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ARTICLE

Control of IgG glycosylation by in situ and real-time estimation of specific growth rate of CHO cells cultured in bioreactor

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Abstract

The cell-specific growth rate (μ) is a critical process parameter for antibody production processes performed by animal cell cultures, as it describes the cell growth and reflects the cell physiological state. When there are changes in these parameters, which are indicated by variations of μ , the synthesis and the quality of antibodies are often affected. Therefore, it is essential to monitor and control the variations of μ to assure the antibody production and achieve high product quality. In this study, a novel approach for on-line estimation of μ was developed based on the process analytical technology initiative by using an in situ dielectric spectroscopy. Critical moments, such as significant μ decreases, were successfully detected by this method, in association with changes in cell physiology as well as with an accumulation of nonglycosylated antibodies. Thus, this method was used to perform medium renewals at the appropriate time points, maintaining the values of μ close to its maximum. Using this method, we demonstrated that the physiological state of cells remained stable, the quantity and the glycosylation quality of antibodies were assured at the same time, leading to better process performances compared with the reference feed-harvest cell cultures carried out by using off-line nutrient measurements.

KEYWORDS

antibody glycosylation, cell culture, in situ dielectric spectroscopy, real-time monitoring, specific cell growth rate

1 | INTRODUCTION

The need for biopharmaceutical protein products, in particular recombinant monoclonal antibodies (mAbs), has been constantly increasing in the last decade (Walsh, 2014). Chinese hamster ovary (CHO) cells are the most commonly used cell lines for mAb production due to their capability of performing human-like posttranslational modifications (PTMs; Cole, Demont, & Marison, 2015). Glycosylation is one of the most important PTMs for mAbs, as it impacts effector functions, pharmacokinetics, antigenicity, safety, stability, and solubility of the mAbs produced (Tharmalingam, Wu, Callahan, & T. Goudar, 2015). Therefore, glycosylation is a critical

quality attribute (CQA) for mAb production processes that should be taken into consideration in all manufacturing steps, as outlined in the quality by design (QbD) approach initiated by the Food and Drug Administration (FDA) in 2004 (ICH, 2017). The QbD initiative emphasizes the importance of using process analytical technologies (PAT) to monitor and control in real-time the critical process parameters (CPPs) which may influence the product CQAs in biopharmaceutical production processes.

Among various CPPs of mAb production bioprocesses, viable cell density (VCD) is undoubtedly one of the most important, as it is often closely related to the cell growth, the mAb production, and overall process performances (Lee, Carvell, Brorson, & Yoon, 2015). With

increasing production potential of these bioprocesses, reliable in-line and real-time measurements of VCD are more and more required. Various PAT tools have been proposed to monitor VCD in real-time, including dielectric spectroscopy (Ansorge, Esteban, & Schmid, 2007; Cannizzaro, Gügerli, Marison, & von Stockar, 2003; Courtès, Ebel, Guédon, & Marc, 2016; Opel, Li, & Amanullah, 2010; Yardley, Kell, Barrett, & Davey, 2000), near-infrared and Raman spectroscopies (Abu-Absi et al., 2011; Cervera, Petersen, Lantz, Larsen, & Gernaey, 2009), in situ microscopy (Guez, Cassar, Wartelle, Dhulster, & Suhr, 2004), acoustic resonance densitometry (Kilburn, Fitzpatrick, Blake-Coleman, Clarke, & Griffiths, 1989), and soft-sensor-based approaches (Kiviharju, Salonen, Moilanen, & Eerikäinen, 2008). Among these technologies, dielectric spectroscopy is probably one of the most reliable methodologies to monitor VCD due to its simplicity, robustness, and its capability of in situ fast signal acquisition which is noninvasive and nondestructive for cell cultures. The dielectric spectroscopy is also insensible to air bubbles and cell debris which often cause problems for other methods (Justice et al., 2011). Dielectric spectroscopy is based on measurements of the ability of viable cells to store electrical charge as a function of the frequency of the applied electrical field. The basic theoretical background of dielectric properties of biological cells was described elsewhere (Yardley et al., 2000). However, on-line monitoring of VCD alone gives an incomplete vision of cell growth and cell physiological state. To date, it remains challenging to monitor in real-time the cellular physiological state due to the complexity of animal cell system (Henry, Kamen, & Perrier, 2007).

The specific cell growth rate (μ) is one of the most direct indicators of cell physiological state. When cells are in their active growth stage (μ close to its maximum, μ_{\max}), they are able to produce enough energy for energy-consuming actions such as cell division and recombinant protein synthesis. On the contrary, under nutrients depletion, toxins accumulation, or when other physicochemical stresses appear, energy production of cells may be negatively influenced, resulting in a decrease in μ (Kondo et al., 2000). In this case, not only the quantity but also the quality of the recombinant proteins produced could be affected, since noncorrectly performed PTMs associated to mAb production could appear during the process (Kochanowski et al., 2008). Therefore, μ appears as an essential parameter for mAb production bioprocesses, which should be carefully monitored and controlled throughout the process to ensure the quantity and the quality of the mAb synthesized. Classically, μ is often calculated indirectly from off-line measurements of cell densities, or by macroscopic kinetic modeling approaches, which could be inaccurate or provide only estimated values, resulting in delayed information which is not adequate for control strategies (Xiong, Guo, Chu, Zhuang, & Zhang, 2015).

In the present work, a novel approach of real-time estimation of μ was developed based on on-line monitoring of VCD by using in situ dielectric spectroscopy and mass balance equations. This approach was then applied on a recombinant immunoglobulin G (IgG) production process performed by CHO cells cultured in a bioreactor in feed-harvest mode to investigate its potential for the improvement of the process control. Quality of the IgG produced was assessed by analyzing the macroheterogeneity of IgG glycosylation which is the glycosylation site occupancy. Overall, the results of this study demonstrate that μ is an

important process parameter allowing to detect early cell physiological state changes. On-line estimation of μ by using dielectric spectroscopy leads to better process control for the feed-harvest cultures, and as a result, the antibody quantity and the quality concerning the glycosylation were assured.

