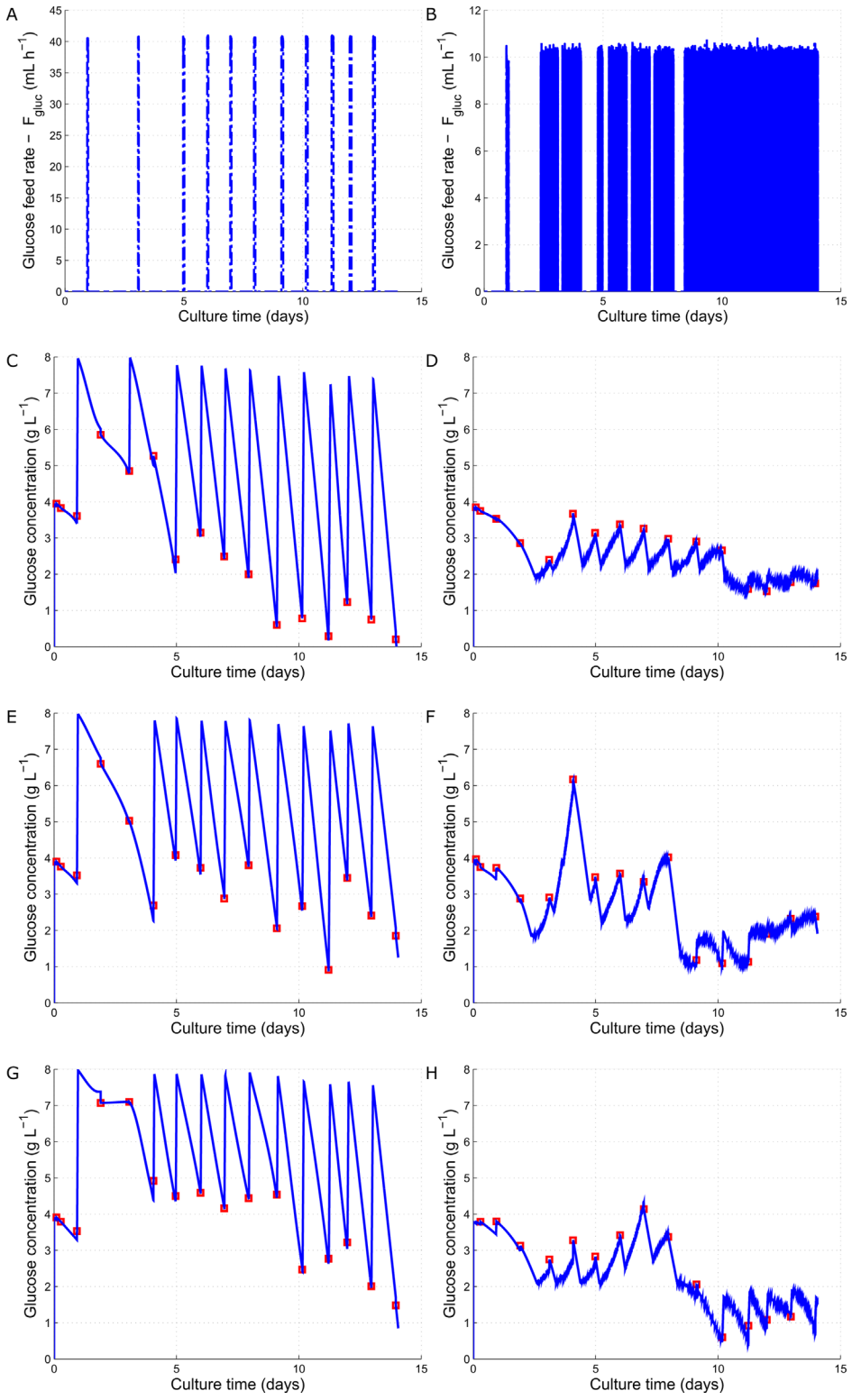


Figure 3. Feed additions of glucose (F_{Gluc}) for (A) the bolus fed-batch strategy for cell culture run 1 and (B) the on-line glucose APC strategy for cell culture run 2. Glucose concentrations for cell culture runs 1 (C), 3 (E), and 5 (G) controlled by fed-batch control and for cell culture runs 2 (D), 4 (F), and 6 (H) controlled by the online glucose APC strategy. The off-line concentrations are represented by the red squares and the calculated glucose concentration is represented by the solid blue lines based on a subsequent mass balance.

