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BIOSCIENCES

CULTURE BIOSCIENCES' 250ML CLOUD BIOREACTOR IS A REPRODUCIBLE AND SCALABLE SYSTEM FOR CHO CELL CULTURE

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INTRODUCTION

The bench-scale bioreactor has been the workhorse of CHO cell culture process development for decades. It serves as an indispensable tool for evaluating cell lines after static and/or shaker screening, process parameter optimization, media formulation development, troubleshooting issues at scale, and demonstration of process understanding to regulators.[1]

However, running benchtop bioreactors - while clearly more economical than optimization at scale - still requires significant resources, infrastructure, and staffing.[2] Peripheral tubing assembly, sterilization, calibration, and other set-up activities can take hours to days. Tear-down, cleaning, and deactivation are similarly burdensome. There are potentially large associated costs to prepare or purchase media, carry seed trains, and maintain facilities.

The industry trend toward miniaturization seeks to address the costs of small scale development by reducing media volumes, seed culture volumes, sophistication of facilities, and the lab footprint required. While miniaturization improves operational efficiency, there is more ground to be gained. As long as process development experiments are executed on site, costs, disruptions, and inefficiencies have to be managed. Hardware failures and facility issues must be prepared for and resolved. Operational complications such as contaminations, protocol errors, and staffing disruptions can add rework or delay projects already competing for bioreactor capacity. Most critically, running bioreactors may be an inefficient use of staff hired to focus on innovation, experiment design, and complex data analysis — not for daily sampling and monitoring trends. At the same time, consistent and expert laboratory operations remain essential to generating reproducible and reliable data.

To address these challenges, Culture Biosciences built a 250 mL bioreactor system that runs off site and in the cloud. In this paradigm:

Customers

- provide process parameters, vials, and media
- receive samples for analysis and storage
- perform unique assays and upload data to the cloud
- analyze all data and generate graphs in Culture's centralized Bioprocessing Console

Culture Biosciences

- thaws vials
- carries seed trains
- performs studies
- uploads online, at-line, and platform offline data to the cloud
- ships retention samples to customer for unique assay analysis
- ships harvests to customers for downstream processing if needed

To demonstrate the scalability and reproducibility of Culture Biosciences' bioreactors, two CHO cell lines from one customer were grown and evaluated. In the first phase, reproducibility of Culture's cloud bioreactor was demonstrated alongside internal development of automation and streamlining operations. In the second phase, scalability was demonstrated by successful scale-down of the customer's 1L glass benchtop bioreactor to Culture's cloud system.

METHODS

EQUIPMENT

Bioreactors

Culture Biosciences' 250 mL cloud bioreactor is suitable for both cell culture and fermentation applications.[3] During development of mammalian cell culture capability at Culture Biosciences:

- four total mass flow controllers (MFC) were incorporated to enable independent control of air, oxygen, and carbon dioxide flow through the sparge line, as well as air flow to the bioreactor headspace
- gas lines betweens MFCs and reactors were plumbed to minimize deadlegs for faster control response times
- a minimum air sparge of 1 sccm was implemented to enable smooth delivery of oxygen and/or carbon dioxide and protect the sparge line from backflow
- a PID control strategy was developed to enable control of top (carbon dioxide) and bottom (liquid base) pH deadbands
- a PID control strategy was developed to enable control of dissolved oxygen

Customer data were generated in 1L glass Sartorius Biostat Qplus benchtop bioreactors.

Analytical Instrumentation

Viable cell density and cell viability were measured daily on a Beckman Coulter Vi-CELL BLU instrument. Customer data were collected on a Beckman Coulter Vi-CELL XR instrument. A thorough assessment of image analysis parameters was performed to ensure comparable cell counts.

Offline pH, gases, metabolites, chemistry, and osmolality were measured daily on a BioProfile® FLEX2™ instrument. Customer data were collected on the same type of instrument.

IgG titer samples were collected on selected days and analyzed in real time on a Cedex BioHT system. Customer data for cell line A were collected by Protein A HPLC analysis. Customer data for cell line B were generated on a ForteBio Octet instrument. While cell line B does not produce a standard monoclonal antibody, the product contains the structural motif required for Cedex BioHT IgG assay compatibility, and relative titer between runs could be assessed at Culture Biosciences in real time.

CELL CULTURE

Cell Lines and Seed Trains

CHO cell lines A and B were thawed from cryovials and carried at 37°C in shake flasks in an INFORS HT Multitron humidified shaker with a 5% carbon dioxide atmosphere. Thaw, alternating 3-day and 4-day passaging, and scale-up used a single proprietary seed train maintenance media provided in liquid form by the customer. Multiple thaws were carried out to control cell age range at inoculation, which ranged from 13-20 days for all experiments.