2 | MATERIALS AND METHODS

2.1 | Cell culture

A genetically modified DG44 CHO cell line (CHO M250-9) kindly provided by Bioprocessing Technology Institute (Singapore) was used in this study. The culture medium used during this study was a protein-free medium mixture consisting of a 1:1 ratio of PF-CHO (GE Healthcare, Velizy-Villacoublay, France) and CD-CHO (Thermo Fisher Scientific, Villebon-sur-Yvette) supplemented with 4 mM L-glutamine (Sigma-Aldrich, Saint-Quentin Fallavier), and 0.1% Pluronic F-68 (Sigma-Aldrich). Cells were initially cultured in shake flasks (Thermo Fisher Scientific) and were incubated in an agitated incubator (Kuhner, Birsfelden, Switzerland) at 37°C, 5% CO₂, 80% humidity with an agitation rate of 70 rpm. Bioreactor cell culture of CHO cell were performed in 2 L bench-top bioreactors (Pierre Guérin, Mauzé-sur-le-mignon, France). The exponentially growing cells were seeded at about 3×10^5 cell/ml in 1.5 L of working volume. Culture temperature was maintained at 37°C and the pH was regulated at 7.2 using 0.5M sodium hydroxide and CO₂. Dissolved oxygen was controlled at 50% of air saturation by delivering oxygen through the sparger. Agitation rate was fixed at 90 rpm throughout the culture.

2.2 | Off-line analysis

Samples were taken 3–4 times per day. VCD, viability, and average diameter of cells were measured by Vi-CELL™ cell counter (Beckman Coulter, Villepinte) on the basis of trypan blue dye exclusion of the viable cells. An automated photometric analyzer Gallery™ (Thermo Fisher Scientific) was used to measure glucose, lactate, glutamine, ammonium ions, and IgG titer.

For glycosylation analysis, IgGs were precipitated by cold acetone and were incubated at –20°C for 2 hr. Centrifugation for 5 min at 13,000g was carried out to recover the IgGs. Recovered IgGs were dissolved in 500 μ l of 50 mM ammonium hydrogen carbonate buffer and were then heated to 95°C for 10 min to be denatured. Next, 20 μ l of prepared trypsin solution (Sigma-Aldrich) were added in the cooled IgG samples at a concentration of 1 g/L. Samples were incubated overnight at 37°C, and the reaction was stopped by adding 15 μ l of formic acid (98%; Sigma-Aldrich). Analysis of peptides compounds from IgG hydrolysate was performed on a UHPLC-MS system (Thermo Fisher Scientific) connected to a photodiode array detector and a linear trap quadrupole mass spectrometer equipped with an atmospheric pressure ionization interface operating in positive electrospray mode (ESI+). Details of the mass spectrometer (MS) analyses have been described previously (Li et al., 2018).

The abundance of nonglycosylated IgG form was obtained by dividing its MS peak integral to the total MS glycopeptide signal. Concentration of nonglycosylated IgGs was calculated by multiplying the percentage of nonglycosylated form of digested IgGs by total IgG titer measured by Gallery™ device.

2.3 | In situ dielectric spectroscopy

The cell culture permittivity was measured in real-time using a sterilizable capacitance probe connected to an Evo 200 iBiomass system (Hamilton, Bonaduz, Switzerland) at a working frequency of 1,000 kHz. Measurements were performed every 12 min and the baseline was set by recording the permittivity of the cell-free medium, before cell seeding. The permittivity can be related to VCD using a linear relationship (Courtès et al., 2016):

$$\varepsilon = \alpha \times \text{VCD} + \beta. \quad (1)$$

In this study α equals to 13 and β equals to 3.

Based on VCD on-line estimation and mass balance equation, μ can be obtained in real-time by calculating the slope of a moving window linear regression on the logarithmic values of VCD in an interval of time (Equation (2)).

$$\mu = \frac{d\text{VCD}}{dt} \times \frac{1}{\text{VCD}} = \frac{\Delta \ln(\text{VCD})}{\Delta t}. \quad (2)$$

To reduce the noise of data collected from dielectric spectroscopy, moving average filters were used to have a more stable on-line μ estimation. Meanwhile, the distance residual (R) was calculated in real-time to evaluate the variations of the μ values with respect to the value of μ_{\max} (Equation (3)).

$$R = (\mu_{\max} - \mu_t)^2. \quad (3)$$

Here μ_{\max} is a predefined value of the maximum value of μ at the exponential growth phase obtained from previous cultures, and the μ_t is the on-line estimation of μ at the instant t .

Moreover, using multifrequency dielectric measurements and off-line cell diameter measurements, a cell-specific dielectric property parameter, the intracellular conductivity (σ_i) can be calculated with Equations (4-6):

$$p_m = \frac{4}{3} \cdot \pi \cdot \left(\frac{d}{2}\right)^3 \cdot \text{VCD}. \quad (4)$$

$$\sigma_a = \frac{\sigma}{(1-p_m)^{1.5}}. \quad (5)$$

$$\sigma_i = \frac{8 \cdot \pi \cdot f_c \cdot \Delta \varepsilon \cdot \sigma_a}{9 \cdot p_m \cdot \sigma_a - 4 \cdot \pi \cdot f_c \cdot \Delta \varepsilon}. \quad (6)$$

Where p_m is the viable biovolume fraction, and σ_a (S/m) is the conductivity of the medium. The values used in these calculations are the critical frequency f_c (Hz), the magnitude of the β -dispersion drop $\Delta \varepsilon$ (F/m), and the suspension conductivity σ (S/m) measured by the

dielectric spectroscopy; the average cell diameter d (m) measured off-line by Vi-CELL™ cell counter.

2.4 | Statistical analysis

The accuracy of the developed method was evaluated by the root mean square error (RMSE), calculated as follows:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}}. \quad (7)$$

where y_i is the off-line measured values (VCD or μ), \hat{y}_i is the on-line predicted values (VCD or μ), and n the number of samples under consideration.