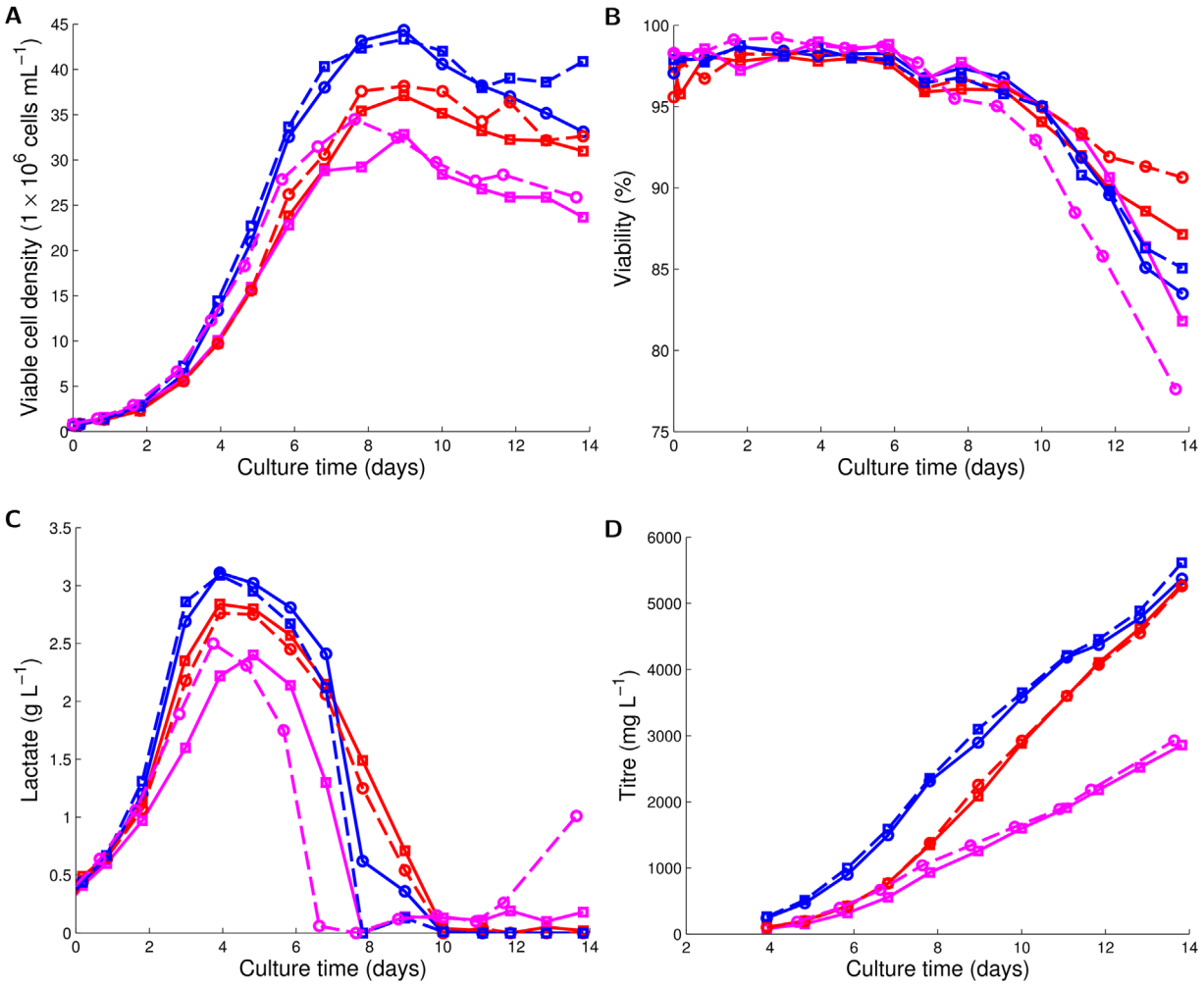


Figure 4. Comparison of kinetic profiles of three different mAb products (cell culture runs 1–6) controlled through bolus fed-batch glucose additions (solid lines) and the on-line glucose APC strategy (dashed lines) for the off-line variables (A) viable cell density, (B) viability, (C) lactate and (D) titre. The cell lines A, B, and C are represented by the blue, red, and pink lines, respectively.

cell line D were examined. These were measured on days 8, 10, 12, and 14 for cell culture runs that were controlled through fed-batch and APC feeding strategies. Both glucose control methods were found to adequately maintain consistent glycosylation patterns throughout the cell culture run as shown in S1 (supporting information) A, B, C, and D. Although the average glucose concentration in the fed-batch bolus controlled runs is almost twice as high as the APC strategy, no significant changes to the glycosylation profiles were observed for the particular cell line investigated. However, these cell lines had been screened using a fed-batch glucose protocol and selected specifically for robustness and product quality. Further process yield improvements could be potentially observed through incorporation of this on-line glucose APC strategy into the cell line selection protocol. Gagnon et al.[18] reported similar findings and found no difference in glycosylation patterns during the comparison of a conventional fed-batch process to an advanced glucose controlled process. They suggested the consistent glycosylation patterns were due to the ability of mammalian cells to regulate their intercellular levels of glycosylation-related precursor molecules at transient levels of glucose. The application of this methodology has the potential to improve product quality in cell lines that are less robust or susceptible to large glucose fluctuations. For example, there have been reports where changes to the glucose concentration and the feeding strategy were shown to influence the glycan concentrations for multiple cell lines.[19]