Cell Culture Fed-Batch Production Experiments

Cell lines A and B were evaluated in two processes, with different goals. Process 1 was the first experience with this customer's process at Culture Biosciences, providing an opportunity to evaluate reproducibility, identify any gaps in the system, and further tune PID control of oxygen and carbon dioxide sparging.

Process 2 was updated to a revised version of the customer's process, utilized updated automation and streamlined operations, and demonstrated scalability from their 1L glass benchtop system to Culture Biosciences' 250mL cloud bioreactor. Additionally, trend comparison of Process 1 vs. Process 2 was a chance to observe the impact of process variables on cell culture performance in 250mL cloud bioreactor.

Table 1 describes how each of the two cell culture processes was executed. Within each process, cell lines A and B differed by pH target before the 24-hour pH shift (Table 1). Process 1 and Process 2 differed by seed train duration preceding production inoculation (3 vs. 4 days), and Process 2 incorporated other improvements related to consistency and control.

The CHO cell culture processes were 14-day fed-batch cell cultures, with nutrient feeds occurring every other day after a trigger of growth and/or duration. If the concentration of glucose fell below or was approaching 2 g/L after daily sampling, the culture was supplemented with 45% glucose to a target of 6 g/L in culture. pH was controlled by addition of sodium carbonate (Na_2CO_3) or sparging of carbon dioxide. Online pH was compared daily to an offline reading, and corrected to the offline value if the difference was >0.03 pH units. Dissolved oxygen was maintained at 30% of air saturation by variable sparging of pure oxygen, with a constant minimum air sparge of 1 sccm. Temperature was maintained at 37°C throughout. Antifoam was added to control foam buildup as required. All media and chemical reagents were supplied by the customer, and were nominally identical to those used in the customer's 1L glass benchtop bioreactor.

		Process 1		Process 2	
		A	B	A	B
Parameters	Seed Train Duration Preceding Production Inoculation	3 days		4 days	
	Initial pH	7.3	7.2	7.3	7.2
	Initial pH Deadband	+/- 0.03			
	pH Shift Time	24 hours			
	Post-Shift pH	6.9			
	Post-Shift pH Deadband	+/- 0.05			
	Initial Volume (V_i)	200 mL			
	Final Volume (V_f)	250 mL*			
	Initial Feed Trigger	2 x 106 viable cells/mL or day 3			
	Feed Volume	5% V_f			
	Feed Timing	every other day upon and after feed trigger			
	Glucose Feed Criteria	If [glucose] < 2 g/L after daily sample, feed up to 6 g/L in culture			
	Dissolved Oxygen Setpoint	30%			
	Temperature Setpoint	37°C			
	Seed Density	0.5 x 106 viable cells/mL			
	Run Duration	14 days			

Table 1. Process parameters summary. Process 1 was used to initially evaluate reproducibility in Culture's cloud bioreactor. Process 2 was used to evaluate scalability from the customer's 1L glass benchtop bioreactor to Culture's cloud bioreactor, and was performed similarly at each site.

*V_f approached steadily over run duration by managing sample/feed volumes.

Four independent experiments were run according to Process 1 and Process 2, with system improvements implemented along the way (Table 2). Cell line A was not included in the first Process 2 experiment.

While the only process parameter changed between Process 1 and Process 2 was the duration of the seed train passage before inoculation, various technical improvements were incorporated with each experiment.

Process	Inoculating ST Passage Duration	Replicates for each Cell Line	Programed shifts	Improvements from Previous Process
1.1	3 days	A: 2 B: 2	--	--
1.2	3 days	A: 2 B: 1	--	PID tuning
2.1	4 days	A: -- B: 1	--	PID tuning, Streamlined inoculation
2.2	4 days	A: 4 B: 2	pH shift	Streamlined inoculation, Task scheduling

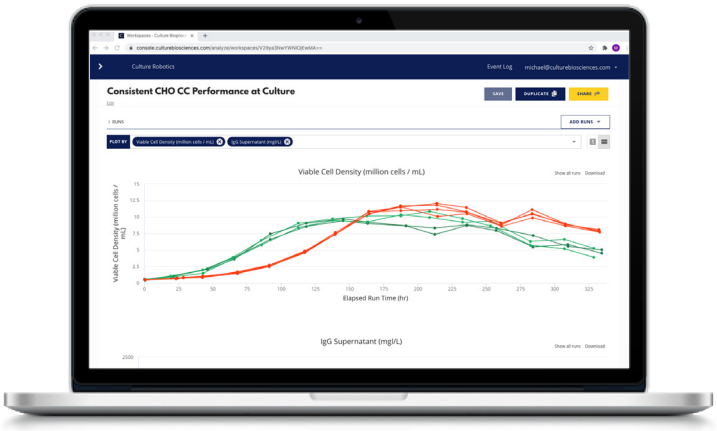
Table 2. Process version details

DATA VISUALIZATION

Culture Biosciences' Website

Time-series graphs were generated through the Culture Biosciences Console, the customer website for accessing and analyzing process data.