3 | RESULTS AND DISCUSSION

3.1 | Cell growth, metabolism, and IgG production kinetics in batch culture

To assess the influence of cell growth on IgG glycosylation during batch cultures, CHO cells were cultured in a 2-L bioreactor, and cell density, viability, nutrients and by-products concentration, IgG production, and IgG glycosylation were monitored off-line. In addition, the off-line values of μ were calculated at the end of the culture using VCD values measured off-line. Although cells displayed excellent viabilities (>95%) until 127 hr, the value of μ started to decrease after about 100 hr of culture (Figure 1a). This decrease of μ is probably due to the glutamine depletion (Figure 1b), since glutamine, in addition to its energetic substrate function, is proved to be one of the most important key-precursors in several biosynthetic pathways of CHO cells. Its availability can affect the purine and pyrimidine synthesis rate (Hayter et al., 1991). Alternatively, it is possible that the accumulation of lactate and ammonia inhibits the growth of cells according to other authors (Glacken, Fleischaker, & Sinskey, 1986; Hassell, Gleave, & Butler, 1991; Yang & Butler, 2000). However, in our case, the concentration of ammonium ions and lactate were relatively low (<5 and <20 mM, respectively; Figure 1b), which were shown to have little or negligible effect on the growth of cells (Hayter et al., 1991; Lao & Toth, 1997). The intracellular conductivity (σ_i), which measures the ability of the cell's intracellular environment to conduct electrical current, was relatively stable during the first 95 hr of culture, followed by a light decrease until 103 hr before a significant increase after this period. This observation indicates potential changes in cell physiological state, which is demonstrated by the variations of σ_i (Figure 1c). As suggested by some authors, the decrease of this parameter often corresponds to nutrients limitations and depletions (Ansoorge, Esteban & Schmid, 2009; Opel et al., 2010). In our case, the decrease of σ_i is associated with glutamine exhaustion. In addition, the final increase of σ_i was reported to be linked to cell death since alteration in cell membrane integrity could result in the entry of medium ions into cells, leading to significant changes in the intracellular environment (Opel et al., 2010). As for IgG production, Figure 1d shows that almost all the

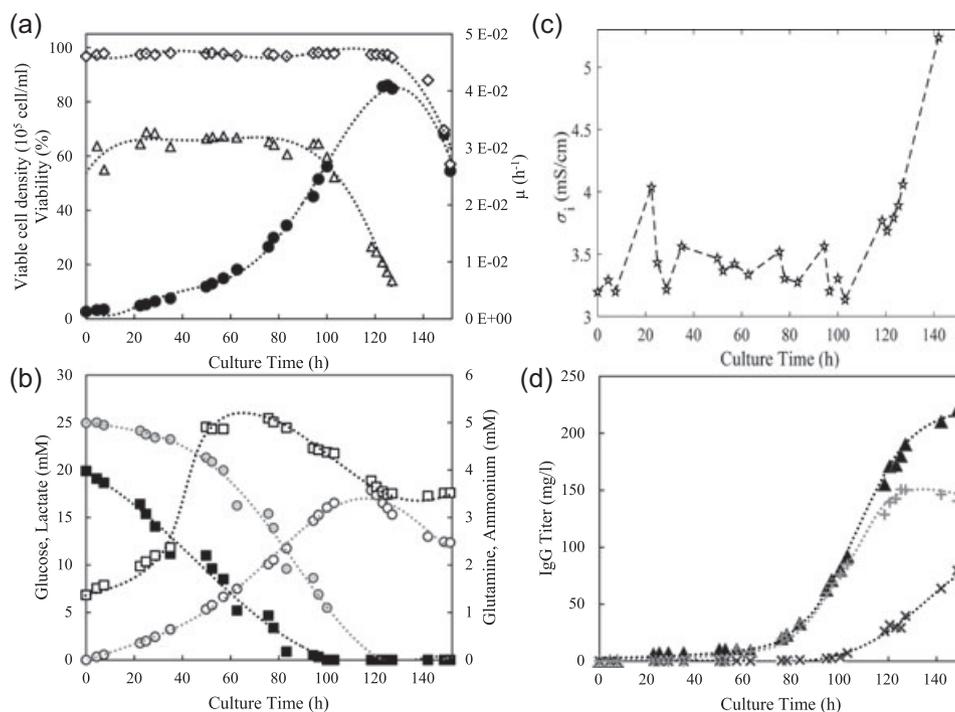


FIGURE 1 (a) Kinetics of cells growth: viable cell density (●), viability (◇), and specific growth rate (μ ; △). (b) Nutrients consumption and by-products accumulation: glucose (●), glutamine (■), lactate (○), and ammonium ions (□). (c) Cell physiological state: intracellular conductivity (σ_i ; ☆). (d) IgG production: total IgG (▲), glycosylated IgG (+), nonglycosylated IgG (×) throughout a representative batch culture of CHO cell in bioreactor. IgG: immunoglobulin G

IgGs produced were fully glycosylated until 100 hr of culture, then nonglycosylated IgGs began to accumulate in cell culture and reached about 30% of the total IgGs, resulting in an important product quality decrease.

It seems that the diminution of μ coincided with the accumulation of nonglycosylated IgG form, which could be explained by an insufficient energy production of CHO cells due to the glutamine exhaustion after about 100 hr, as suggested by Xie and Wang (1996). This decrease of μ led to cell physiological state changes as showed by σ_i calculations. Consequently, the recombinant protein production was also affected, resulting in a loss of the ability for cells to perform correctly the PTMs for the proteins produced because of less available energy (Hayter et al., 1993). In this study, we demonstrated that the global glycosylation of IgGs was influenced by the growth of cells, since the nonglycosylated IgG level increased abundantly after the decrease of μ , leading to a significant decrease in the final product quality at the end of the culture.

Therefore, as a direct indicator of the cell physiological state, μ is a key parameter of cell culture processes and should be accurately monitored and controlled as rapidly as possible, to limit the physiological changes of cells, and thus make sure that the glycosylation of IgGs produced is not altered.

3.2 | In situ and real-time estimation of μ

To estimate in real-time the value of μ , an in situ capacitance probe connected with dielectric spectroscopy was used. The permittivity

was measured every 12 min during cell cultures, and the on-line VCD was calculated from permittivity measured, by using a linear correlation. Estimation of on-line VCD is presented alongside with off-line VCD measurements in Figure 2a. The values of VCD predicted were in good agreement with the off-line VCD values (RMSE = 3.2×10^5 cell/ml).

