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Transfer-rate Online Measurement (TOM) system for analyzing the respiratory activity of a Chinese Hamster Ovary (CHO) cell pool expressing SARS-CoV-2 Spike Protein in a Fed-Batch process

ABSTRACT

Shake flasks are a widely used efficient vessel for the maintenance and rapid scale-up of mammalian cell bioprocesses; however, noninvasively monitoring the respiratory activity of a culture in these vessels can be a challenge. Here we examine the applicability of a new instrument, the Kuhner Transfer-rate Online Measurement (TOM) system, for monitoring the respiratory activity of a CHO cell pool Fed-Batch process in real-time. The results indicate that the TOM can characterize process events as well as describe the respiratory behaviour throughout the culture run. This characterization tool is further applied in the validation of a new cell bank to ensure its behavior is inline with historical data. The results also demonstrate that the Oxygen Transfer Rate (OTR) is highly correlated with cell growth and glucose uptake rates indicating that it can serve as a soft sensor for glucose consumption and biomass monitoring.

INTRODUCTION

The respiratory activity of mammalian cells can be directly linked to their growth and metabolic function. As O_2 is consumed by the cells, the concentration of O_2 inside the vessel is reduced and this can be monitored using an off-gas analyzer [1, 2]. A similar approach can also be used to monitor the change in the CO_2 concentration. The Kuhner TOM system is a new instrument capable of monitoring this activity and operates in a similar capacity to the Respiration Activity Monitoring System (RAMOS) [3]. This technology utilizes a closed system that simulates the aeration of an open vessel, and it is important to confirm that this approach does not negatively impact cell growth, metabolism, and production [3]. It is also important to confirm that the results from the TOM align with the activity of the culture and that this technology is compatible with a mammalian cell process.

MATERIALS AND METHODS

CHO stable pool cells expressing SARS-CoV-2 trimeric spike protein (SmT1) were grown in BalanCD CHO Growth A medium (Fujifilm/Irvine Scientific) with 50 µM MSX (L-Methionine sulfoximine, Sigma-Aldrich) in a 250-mL Corning shake flask. The flasks (12 total) were shaken in parallel at 120 rpm (25 mm orbital diameter) in a Kuhner ISF1-X at 37ºC, 5% CO_2, and 75% relative humidity. Initially 4 flasks were connected to the TOM via the Corning adapters. Each flask was seeded at 0.4 x 10^6 cells/mL and cultivated for 17 days. Temperature shift (37ºC to 32ºC) was realized 3 days after seeding. Induction was realized with 4-Isopropylbenzenecarboxylate (Cumate, ArkPham), concomitantly with the temperature shift. Cultures where fed with BalanCD CHO Feed 4 (Fujifilm/Irvine Scientific) and supplemented with glucose as to maintain the concentration above 17 mM. 1-mL samples were taken from the flasks on days -3, -2, -1, 0, 3, 5, 7, 10, 12, and 14 dpi (day post-induction) for off-line analysis, while feeding was realized from 0 dpi onward. Cell density, viability, metabolites (glucose, lactate, ammonia), were measured utilizing the previously reported methodology [4, 5]. Total sampling duration was 20 minutes during every sampling day and samples were always taken in the same order (1-4 in numbered flasks for the exploratory experiment and 1-8 numbered flasks in the validation experiment). The TOM gathers the OTR and CTR online data every 1.5 hours. After an exploratory Fed-Batch experiment (n=4), 2 cell banks were compared. Each bank with n=4 replicates of shake flasks connected to the TOM following the same protocol as detailed above.

Results

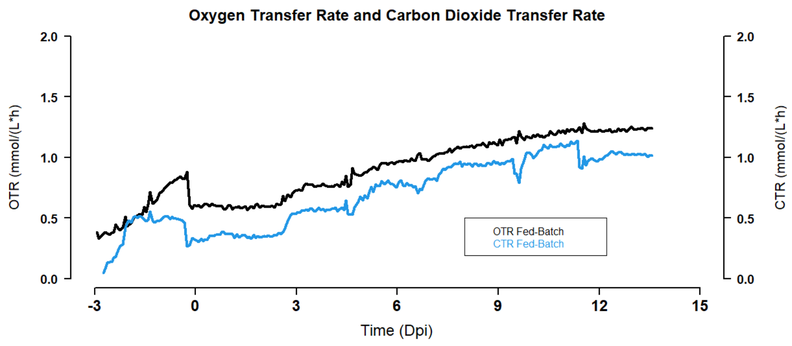

Figure 1. Respiratory results of a Fed-Batch process

As it can be seen from figure 1, the OTR mirrors the initial exponential growth phase of a cell culture until 0 dpi, which corresponds to induction and temperature shift. A sudden drop is observed at 0 dpi, which is likely associated with the 5ºC temperature reduction. Subsequent feeding days can be visualized on the graph as noticeable increases in respiratory activity. The sharp reduction in CTR prior to CTR increases in feeding days are most likely due to perturbations of the CO_2 atmosphere in the incubator. Additionally, a stark difference in respiratory profile is observed post induction (production phase) when compared to pre induction (growth phase), which could be related to increased protein synthesis activity given that OTR requirements are almost doubled in the production phase (Table 1). Additionally, TQ (ratio CTR/OTR) was observed to oscillate around one (data not shown), which indicates full oxidation of carbon substrate and is inline with other CHO results in the literature.