4. Discussion

4.1. Strength of Correlation Between *OTR* and Glucose Consumed

The application of the *OTR* to be utilized as a soft-sensor for on-line glucose concentration is demonstrated by the highly linear relationship between the cumulative values of the *OTR* and glucose consumed for a wide range of cell lines shown in Figure 2A. The strength of this linear relationship is fundamental to the APC control strategy outlined in Figure 1

and enables real-time predictions of the glucose concentration during the manufacture of various therapeutic proteins. Similar linear relationships have been reported between the *OUR* and glucose consumed for mammalian cell cultures.[28,29,41] However, the implementation of the *OTR* as an on-line predictive tool offers significant advantages in comparison to previous work utilizing the dynamically calculated *OUR* for advanced control applications.[28,29,32,42] To calculate the *OUR* in these previously described studies significant process deviations involving dissolved oxygen shifts between 30 and 60% of saturation and off-line data analysis are required. These complications in addition to the potential mixing heterogeneities caused by the dissolved oxygen fluctuations limit the wide-spread application of this method. Therefore, the on-line glucose APC strategy proposed here involving the *OTR* is highly advantageous as it requires no process deviations or set-point changes. Furthermore, the method acts completely unsupervized and only requires operator input to add in the off-line glucose concentration with all other process measurements automatically acquired enabling the on-line predictions and control actions to be implemented autonomously.