To calculate the value of μ , VCD predicted was first converted to a logarithmic form ($\ln(\text{VCD})$), then the slope of the moving window linear regression of $\ln(\text{VCD})$ as a function of culture time was calculated to obtain the value of μ , as illustrated by Equation (2). However, data collected from capacitance probe presented high level of noise, so smoothing filters were applied to make data more stable for μ estimation. In this study, two moving average filters were implemented to smooth the VCD predicted and the $\ln(\text{VCD})$ calculated. The on-line values of μ estimated are presented in Figure 2b, which are validated by the off-line values of μ calculated at the end of the culture, with a RMSE equal to 0.008 hr^{-1} , indicating that the order of magnitude and the general trends of μ were correctly predicted, which allows validating our developed method.

Variations of μ , with respect to μ_{\max} , estimated in real-time were highlighted by the calculation of R (Equation (3); Figure 2b). Nevertheless, these variations of μ could be caused by physiological changes of cells and also signal instabilities. The signal instabilities were evaluated during the middle stage of exponential cell growth phase (between 40 and 70 hr in this study). The average values and the derivatives of R were calculated and were defined as the threshold since μ was supposed to be constant during this phase;

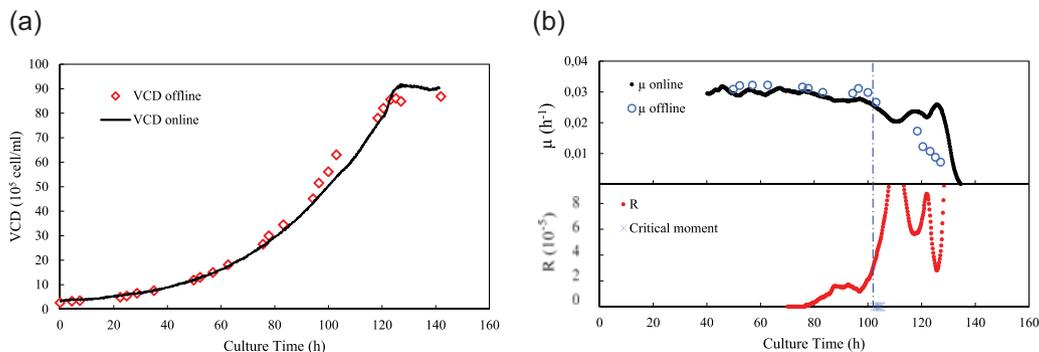


FIGURE 2 (a) On-line VCD prediction: VCD predicted (—) versus VCD measured (\blacklozenge). (b) On-line μ estimation: μ estimated (\bullet) versus μ calculated off-line (\circ), on-line calculation of R (\bullet), and on-line critical moment (\times) detection (blue dotted line). VCD: viable cell density [Color figure can be viewed at wileyonlinelibrary.com]

therefore, the variations of R were mainly caused by signal instabilities. When both the average values and the derivatives of R exceeded the threshold value during at least 36 min (three recorded dielectric signals), the critical moment when the cell growth begins to slow down due to cell physiological changes can be determined. Here, the critical moment was detected at 102 hr (blue dotted line). It should be noticed that after the critical moment has been identified, the culture, and in particular the physiological state of cells, could be altered as long as no interventions have been carried out.

These results demonstrated the potential of using on-line estimation of μ to have better understandings of animal cell culture bioprocesses, which could lead to a better process control by rapid interventions at the critical moment to maintain an appropriate cell physiological state.

3.3 | Application of the on-line estimation of μ in feed-harvest cell cultures

To demonstrate the interest of using on-line estimations of μ in the animal cell process control, the approach described previously in this study was used to control feed-harvest cell cultures. This culture mode could extend the duration of the cell culture, increase the antibody production level, and could also maintain an appropriate cells physiological state associated with fully glycosylated IgG production by restoring the nutrients and removing the cell wastes at appropriate moments. In this study, the feedings were performed by punctual medium renewals of two-thirds of total medium volume each time. Classically, medium renewals are usually carried out at fixed time points or by off-line measurements of nutrients (glucose and glutamine) with little considerations of cell physiological states. However, without a perfect knowledge of cell metabolism, it is extremely difficult to predict the exact moment when medium renewals are needed. Although using off-line measurements could help in understanding cell metabolic behaviors, there would still be risks of missing the moment for efficient medium renewals, as off-line measurements are performed only 2–3 times per day and the kinetics of nutrients consumption could vary rapidly especially at high cell concentrations. Consequently, when medium renewals are not performed at the right time, there could be either nutrients depletion, resulting in a

decrease in cell energy production, or in changes in cell physiological states, that may affect the culture process and the production of antibodies as well as their glycosylation.

Three feed-harvest cell cultures were carried out in this study using different strategies for medium renewals. The first cell culture using an off-line strategy was designed to simulate late medium renewals after total glucose and glutamine depletion (Figure 3b, left). For the second strategy, medium renewals were performed at the time when the concentration of glucose or glutamine reached their threshold (5 and 1 mM, respectively; Figure 3b, middle), to simulate early medium renewals. These concentrations were set up as more than 10 times of their Monod's half rate constants for the cell line studied (Xing, Bishop, Leister, & Li, 2010). The last cell culture was performed using real-time estimations of μ , the medium renewals were carried out each time when a critical moment was detected by the on-line developed method (Figure 3, right).