Table 1. Respiratory profiles summary.

	Growth Phase	Production Phase
OTR (mmol/(L*H))	*0.58*	*0.96*
CTR (mmol/(L*H))	*0.38*	*0.76*
TQ	*0.61*	*0.78*
OUR (mmol/(L*H)) ≈	*0.58*	*0.95*
CER (mmol/(L*H)) ≈	*0.45*	*0.75*

Once exploratory Fed-batch results were realized, a validation between two cell banks (Beta variant B.1.351 CHO pool, abbreviated SA in the graphs) was carried out to determine if they behaved the same. Cell bank A refers to the original cell bank while cell bank B refers to the new cell bank. Cell viability and metabolites were measured throughout the Fed-Batch process (Figures 2 & 3). When compared between original and new cell bank, no discernible variation on culture growth or metabolic profile was observed. The error bars represent ±one standard deviation of n=4 replicates.

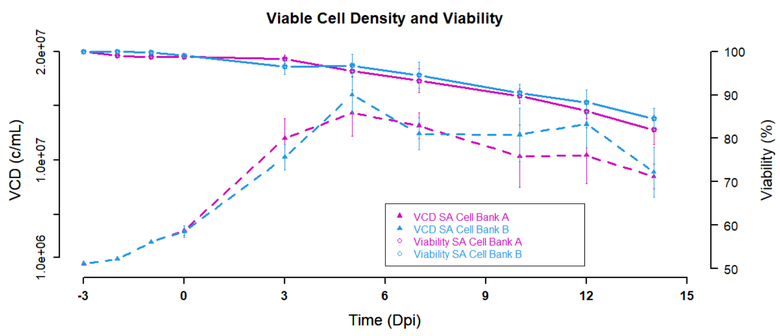

Figure 2. Cellular growth profile

Off-line measurements of Viable Cell Density and Viability over the Fed-Batch process.

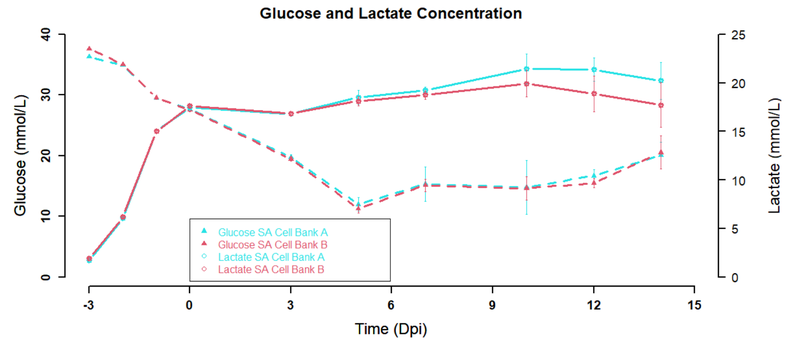

Figure 3. Metabolic profile

Off-line measurements of glucose and lactate over the Fed-Batch process.

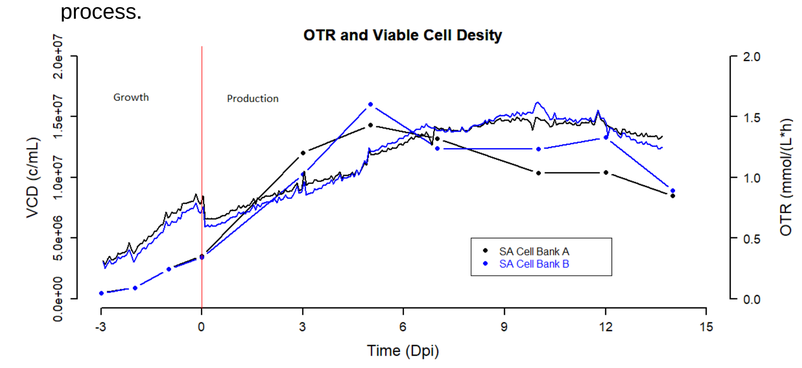

Figure 4. Overlay OTR and Cell Growth (VCD) data

Viable cell density and oxygen transfer rate profiles: No differences were observed in the OTR profiles between the two cell banks.

As the biomass increases, so will the demand for oxygen in the culture; consequently, the OTR should correlate with cell growth. It should also give indirect information about the metabolic demand of the cells in the culture. As it can be seen from figure 4, the OTR profile is able to mirror the initial exponential growth phase. Interestingly, it can also mirror the secondary growth phase after the temperature shift and the plateau and decline phase from 7 dpi to 14 dpi.