The Console visualization suite enables time-series overlay of run data within and across experiments, color customization, grouping and multi-axis zoom.



RESULTS AND DISCUSSION

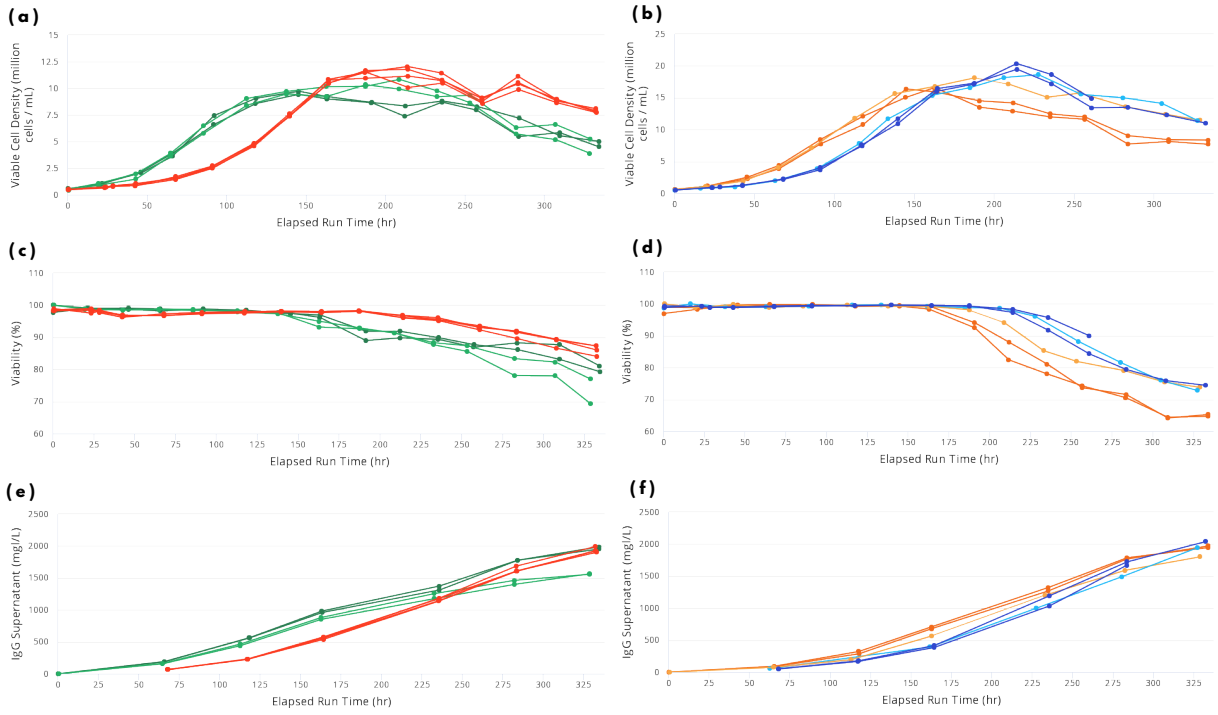
RESULTS INTRODUCTION

Execution of Process 1 and Process 2 demonstrated reproducible outcomes in Culture's cloud bioreactors and successful scale-down from the customer's 1L glass benchtop system. Comparing KPIs, control trends, and process indicators such as metabolites and chemistry between Process 1 and Process 2 showed reproducibility between and within experiments. Comparing results from Process 2 in Culture's cloud bioreactors to the customer's 1L glass bioreactors demonstrated scale-down from a typical benchtop system.

REPRODUCIBILITY

Key Process Indicators (KPIs): Growth, Viability, and Titer

Within each of Process 1 and Process 2, tight clustering of viable cell density (VCD), viability, and titer was observed over the run duration (Figure 1). The slightly lower titer accumulation observed for cell line A in Process 1.2 is likely due to the late manual pH shift (see Figure 2.c). This could have impacted the process by altering metabolism, raising osmolality due to early base addition, increasing initial nutrient uptake rates leading to an unknown depletion, or some combination. As detailed in Table 2, automated pH shifts were scheduled for exactly 24 hours post-inoculation in Process 2.2.