3.3.1 | Feed-harvest cell culture using off-line strategy with late medium renewals

In the first cell culture process, two medium renewals were performed at 159 and 215 hr (red lines), when off-line measurements indicated that there was no more glucose, nor glutamine in the culture medium (Figure 3, left), leading to a total cumulative production of 1 g IgG at the end of the culture (312 hr; Figure 4). However, on-line monitoring of μ and the calculation of R showed that the critical moments (blue dotted lines) were 130, 200, and 250 hr of culture, respectively, (Figure 3, left). As highlighted by the calculation of R , the value of μ began to decrease significantly after every critical moment, as nutrients depletions could cause nonsufficient energy production in cells to support the cell exponential growth and an appropriate cell physiological state for fully glycosylated IgG production. Indeed, as showed in Figure 5a, the σ_i of the cells increased after each critical moment, and decreased after the medium renewals, indicating that changes have appeared in cell physiological state. Moreover, the nonglycosylated form of IgG increased dramatically after the critical moments (Figure 6a). Similar phenomenon has been observed in many other studies, where

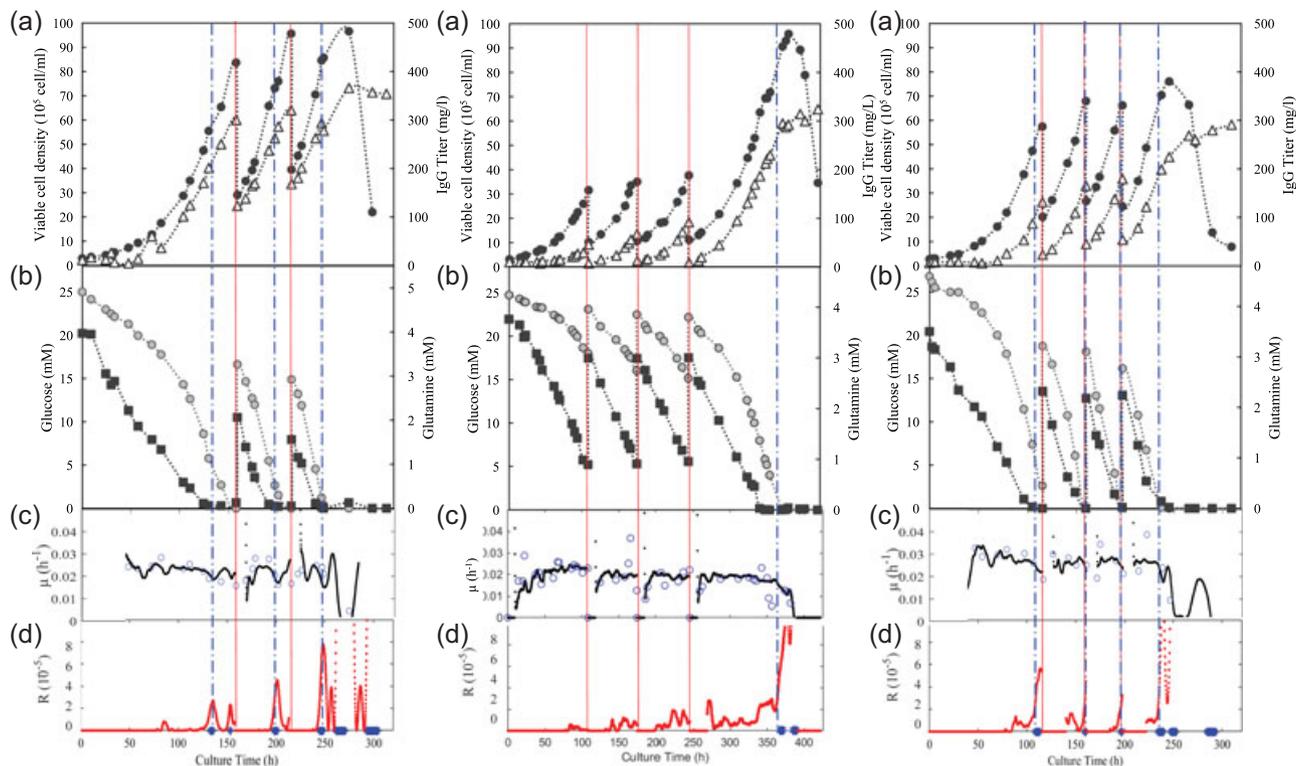


FIGURE 3 Kinetics of feed-harvest cell cultures using off-line late (left), off-line early (middle), and on-line (right) medium renewal strategies. (a) Viable cell density (●) and IgG titer (△). (b) Glucose (○) and glutamine (■) concentration. (c) On-line μ estimation (●) and off-line μ calculated (○). (d) On-line R calculation (●) and critical moments detected (×). Blue dotted lines indicate critical moments and red solid lines indicate moments of medium renewals. IgG: immunoglobulin G [Color figure can be viewed at wileyonlinelibrary.com]

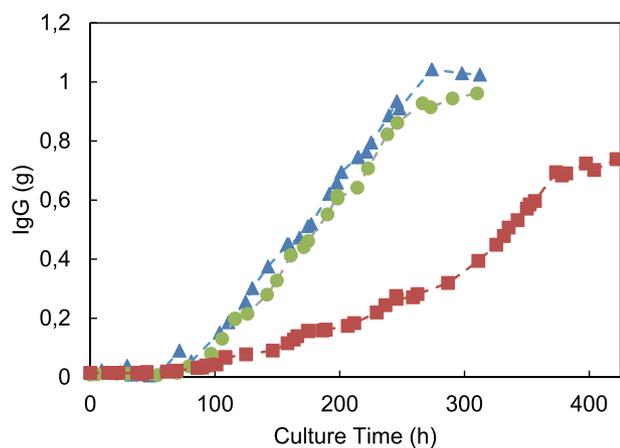


FIGURE 4 Cumulated antibody production in feed-harvest cell cultures using off-line late (▲), off-line early (■), and on-line (●) medium renewal strategies. IgG: immunoglobulin G [Color figure can be viewed at wileyonlinelibrary.com]

changes in protein glycosylation occurred at the end of the exponential cell growth phase (Castro, Ison, Hayter, & Bull, 1995; Hooker et al., 1995; Liu et al., 2014). They suggested that the depletion in nutrients or in glycosylation precursors in the medium could be responsible for the change in the glycosylation pattern. However, another study suggested that protein glycosylation was not dependent on limitation in glucose, nor glutamine, but highly dependent on the energetic status of the cells (Kochanowski et al.,

2008). Therefore, when there were alterations in cell physiological state as indicated by the decreases of μ and confirmed by the variations of σ_i , the nonglycosylated antibody level increased, although this level was reduced slowly after medium renewals.