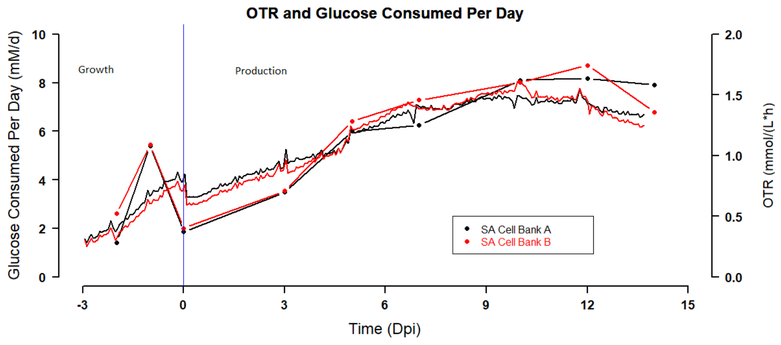

Figure 5. Overlay OTR and Glucose consumed per day rate

Glucose consumed per day and oxygen transfer rate profiles:

By observing figure 5 it is clear that no differences were observed in the OTR or glucose consumption profiles between the two cell banks. Importantly, it is expected that as metabolic demand increased, respiratory activity follows. Indeed, OUR has been used as a predictor for glucose consumption in bioreactors given its highly linear correlation [6].

This linear correlation may be explained by the resulting flux from glycolysis into the TCA cycle which is highly aerobic. Importantly increased TCA cycle activity has been associated with increased protein synthesis and thus higher oxygen consumption [6]. In Figure 5, it is clear that in the production phase of the culture, the glucose consumption is increased with respect to the growth phase, this is also reflected in the OTR curve. This could hint at energy requirements of protein synthesis within the culture process. Interestingly the observed co-linearity could imply that glucose addition to the cultures could be realized without off-line measurements thus opening the doors for on-line glucose soft sensing [6].

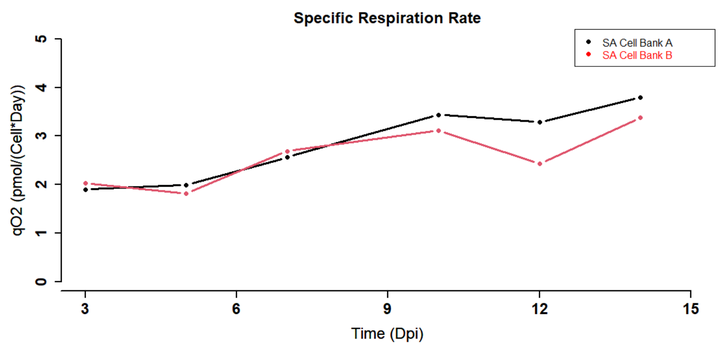

Figure 6. Specific Respiration Rate profile.

The specific respiration rates (OTR/VCD = qO_2) are plotted for each cell bank from 3 dpi to 14 dpi.

From figure 6 it is clear that that the respiration rate shows little variation between the cell banks. Specific respiration rates (qO_2) report the individual oxygen consumption of individual cells and have been shown to correlate highly with metabolic activity and proteins production [7]. Although qO_2 is constant during the growth phase up to 5 dpi (Fig. 6), here we observed an increase in qO_2 during the plateau and production phases, suggesting that the qO_2 can function as a reporter for various biological activity (e.g., biomass, protein production, etc.). Notably, the qO_2 aligned with the previously reported values of 1-5 pmol/(Cell*Day) [8].

CONCLUSION

We successfully implemented the TOM system into our CHO cell culture production platform at the NRC for a Fed-Batch process. The OTR and CTR data collected from the TOM align with the expected cell growth and production behavior that is typically observed for these cells; this includes the distinctive lag, exponential growth, plateau, and deceleration phase over a 17-day Fed-Batch process. In the initial exploratory experiments, a maximal OTR value of ~1.25 mmol/L/H was recorded for this cell pool. A peak CTR of ~ 1 mmol/L/H was registered after ~14 days after induction of culture. Given the knowledge of how a standard Fed-Batch process is reflected on the OTR/CTR profiles, the TOM was then applied in the validation of a new cell bank to ensure its growth profiles, metabolic profiles, respiratory profiles and final titer (~ 620 mg/L yield in cell bank A and ~718 mg/L yield in cell bank B) behaved the same. The experiment demonstrated the applicability of the TOM in characterization of mammalian pools through respiratory profiles. Indeed, ensuring similar respiratory activity is key for validating newly developed cell banks as cellular respiration can not only be a gauge for cell growth profiles but for metabolic and production activity as well. In fact, during the validation process, it was observed that the correlation between glucose consumption rates and oxygen consumption rates was very high. Thus, indicating that the TOM may be applied in developing a glucose soft-sensor. Such soft sensor would allow for online monitoring of glucose in the media without the need of off-line sampling [6]. With this increased sampling rate, the development of dynamic feeding strategies can become possible.

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