4.2. Implementation of On-Line Glucose APC Strategy

The on-line glucose APC strategy outlined in this work is highly robust and was successfully applied to three different scales, 15 mL, 7 L, and 50 L and multiple cell lines as demonstrated in Table 1B. The algorithm manipulated the glucose feed pump based on a simple "if-statement" that continuously switched the feed pump on and off depending on the predicted on-line glucose concentration. The feed pump rate set-point was $10\,mL\,h^{-1}$ and the execution time of the algorithm was every 10 s for the 7 L bioreactors used in this work as shown in Figure 3B. The feed pump rate and execution time of the algorithm were carefully chosen to ensure the glucose concentration was adequately maintained throughout the culture run. Furthermore, the glucose concentration set-point of $2\,g\,L^{-1}$ was chosen to take in account any potential inaccuracies in the off-line glucose concentration measurements and thus ensure that glucose limitation did not occur. These parameters were found to be suitable for the varied glucose consumption rates of the cell lines investigated here. The glucose consumption rates ranged from cell line B with a peak glucose consumption of 0.2 g of glucose per million cells to cell line A with a glucose consumption rate of 0.5 g of glucose per million cells. The high glucose requirements of these cell lines posed a significant control challenge for on-line glucose APC strategy and demonstrated the robustness of the proposed strategy. The control algorithm is highly adaptive and calculates the stoichiometric ratio of glucose to oxygen requirement after each off-line measurement of glucose is recorded. The calculated correlation utilizes the three most recent values of the cumulative glucose consumed and corresponding cumulative *OTR* measurements thus minimizing any potential model-mismatch as a result of inaccuracies in the off-line measurement of glucose or process deviations influencing the *OTR* calculation. The modifications necessary to implement the algorithm on multiple scales are the bioreactor dimensions and the k_La coefficients that were easily calculated for the three different vessel set-ups used in this work. To ensure that cellular oxygen demand could be met across all scales a similar power per unit volume (P/V) was used. This strategy enables the method to be implemented across different bioreactor scales in addition to a wide variety of cell lines with both high and low glucose demands.

5. Conclusions

Current on-line methods to control glucose require expensive process analytical technology (PAT) devices that have proven to be difficult to validate and are not commonly incorporated in industrial biopharmaceutical facilities. One of the key requirements in the development of the on-line glucose APC strategy for this study was to ensure that no additional sensors, off-line analyzes, or significant changes to standard operating procedures (SOPs) were needed to enable its wide-spread application. This simple to implement control strategy generates a correlation between the cumulative oxygen transfer rate and the cumulative glucose consumed enabling enhanced glucose control. In comparison to conventional fed-batch bolus glucose control, the on-line glucose APC strategy demonstrated similar growth characteristics and product quality characteristics. Thus the strategy is highly applicable and is suitable for integration into biopharmaceutical facilities currently utilizing conventional fed-batch control. The algorithm allows for real time visualization of glucose concentration, enabling enhanced understanding of the metabolic uptake rates of this critical process component. This presents opportunities for more advanced fault detection capabilities through real time analysis of glucose uptake rates and opportunities to adjust glucose concentrations on-line to maximize protein production and minimize inhibitory concentrations. The proposed method is highly robust and flexible and was verified at multiple scales and across multiple cell lines with significantly varied glucose uptake rates.

Nomenclature

a, a, b, oxygen mass transfer coefficients (−); C_{Gluc}, concentration of glucose in feed solution ($g\,L^{-1}$); DO_2, dissolved oxygen ($mg\,L^{-1}$); DO^*_2, dissolved oxygen at maximum saturation ($mg\,L^{-1}$); D_{imp}, impeller diameter (m); F_{gluc}, glucose feed flowrate ($L\,h^{-1}$); F_{gas}, inlet gas flowrate ($s\,h^{-1}$); *Gluc*, off-line glucose concentration ($g\,L^{-1}$); $Gluc_{S.P}$, off-line glucose concentration set-point ($g\,L^{-1}$); k_La, oxygen mass transfer rate (s^{-1}); k, off-line measurement time-point; Lact, off-line lactate concentration ($g\,L^{-1}$); n, on-line measurement time-point; OTR, oxygen transfer rate ($mg\,L\,h^{-1}$); P_{ag}, agitator power (kW); Po, unaerated power number (−); r, bioreactor radius (m); RPM, agitator rate (rmp); *V*, bioreactor volume (L); V_{gluc}, volume of glucose added (L); VCD, viable cell density (cells $\times 10^6$ mL^{-1}); V_s, superficial gas velocity ($m\,s^{-1}$); ρ, density ($kg\,L^{-1}$).