3.3.2 | Feed-harvest cell culture using off-line strategy with early medium renewals

In another feed-harvest cell culture, three medium renewals were performed when the concentration of glucose or glutamine reached 5 or 1 mM, respectively. As indicated by the on-line calculation of μ , there was no critical moment detected until about 365 hr of cell culture, suggesting that the medium renewals were performed too early, before the critical moments when they become necessary. Although the values of μ remained relatively stable all along the cell culture, short lag phases can be observed each time after the medium renewal. Consequently, the values of μ dropped immediately after medium renewals and regained its maximum value about 10 hr after (Figure 3c, middle), indicating that this phenomenon could be considered as a sign of an instable cell physiological state during these periods. This hypothesis can be confirmed by the decreases of σ_i every time when a medium renewal was performed (Figure 5b). Concerning the IgG production, whereas there was no negative effect for the IgG glycosylation as the level of nonglycosylated IgG remained low all along the cell culture, IgG produced in this culture appeared much lower in quantity and

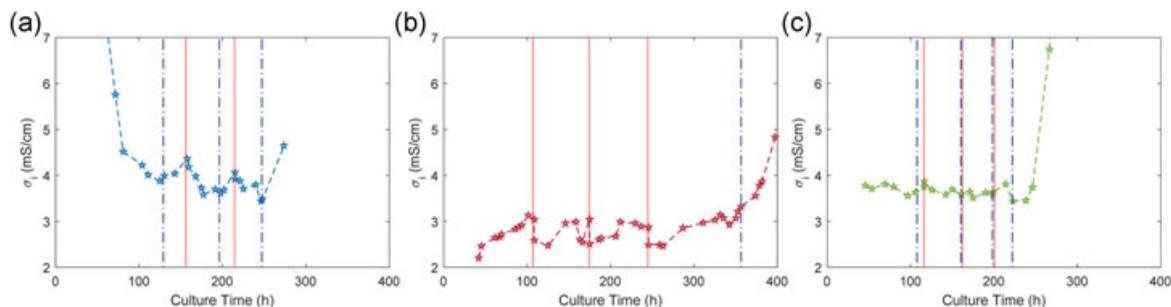


FIGURE 5 Profile of cell intracellular conductivity (σ_i) in feed-harvest cell cultures using off-line late (a), off-line early (b), and on-line (c) medium renewal strategies. Blue dotted lines indicate critical moments and red solid lines indicate moments of medium renewals [Color figure can be viewed at wileyonlinelibrary.com]

accumulated in a longer period, with a total of 0.74 g produced in 422 hr. This could be explained by the fact that the medium renewals were performed too early, and cells did not have enough time for cell division before being diluted, which may cause the lag phases. Therefore, with less cells and longer lag phases, the IgG productions were negatively affected in these experimental conditions.

3.3.3 | Feed-harvest cell culture using on-line estimation of μ

The developed approach of on-line estimation of μ was used in a feed-harvest culture to perform medium renewals at critical moments, at about 110, 160, and 200 hr of the culture, as indicated by the on-line calculation of R (Figure 3b, right). By using this on-line strategy, the decreases of μ were limited by timely performed medium renewals, and the value of μ remained nearly constant during the whole culture process before the final cell death phase. Moreover, there was no observation of lag phases after each medium renewal, and the values of μ remained close to its maximum. Consistently, a constant value of σ_i was maintained all along the cell culture before the final death phase (Figure 5c), indicating that cell physiological state remained stable, which led to better process performance regarding antibody quantity as well as antibody quality. As demonstrated in Figure 4, the total cumulative production of IgG was 0.96 g in 310 hr, which was more important than that produced in the cell cultures using off-line (early) strategy, and similar to that produced in the cell culture using off-line (late) strategy. Most

importantly, the quality of the produced IgG was maintained, as the level of nonglycosylated form of IgG produced was kept low (~5%) throughout the culture using this on-line strategy (Figure 6c).

Moreover, it is noteworthy that the critical moment would also be a good indicator for final culture harvesting, as it allows to harvest the cell culture before the accumulation of nonglycosylated antibodies due to the final cell death stage. Indeed, for the cell culture using on-line strategy if the harvest is performed at that moment, then the final level of the nonglycosylated IgG would be about 2% of the total IgG produced, leading to 0.79 g IgG correctly glycosylated. However, for cell cultures using off-line strategies, the final harvest could only be done based on off-line measurements of the cell viability. Therefore, for cell cultures using off-line late and early strategies, the final harvest would be performed at 274 and 382 hr when cell viability was inferior to 80%, which would lead to only 0.69 and 0.55 g correctly glycosylated IgG, respectively.

Using on-line estimation of μ , these results suggest that when the growth rate of cells is kept stable by medium renewals, correctly and timely performed, both the quantity and the quality of the antibody produced can be assured, leading to better process performances with minimum operator intervention.

3.3.4 | Statistical analysis

As for statistical analysis, the RMSE of VCD prediction for the three feed-harvest strategies (off-line late, off-line early, and on-line) was 3.4 , 2.7 , and 2.6×10^5 cell/ml, respectively. These low relative errors

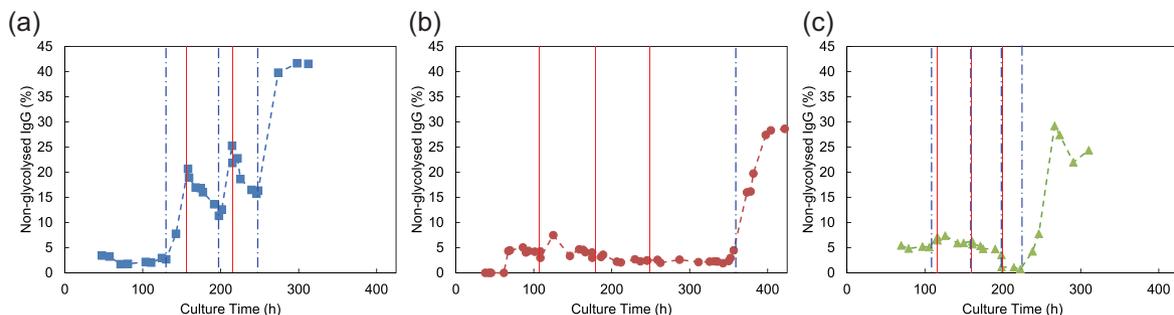


FIGURE 6 Relative abundance of nonglycosylated IgG produced in feed-harvest cell cultures using off-line late (a), off-line early (b), and on-line (c) medium renewal strategies. Blue dotted lines indicate critical moments and red solid lines indicate moments of medium renewals. IgG: immunoglobulin G [Color figure can be viewed at wileyonlinelibrary.com]