Cell Line	Process	Inoculating ST
A	1.1	3
A	1.2	3
A	2.2	3

Cell Line	Process	Inoculating ST
B	1.1	3
B	1.2	3
B	2.1	4
B	2.2	4

Figure 1. VCD, Viability, and Titer trends from cell lines A and B evaluated in Process 1 and 2

Control: pH, pCO_2, Dissolved Oxygen, and pO_2

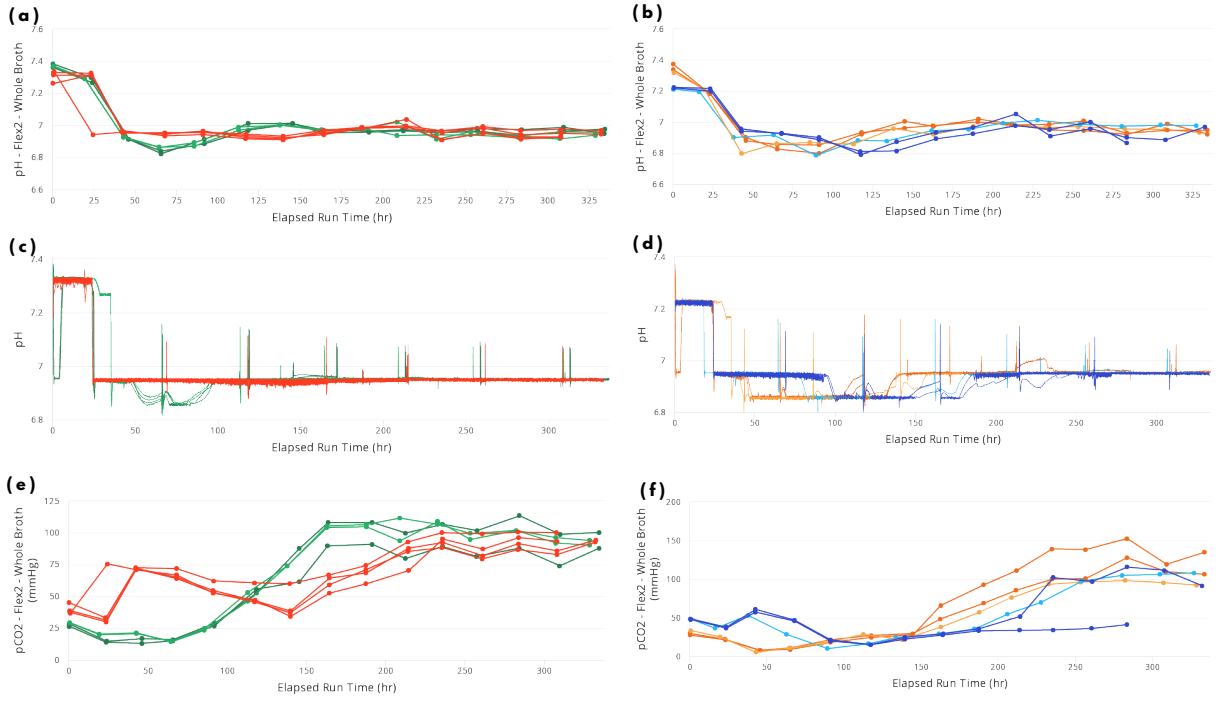
Within each of Process 1 and Process 2, tight clustering of pH control, gas partial pressures, and online control trends was observed over the run duration (Figure 2).

