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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

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 invasiveness and impact of a new instrument, the Kuhner Transfer-rate Online Measurement (TOM) system, for monitoring the respiratory activity of a CHO cell pool growth batch process in real-time. The results indicate that the TOM did not impact the cell density, cell viability, metabolites, or pH of the cell culture after 10 days of monitoring. The results also show an Oxygen Transfer Rate / Carbon Dioxide Transfer Rate (OTR/CTR) profile that aligns with the growth pattern of these cells. These results suggest that the TOM is a non-invasive instrument that can be utilized for monitoring mammalian cell culture processes.

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. 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 or metabolism [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 (16 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. Half of the flasks (8x, TOM condition) were connected to the TOM via the Corning adapters (Figure 1). From these, 4 flasks were subjected to sampling procedures (Sampled Flasks) and 4 other flasks were left unsampled (Un-Sampled Flasks). Conversely, the remaining half of the flasks (8x) were kept as No-TOM control condition; 4 flasks were sampled and 4 were unsampled. Each flask was seeded at 0.2 x 10^6 cells/ml and cultivated for 10 days in a batch mode. 1-ml samples were taken from 4 flasks of each group (TOM and No-TOM) on days 3, 4, 5, 6, 7, and 10 for off-line analysis. Cell density, viability, metabolites (glucose, lactate, ammonia), and pH 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 (1-16 in numbered flasks). The TOM gathers the OTR and CTR online data every 1.5 hours.

TOM Set-up

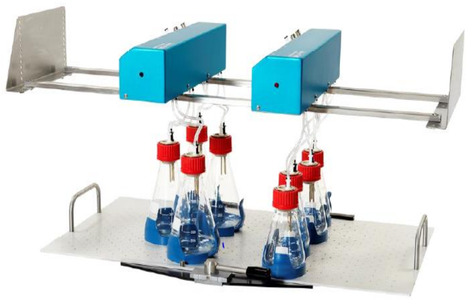

Figure 1. TOM system set-up in shaking incubator

RESULTS

Cell viability, metabolites, and pH were measured throughout the batch cycle (Figures 2 & 3). When compared between TOM and No-TOM conditions the figures demonstrate that the TOM made no discernible impact on culture viability or metabolic profile. The error bars represent ±one standard deviation of n=4 replicates. In Figure 2, cell viability and Viable Cell Density (VCD) are plotted with respect to culture time for the TOM and No-TOM conditions. Low variability between conditions suggests that the TOM has no negative impact on cellular growth and viability. There also appeared to be no difference in the metabolic profile of the TOM and No-TOM conditions (Figure 3).

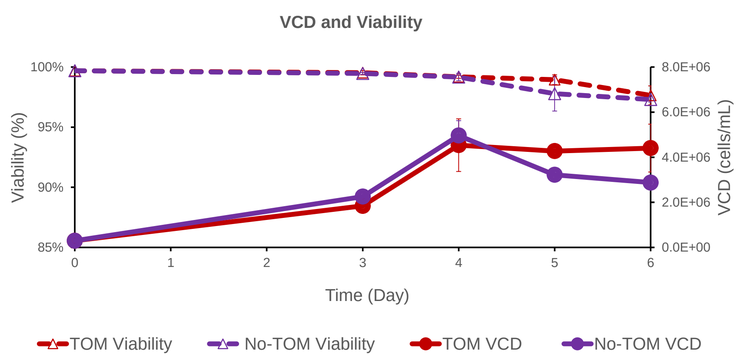

Figure 2. Cellular viability and Viable Cell Density (VCD)

Viability (%) and viable cell density (cells/mL) are plotted over the first 6-days of a 10-day batch process for both the TOM (red) and No-TOM (purple) conditions.