Acknowledgements

S. G. and K. L. contributed equally to this work. The authors would like to thank Christopher Sellick, Neil Birkett, Karen Dickson, and Andrew Smith from MedImmune for their valuable help with analytics on this project. Furthermore, the authors would like to thank Steven Ruddock and Ruchika Bandekar for performing the large scale runs. Additional the

authors would like to thank Mark Berge and Sanjeev Ahuja for their helpful discussions regarding the manuscript. This research is associated with the joint UCL-MedImmune Centre of Excellence for predictive multivariate decision support tools in the bioprocessing sector and financial support from MedImmune and UCL for S. Goldrick is gratefully acknowledged. Furthermore, support from the EPSRC is also greatly appreciated (EP/I033270/1).

Conflict of Interest

The authors declare no commercial or financial conflict of interest.

Keywords

advanced process control (APC), glucose control, mammalian cell culture, oxygen transfer rate, process analytic technology (PAT), soft sensor

Received: September 20, 2017
Revised: November 27, 2017
Published online:

[1] B. B. Yahia, L. Malphettes, E. Heinzle, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 7009.
[2] R. Fike, *BioProcess Int.*, **2009**, *7*, 46.
[3] F. Lu, P. C. Toh, I. Burnett, F. Li, T. Hudson, A. Amanullah, J Li, *Biotechnol. Bioeng.* **2013**, *110*, 191.
[4] Y. T. Luan, R. Mutharasan, W. E. Magee, *Biotechnol. Lett.* **1987**, *9*, 691.
[5] J. J. Priola, N. Calzadilla, M. Baumann, N. Borth, C. G. Tate, M. J. Betenbaugh, *Biotechnol. J.* **2016**, *11*, 853.
[6] J. Van der Valk, D. Brunner, K. De Smet, F. Svenningsen Å, P. Honegger, L. E. Knudsen, T. Lindl, J. Noraberg, A. Price, M. L. Scarino, G. Gstraunthaler, *Toxicol. In Vitro* **2010**, *24*, 1053.
[7] J. Varley, J. Birch, *Cytotechnology.* **1999**, *29*, 177.
[8] F. M. Wurm, M. de Jesus, Biosimilars of Monoclonal Antibodies: A Practical Guide to Manufacturing, Preclinical, and Clinical Development, **2017**, 327.
[9] F. M. Wurm, *Nat. Biotechnol.* **2004**, *22*, 1393.
[10] C. Mohan, Y. G. Kim, J. Koo, G. M. Lee, *Biotechnol. J* **2008**, *3*, 624.
[11] Z. Du, D. Treiber, J. D. McCarter, D. Fomina-Yadlin, R. A. Saleem, R. E. McCoy, Y. Zhang, T. Tharmalingam, M. Leith, B. D. Follstad, B. Dell, *Biotechnol. Bioeng.* **2015**, *112*, 141.
[12] W. M. Miller, C. R. Wilke, H. W. Blanch, *Biotechnol. Bioeng.* **1989**, *33*, 477.
[13] A. Sanfeliu, C. Paredes, J. J. Cairó, F. Gòdia, *Enzyme Microb. Technol.* **1997**, *21*, 421.
[14] B. C. Mulukutla, S. Khan, A. Lange, W. S. Hu, *Trends Biotechnol.* **2010**, *28*, 476.
[15] T. Omasa, K. I. Higashiyama, S. Shioya, K. I. Suga, *Biotechnol. Bioeng* **1992**, *39*, 556.
[16] Y. H. D. Chang, A. J. Grodzinsky, D. I. Wang, *Biotechnol. Bioeng.* **1995**, *47*, 308.
[17] X. Gong, D. Li, X. Li, Q. Fang, X. Han, Y. Wu, S. Yang, B. Q. Shen, *Cytotechnology.* **2006**, *52*, 25.