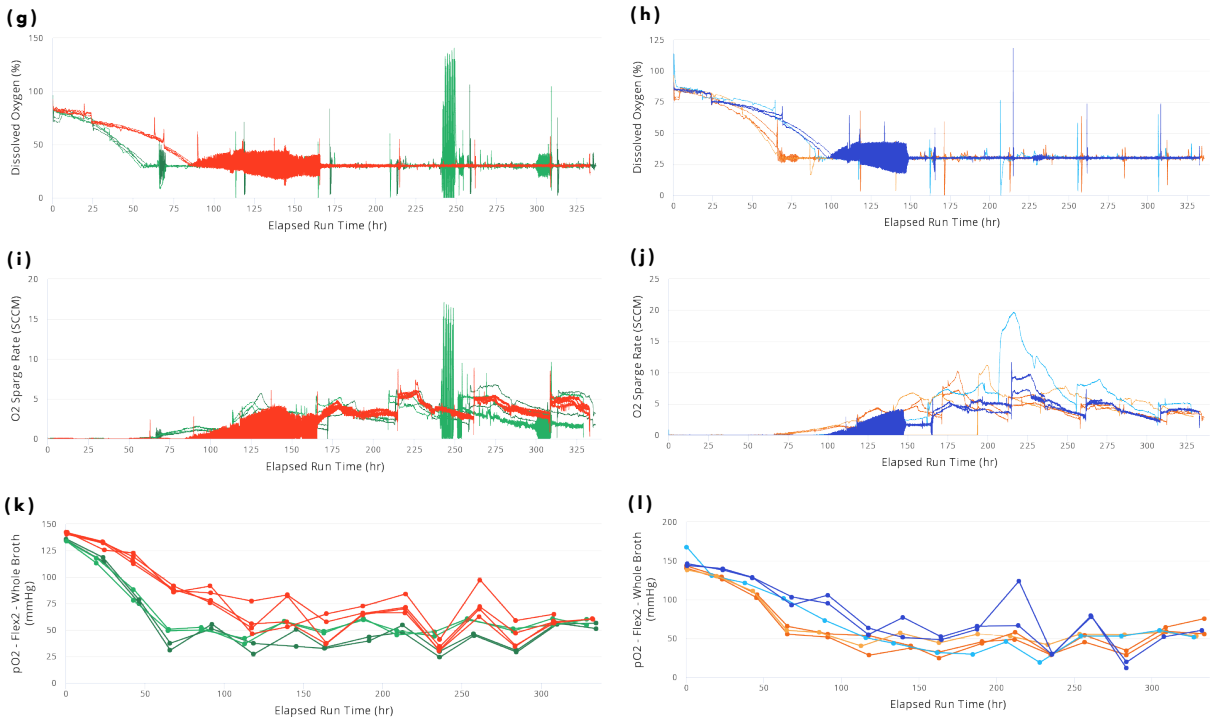
pH Control. pH was controlled within the defined deadbands (see Table 1), and deadband noise was limited to approximately 0.02 pH units for the majority of the runs. As detailed in Table 2, automated pH shifts were scheduled for exactly 24 hours post-inoculation in Process 2.2, which improved consistency of execution. pH trends were consistent within each process. The nutrient feed caused an upward pH spike of about 0.2 pH units upon each bolus addition, and the system returned the culture to within the deadbands in 10-12 minutes, and completely stabilized in 25-30 minutes.

Gas Partial Pressures. pCO_2 and pO_2 trended similarly within each process. The pCO_2 trends reflected time spent controlled at the top deadband.

One replicate of cell line B in Process 2.2 initially appeared to behave differently, but in fact is within the range of customer data (Figure 5.f). The loss of data for this run after day 12 was due to an unrelated software issue that has since been addressed.

Dissolved Oxygen (DO) Control. Dissolved oxygen was maintained at target throughout the runs and reached setpoint in a consistent manner within cell line and process. Process 1 served as an opportunity to better tune oxygen sparge PID settings (see noise in Figure 2.g). Before tuning, increased carbon dioxide sparge rates would strip oxygen when the pH controller responded to the upward pH spike caused by bolus addition of the nutrient feed. In Process 1, DO could momentarily drop to 0%. After implementing enhanced tuning in Process 2, DO would momentarily drop to as low as 10%, and then recover to setpoint quickly. There was a small leak at the outlet of the oxygen MFC for the cell line B Process 2.1 run, which explained the proportional but elevated oxygen sparge rate (Figure 2.j). This resulted in an update to MFC installation and maintenance SOPs.





Cell Line	Process	Inoculating ST
A	1.1	3
A	1.2	3
A	2.2	3

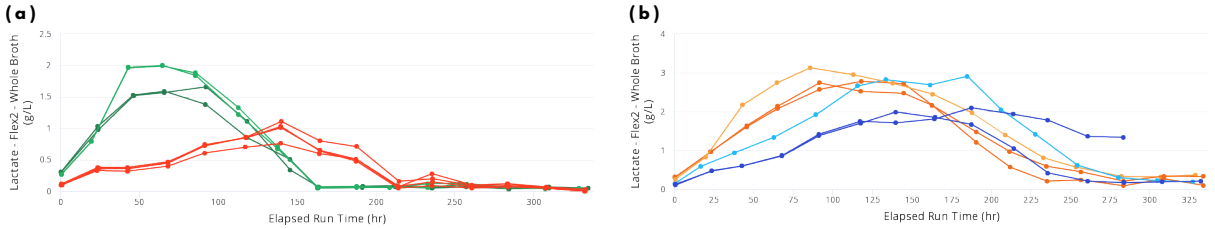
Cell Line	Process	Inoculating ST
B	1.1	3
B	1.2	3
B	2.1	4
B	2.2	4