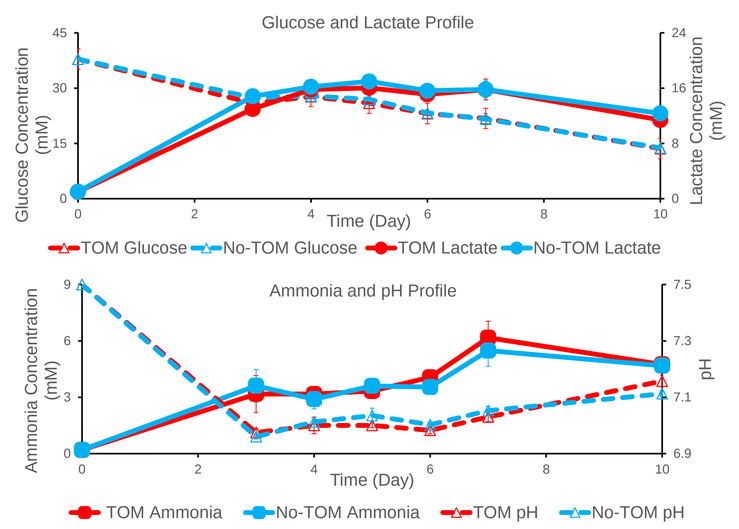

Figure 3. Metabolic profile

Off-line measurements of glucose and lactate or ammonia and pH for the TOM (red) and No-TOM (blue) conditions over the 10-day batch process.

As the cell density increases, so will the demand for oxygen in the media; therefore, the rate of oxygen transferring into the media (OTR) should positively correlate with cell growth. The results in Figure 4 demonstrate this relationship, with the OTR of the sampled flasks increasing in parallel with the viable cell density of the cell culture over time. This indicates that the TOM can offer quantitative information regarding the state of the culture (exponential phase, plateau phase, death phase) without the need for invasive sampling.

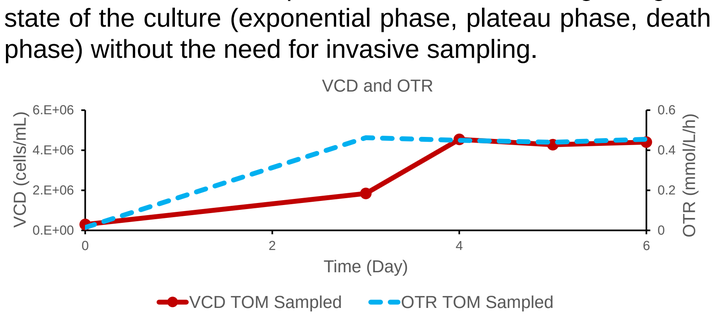

Figure 4. Overlay OTR and Cell Growth (VCD) data

Viable cell density and oxygen transfer rate profiles: The correlation factor between averaged OTR (averaged over 4 data points before and after sampling) and averaged VCD is 0.8 (data not shown).

Comparing the OTR and CTR profiles across the different TOM channels revealed minimal variation between replicates of either condition. Additionally low variation between the sampled and un-sampled conditions was observed (Figure 5). The decrease in volume caused by sampling was taken into account when calculating the OTR and CTR. Consequently, it can be seen that there is no obvious effect of sampling on the course of OTR and CTR.

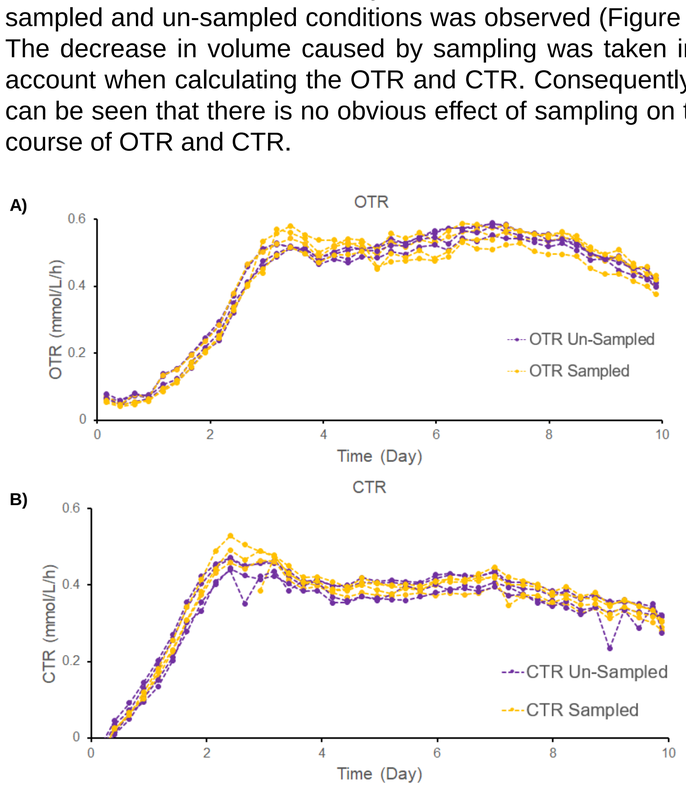

Figure 5. OTR and CTR profiles

OTR (A) and CTR (B) plotted over a 10-day batch process (averaged over 4 data points) for replicates (n=4) of both Sampled (yellow) and Un-Sampled (purple) flasks.