showed good prediction capability of dielectric spectroscopy for the VCD values. The RMSE of the on-line estimation of μ was 0.01, 0.007, and 0.015 hr^{-1} for cell cultures using off-line late, off-line early, and on-line strategies, respectively. The general trends of the on-line and off-line values of μ were in agreement, allowing the identification and the confirmation of the critical moments.

4 | CONCLUSION

Cell-specific growth rate (μ) is a key parameter for antibody-producing animal cell culture processes which reflects the cell physiological state. To optimize the antibody production, and especially, to ensure the quality of the final product, μ should be carefully monitored and controlled in real-time. In this study, a method of on-line estimation of μ was developed based on in situ dielectric spectroscopy measurements. As a result, the variations of μ were monitored in real-time using various mathematical methods to process these data, allowing rapid detection of the critical moment when μ decreased significantly. This on-line detection of the critical moment was successfully implemented in feed-harvest cell cultures, and μ was maintained around its maximum value by performing medium renewals at critical moments. This on-line strategy allowed to maintain an appropriate cell physiological state. Consequently, a better process performance was obtained with both the quantity and the quality (concerning the glycosylation) assured for the antibody produced. When harvested at a critical moment, the nonglycosylated antibody level was kept minimum, leading to the highest recovery of the antibody correctly glycosylated compared with the cell cultures performed with off-line strategies. The method of on-line estimation of μ shows great potentials for antibody production bioprocesses, and future efforts should be aimed at the implementation of this on-line method to fully automated feedback control scheme in animal cell culture processes for antibody quality control.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

- Abu-Absi, N. R., Kenty, B. M., Cuellar, M. E., Borys, M. C., Sakhamuri, S., Strachan, D. J., ..., Li, Z. J. (2011). Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. *Biotechnology and Bioengineering*, 108(5), 1215–1221.
- Ansorge, S., Esteban, G., & Schmid, G. (2007). On-line monitoring of infected Sf-9 insect cell cultures by scanning permittivity measurements and comparison with off-line biovolume measurements. *Cytotechnology*, 55(2-3), 115–124.
- Ansorge, S., Esteban, G., & Schmid, G. (2009). Multifrequency permittivity measurements enable on-line monitoring of changes in intracellular conductivity due to nutrient limitations during batch cultivations of CHO cells. *Biotechnology Progress*, 26(1), 272–283. <https://doi.org/10.1002/btpr.347>
- Cannizzaro, C., Gügerli, R., Marison, I., & von Stockar, U. (2003). On-line biomass monitoring of CHO perfusion culture with scanning dielectric spectroscopy. *Biotechnology and Bioengineering*, 84(5), 597–610. <https://doi.org/10.1002/bit.10809>
- Castro, P. M. L., Ison, A. P., Hayter, P. M., & Bull, A. T. (1995). The macroheterogeneity of recombinant human interferon-gamma produced by chinese-hamster ovary cells is affected by the protein and lipid-content of the culture-medium. *Biotechnology and Applied Biochemistry*, 21, 87–100.
- Cervera, A. E., Petersen, N., Lantz, A. E., Larsen, A., & Germaey, K. V. (2009). Application of near-infrared spectroscopy for monitoring and control of cell culture and fermentation. *Biotechnology Progress*, 25(6), 1561–1581. <https://doi.org/10.1002/btpr.280>
- Cole, H. E., Demont, A., & Marison, I. W. (2015). The application of dielectric spectroscopy and biocalorimetry for the monitoring of biomass in immobilized mammalian cell cultures. *Processes*, 3(2), 384–405. <https://doi.org/10.3390/pr3020384>
- Courtès, F., Ebel, B., Guédon, E., & Marc, A. (2016). A dual near-infrared and dielectric spectroscopies strategy to monitor populations of Chinese hamster ovary cells in bioreactor. *Biotechnology Letters*, 38(5), 745–750. <https://doi.org/10.1007/s10529-016-2036-0>
- Glacken, M. W., Fleischaker, R. J., & Sinskey, A. J. (1986). Reduction of waste product excretion via nutrient control: Possible strategies for maximizing product and cell yields on serum in cultures of mammalian cells. *Biotechnology and Bioengineering*, 28(9), 1376–1389. <https://doi.org/10.1002/bit.260280912>
- Guez, J. S., Cassar, J. P., Wartelle, F., Dhulster, P., & Suhr, H. (2004). Real time in situ microscopy for animal cell-concentration monitoring during high density culture in bioreactor. *Journal of Biotechnology*, 111(3), 335–343. <https://doi.org/10.1016/j.jbiotec.2004.04.028>
- Hassell, T., Gleave, S., & Butler, M. (1991). Growth inhibition in animal cell culture. *Applied Biochemistry and Biotechnology*, 30(1), 29–41.
- Hayter, P. M., Curling, E. M. A., Baines, A. J., Jenkins, N., Salmon, I., Strange, P. G., & Bull, A. T. (1991). Chinese hamster ovary cell growth and interferon production kinetics in stirred batch culture. *Applied Microbiology and Biotechnology*, 34(5), 559–564.
- Hayter, P. M., Curling, E. M. A., Gould, M. L., Baines, A. J., Jenkins, N., Salmon, I., ..., Bull, A. T. (1993). The effect of the dilution rate on CHO cell physiology and recombinant interferon- γ production in glucose-limited chemostat culture. *Biotechnology and Bioengineering*, 42(9), 1077–1085.
- Henry, O., Kamen, A., & Perrier, M. (2007). Monitoring the physiological state of mammalian cell perfusion processes by on-line estimation of intracellular fluxes. *Journal of Process Control*, 17(3), 241–251. <https://doi.org/10.1016/j.jprocont.2006.10.006>
- Hooker, A. D., Goldman, M. H., Markham, N. H., James, D. C., Ison, A. P., Bull, A. T., ..., Jenkins, N. (1995). N-glycans of recombinant human interferon- γ change during batch culture of chinese hamster ovary cells. *Biotechnology and Bioengineering*, 48(6), 639–648. <https://doi.org/10.1002/bit.260480612>
- Justice, C., Brix, A., Freimark, D., Kraume, M., Pfromm, P., Eichenmueller, B., & Czermak, P. (2011). Process control in cell culture technology using dielectric spectroscopy. *Biotechnology Advances*, 29(4), 391–401.