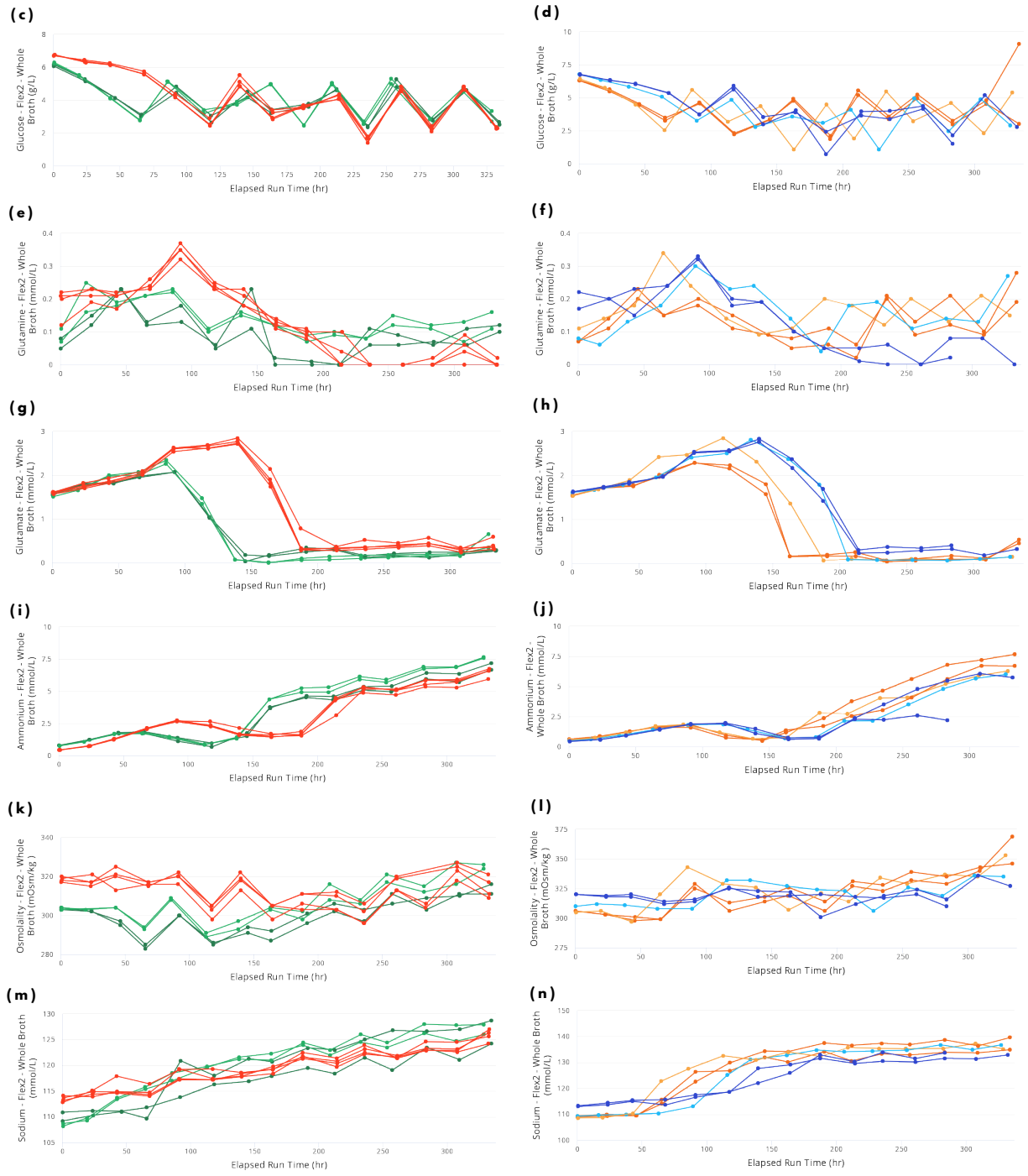
Figure 2. Offline pH, Online pH, pCO_2, DO, O_2 Sparge Rate, and pO_2 trends from cell lines A and B evaluated in Process 1 and 2

Other Process Indicators: Metabolites and Chemistry

Within each of Process 1 and Process 2, tight clustering of all measured metabolites, ions, and osmolality was observed over the run duration (Figure 3). Of particular note, lactate profiles were consistent within each cell line and process (Figure 3.a-b), and glucose levels were well maintained throughout.

Cell line B reached a slightly higher peak lactate concentration in Process 2.1 (Figure 3.b), possibly related to an effect of the earlier manual pH shift on metabolism (Figure 2.d). Cell line B showed split lactate behavior (Figure 3.b) in Process 2, which was consistent with customer experience (Figure 5.d).





Cell Line	Process	Inoculating ST
A	1.1	3
A	1.2	3
A	2.2	3

Cell Line	Process	Inoculating ST
B	1.1	3
B	1.2	3
B	2.1	4
B	2.2	4

Figure 3. Lactate, Glucose, Glutamine, Glutamate, Ammonium, Osmolality, and Sodium trends from cell lines A and B evaluated in Process 1 and 2