It is interesting to note that the daily OTR and CTR pattern throughout the batch also aligned with the typical growth cycle observed for this process. Specifically, an exponential phase (Days 1-3), a deceleration phase (Days 3-4), followed by a plateau phase (Days 4-7), a decay phase (Days 7-10) (Figure 5). The TOM was also able to report minor variations between different conditions for the Transfer Quotient (TQ) (Figure 6); its definition and distinction when compared to the Respiratory Quotient (RQ) is discussed below. This suggests that the TOM may be utilized for the characterization of different process conditions.

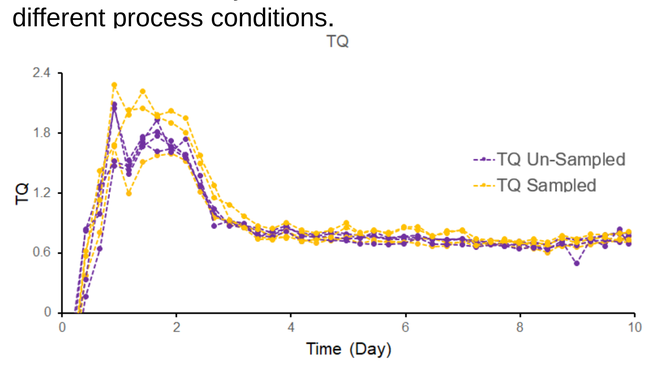

Figure 6. Transfer Quotient (TQ) profile

Transfer Quotient (TQ) plotted over a 10-day batch process with replicates (n=4) of both Sampled (yellow) and Un-Sampled (purple) flasks.

The Transfer Quotient (TQ) is defined as the ratio of CO_2 leaving the media (CTR) and O_2 entering the media (OTR), which is independent from the Respiratory Quotient (RQ) that reflects the ratio of the CO_2 Evolution Rate (CER) and the O_2 Uptake Rate (OUR). The OTR ≈ OUR, but the CTR ≠ CER due to the bicarbonate-based consumption of CO_2 in the media. If the CTR were not influenced by external factors like pH, we would anticipate a TQ ≈ RQ and both ratios ≈ 1. As lactate is produced, the pH of the media decreases (Figure 3) and this influx of H^+ into the media causes a shift towards CO_2 production from carbonic acid, which yields a greater CTR and an increase in TQ (Figure 6). When the drop in pH reverses and begins to steadily increase from Day 3 (Figure 3), the TQ decreases and is held at ~0.8 till the end of the culture, which suggests that the course of the pH is reflected by the TQ [6] and the latter could act as a soft-sensor for pH or [lactate].

CONCLUSION

We successfully implemented the TOM system into our CHO cell culture production platform at the NRC.

The OTR and CTR data collected from the TOM align with the expected cell growth behavior that is typically observed for these cells; this includes the distinctive lag, exponential growth, plateau, deceleration, and decay phase over a 10-day batch process. A maximal OTR value of ~0.6 mmol/L/h was recorded for this cell pool. A peak CTR of ~ 0.5 mmol/L/h was registered after ~2.5 days of culture. In consequence, it was found that the TQ increases to a peak of ~ 2, then decreases and plateaus at ~ 0.8. The sudden increase in CTR with respect to OTR may be due to changes in the pH rather than the CO_2 levels, since the initially buffering of CO_2 entering the media will result in a negative CTR (which happens over the first few hours) while the system stabilizes. Consequently, it was found that the TQ changes with respect to the pH and [lactate] over the course of the process, which could suggest that this ratio could be used to track these parameters. Cellular growth, metabolites (glucose, lactate, ammonia), and pH profiles are not impacted by the TOM system. Sampling did not appear to have any major effect on cell growth or metabolism. In summary, the TOM proved to be non-invasive and fully compatible with a mammalian (CHO) cell process. The online measurements from the TOM align with the cellular behavior and the TOM has the potential to reveal new insight for process development.

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