- Kilburn, D. G., Fitzpatrick, P., Blake-Coleman, B. C., Clarke, D. J., & Griffiths, J. B. (1989). On-line monitoring of cell mass in mammalian cell cultures by acoustic densitometry. *Biotechnology and Bioengineering*, 33(11), 1379–1384. <https://doi.org/10.1002/bit.260331103>
- Kiviharju, K., Salonen, K., Moilanen, U., & Eerikäinen, T. (2008). Biomass measurement online: The performance of in situ measurements and software sensors. *Journal of Industrial Microbiology and Biotechnology*, 35(7), 657–665. <https://doi.org/10.1007/s10295-008-0346-5>
- Kochanowski, N., Blanchard, F., Cacan, R., Chirat, F., Guedon, E., Marc, A., & Goergen, J.-L. (2008). Influence of intracellular nucleotide and nucleotide sugar contents on recombinant interferon- γ glycosylation during batch and fed-batch cultures of CHO cells. *Biotechnology and Bioengineering*, 100(4), 721–733. <https://doi.org/10.1002/bit.21816>
- Kondo, M., Yamaoka, T., Honda, S., Miwa, Y., Katashima, R., Moritani, M., ..., Itakura, M. (2000). The rate of cell growth is regulated by purine biosynthesis via ATP production and G1 to S phase transition. *The Journal of Biochemistry*, 128(1), 57–64.
- Lao, M.-S., & Toth, D. (1997). Effects of ammonium and lactate on growth and metabolism of a recombinant Chinese hamster ovary cell culture. *Biotechnology Progress*, 13(5), 688–691.
- Lee, H. W., Carvell, J., Brorson, K., & Yoon, S. (2015). Dielectric spectroscopy-based estimation of VCD in CHO cell culture. *Journal of Chemical Technology and Biotechnology*, 90(2), 273–282. <https://doi.org/10.1002/jctb.4522>
- Li, M. -Y., Ebel, B., Paris, C., Chauchard, F., Guedon, E., & Marc, A. (2018). Real-time monitoring of antibody glycosylation site occupancy by in situ Raman spectroscopy during bioreactor CHO cell cultures. *Biotechnology Progress*, 34(2), 486–493. <https://doi.org/10.1002/btpr.2604>
- Liu, B., Spearman, M., Doering, J., Lattová, E., Perreault, H., & Butler, M. (2014). The availability of glucose to CHO cells affects the intracellular lipid-linked oligosaccharide distribution, site occupancy and the N-glycosylation profile of a monoclonal antibody. *Journal of Biotechnology*, 170, 17–27. <https://doi.org/10.1016/j.jbiotec.2013.11.007>
- Opel, C. F., Li, J., & Amanullah, A. (2010). Quantitative modeling of viable cell density, cell size, intracellular conductivity, and membrane capacitance in batch and fed-batch CHO processes using dielectric spectroscopy. *Biotechnology Progress*, 26(4), 1187–1199.
- ICH (2017). Quality guidelines. Retrieved from: <http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html>
- Tharmalingam, T., Wu, C.-H., Callahan, S., & T. Goudar, C. (2015). A framework for real-time glycosylation monitoring (RT-GM) in mammalian cell culture. *Biotechnology and Bioengineering*, 112(6), 1146–1154. <https://doi.org/10.1002/bit.25520>
- Walsh, G. (2014). Biopharmaceutical benchmarks 2014. *Nature Biotechnology*, 32(10), 992–1000. <https://doi.org/10.1038/nbt.3040>
- Xie, L., & Wang, D. I. C. (1996). Energy metabolism and ATP balance in animal cell cultivation using a stoichiometrically based reaction network. *Biotechnology and Bioengineering*, 52(5), 591–601. [https://doi.org/10.1002/\(SICI\)1097-0290\(19961205\)52:5<591::AID-BIT6>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0290(19961205)52:5<591::AID-BIT6>3.0.CO;2-E)
- Xing, Z., Bishop, N., Leister, K., & Li, Z. J. (2010). Modeling kinetics of a large-scale fed-batch CHO cell culture by Markov chain Monte Carlo method. *Biotechnology Progress*, 26(1), 208–219. <https://doi.org/10.1002/btpr.284>
- Xiong, Z.-Q., Guo, M.-J., Chu, J., Zhuang, Y.-P., & Zhang, S.-L. (2015). On-line specific growth rate control for improving reduced glutathione production in *Saccharomyces cerevisiae*. *Biotechnology and Bioprocess Engineering*, 20(5), 887–893. <https://doi.org/10.1007/s12257-015-0018-z>
- Yang, M., & Butler, M. (2000). Effects of ammonia on CHO cell growth, erythropoietin production, and glycosylation. *Biotechnology and Bioengineering*, 68(4), 370–380. [https://doi.org/10.1002/\(SICI\)1097-0290\(20000520\)68:4<370::AID-BIT2>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0290(20000520)68:4<370::AID-BIT2>3.0.CO;2-K)
- Yardley, J. E., Kell, D. B., Barrett, J., & Davey, C. L. (2000). On-line, real-time measurements of cellular biomass using dielectric spectroscopy. *Biotechnology and Genetic Engineering Reviews*, 17(1), 3–36.

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