SCALABILITY

Customer data were generated in 1L glass benchtop bioreactors running Process 2 (Table 1). While both systems targeted the same parameters, pH trended high when controlled at the top deadband in the 1L glass benchtop system (Table 3). The 250mL cloud bioreactors at Culture Biosciences maintained pH within the prescribed limits. The impacts of this — all slight — were higher initial growth rate, higher integrated cell density, higher yet overlapping titer, and higher or early initial lactate production observed in the 1L benchtop bioreactor. Within this context, moreover, the general result was that **compared to their respective customer data, cell lines A and B grew similarly whether cultured in Culture Biosciences' 250mL cloud bioreactor or the customer's 1L glass benchtop bioreactor (Figures 4-6).**

Cell Line	Process	Replicates	Pre-Shift pH Control	Post-shift pH Control
A	Cloud 2.2	4	Achieved 7.3 target	Within deadbands throughout
	benchtop A.1	4	pH high near 7.4	Drifted high in second half of run
	benchtop A.2	1	pH low near 7.0	Drifted high in second half of run
B	Cloud 2.1 & 2.2	1 & 2	Achieved 7.2 target	Within deadbands throughout
	benchtop B.1	2	pH high near 7.3	Drifted high in second half of run

Table 3. Summary of subtle pH-control differences between cell culture executed in Culture Biosciences' 250mL cloud bioreactors and the customer's 1L glass benchtop bioreactors, despite sharing identical target conditions

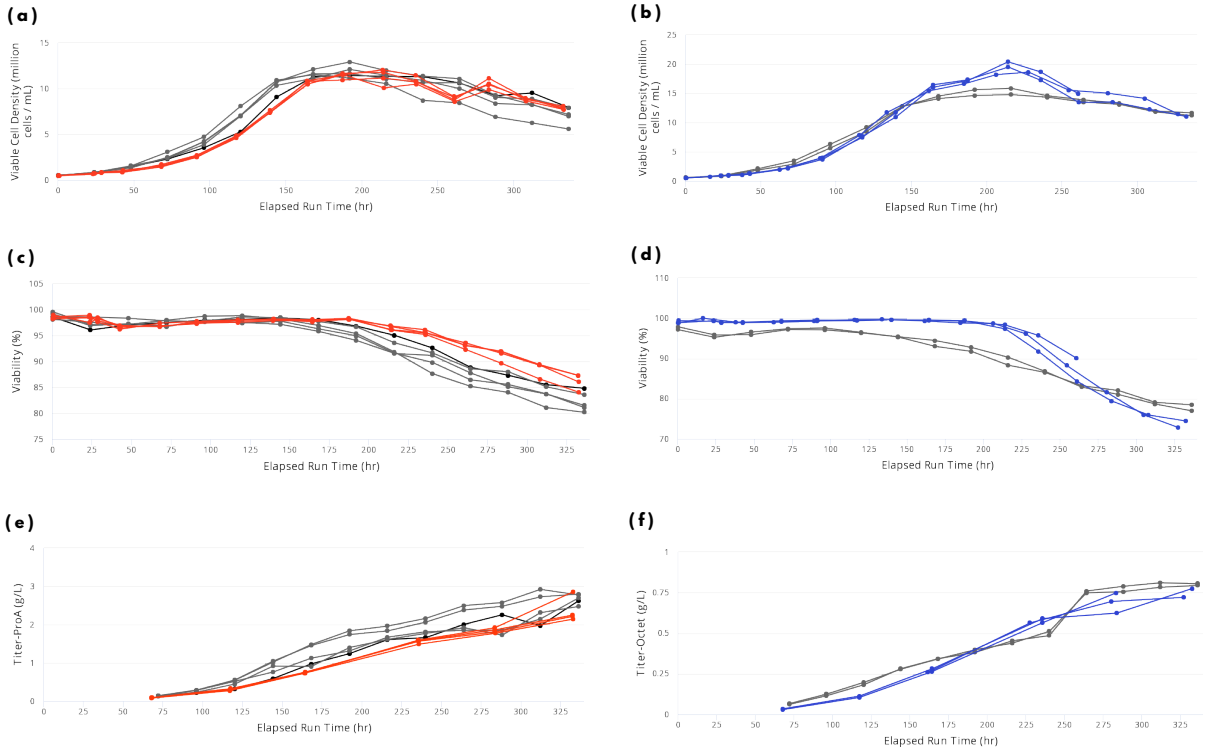
One replicate of cell line A data ("benchtop A.2") was run at a different initial pH setpoint, and is included as an example of the potential range performance of that line.

Key Process Indicators (KPIs): Growth, Viability, and Titer

Compared to their respective customer data, cell lines A and B grew similarly and were similarly productive whether cultured in Culture Biosciences' 250mL cloud bioreactors or the customer's 1L glass benchtop bioreactors.

VCDs were comparable, especially given the slightly higher initial pH in the benchtop bioreactor (Figure 4.a-b).