[18] M. Gagnon, G. Hiller, Y. T. Luan, A. Kittredge, J. DeFelice, D. Drapeau, *Biotechnol. Bioeng* **2011**, *108*, 1328.
[19] Y. Fan, I. Jimenez Del Val, C. Müller, J. Wagtberg Sen, S. K. Rasmussen, C. Kontoravdi, D. Weilguny, M. R. Andersen, *Biotechnol. Bioeng.* **2015**, *112*, 521.
[20] Food and Drug Administration US. Guidance for industry: PAT: A framework for innovative pharmaceutical, development, manufacturing, and quality Assurance. Rockville, MD. Sep 2004.
[21] J. Glassey, K. V. Gernaey, C. Clemens, T. W. Schulz, R. Oliveira, G. Striedner, C. F. Mandenius, *Biotechnol. J* **2011**, *6*, 369.
[22] W. Sommeregger, B. Sissolak, K. Kandra, M. Stosch, M. Mayer, G. Striedner, *Biotechnol. J.* **2017**, *12*, 1600546(1-7).
[23] S. A. Arnold, J. Crowley, N. Woods, L. M. Harvey, B. McNeil, *Biotechnol. Bioeng.* **2003**, *84*, 13.
[24] M. Rhiel, P. Ducommun, I. Bolzonella, I. Marison, U. Von Stockar, *Biotechnol. Bioeng.* **2002**, *77*, 174.
[25] S. Craven, J. Whelan, B. Glennon, *J. Process Control* **2014**, *24*, 344.
[26] N. R. Abu-Absi, B. M. Kenty, M. E. Cuellar, M. C. Borys, S. Sakhamuri, D. J. Strachan, M. C. Hausladen, Z. J. Li, *Biotechnol. Bioeng.* **2011**, *108*, 1215.
[27] L. Ashton, Y. Xu, V. L. Brewster, D. P. Cowcher, C. A. Sellick, A. J. Dickson, G. M. Stephens, R. Goodacre, *Analyst* **2013**, *138*, 6977.
[28] S. M. Mercier, B. Diepenbroek, R. H. Wijffels, M. Streefland, *Trends Biotechnol.* **2014**, *32*, 329.
[29] W. Zhou, W. S. Hu, *Biotechnol. Bioeng.* **1994**, *44*, 170.
[30] W. Zhou, J. Rehm, W. S. Hu, *Biotechnol. Bioeng.* **1995**, *46*, 579.
[31] CM Kussow, W Zhou, DM Gryte, WS Hu, *Enzyme Microb. Technol.* **1995**, *17*, 779.
[32] A. Casablancas, X. Gámez, M. Lecina, C. Solà, J. J. Cairó, F. Gòdia, *J. Chem. Technol. Biotechnol.* **2013**, *88*, 1680.
[33] K. Eyer, A. Oeggerli, E. Heinzle, *Biotechnol. Bioeng.* **1995**, *45*, 54.
[34] M. Aehle, A. Kuprijanov, S. Schaepe, R. Simutis, A. Lübbert, *Biotechnol. letters.* **2011**, *33*, 2103.
[35] S. Winckler, R. Krueger, T. Schnitzler, W. Zang, R. Fischer, M. Biselli, *Bioprocess Biosyst. Eng.* **2014**, *37*, 901.
[36] S. Goldrick, W. Holmes, N. Bond, G. Lewis, M. Kuiper, R. Turner, S. S. Farid, *Biotechnol. Bioeng.* **2017**, *114*, 2222.
[37] K. Van't Riet, *Ind. Eng. Chem. Process Des. Dev.* **1979**, *18*, 357.
[38] J. Li, C. L. Wong, N. Vijayasankaran, T. Hudson, A. Amanullah, *Biotechnol. Bioeng.* **2012**, *109*, 1173.
[39] Food and Drug Administration US. Guidance for industry CMC postapproval manufacturing changes to be documented in annual reports. Rockville, MD, April 2014.
[40] P. Hossler, S. F. Khattak, Z. J. Li, *Glycobiology.* **2009**, *19*, 936.
[41] Y. S. Kyung, M. V. Peshwa, D. M. Gryte, W. S. Hu, *Cytotechnology.* **1994**, *14*, 183.
[42] O. T. Ramirez, R. Mutharasan, *Biotechnol. Bioeng.* **1990**, *36*, 839.