Viability was higher for more of the culture duration in the cloud bioreactor, but followed the benchtop trends (Figure 4.c-d). Titer was similar between benchtop and cloud bioreactor scales (Figure 4.e-f). Consistent with the VCD trend related to initial pH offsets, cell line A titer generated in the cloud bioreactor fell in the lower range of the customer benchtop data (Figure 4.e).



Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
A	Cloud 2.2	Achieved 7.3 target	Within deadbands throughout
A	Benchtop A-1	pH high 7.4	Drifted high in second half of run
A	Benchtop A-2	pH low 7.0	Drifted high in second half of run

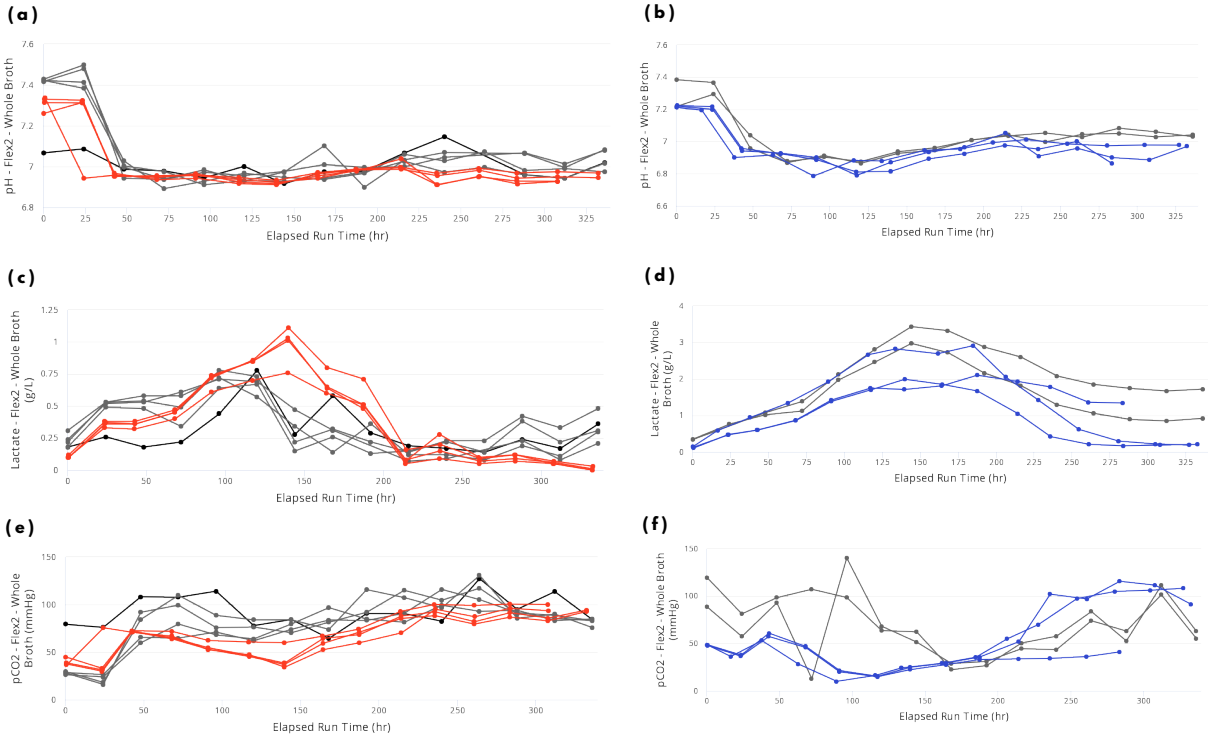
Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
B	Cloud 2.1 & 2.2	Achieved 7.2 target	Within deadbands throughout
B	Benchtop B-1	pH high 7.3	Drifted high in second half of run

Figure 4. VCD, Viability, and Titer trends from cell lines A and B evaluated in Process 2 in either Culture Biosciences' 250mL cloud bioreactor or the customer's 1L glass benchtop bioreactor

Control: pH, Lactate, and pCO_2

pH and gas partial pressures trended similarly for culture grown in Culture Biosciences' 250mL cloud bioreactors compared to the customer's 1L glass benchtop bioreactors. As a response to the initial pH differences (Figure 5.a-b), lactate accumulation was slightly delayed (Figure 5.c) or lessened (Figure 5.d), depending on cell line characteristics.

Despite likely over-sparging of carbon dioxide in the benchtop system as it attempted to achieve setpoint, pCO2 trended similarly to the cloud bioreactor, if only slightly higher early in the run (Figure 5.e-f). pCO2 aligned between scales in the second half of the runs, suggesting similar gas stripping kinetics between systems.



Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
A	Cloud 2.2	Achieved 7.3 target	Within deadbands throughout
A	Benchtop A-1	pH high 7.4	Drifted high in second half of run
A	Benchtop A-2	pH low 7.0	Drifted high in second half of run

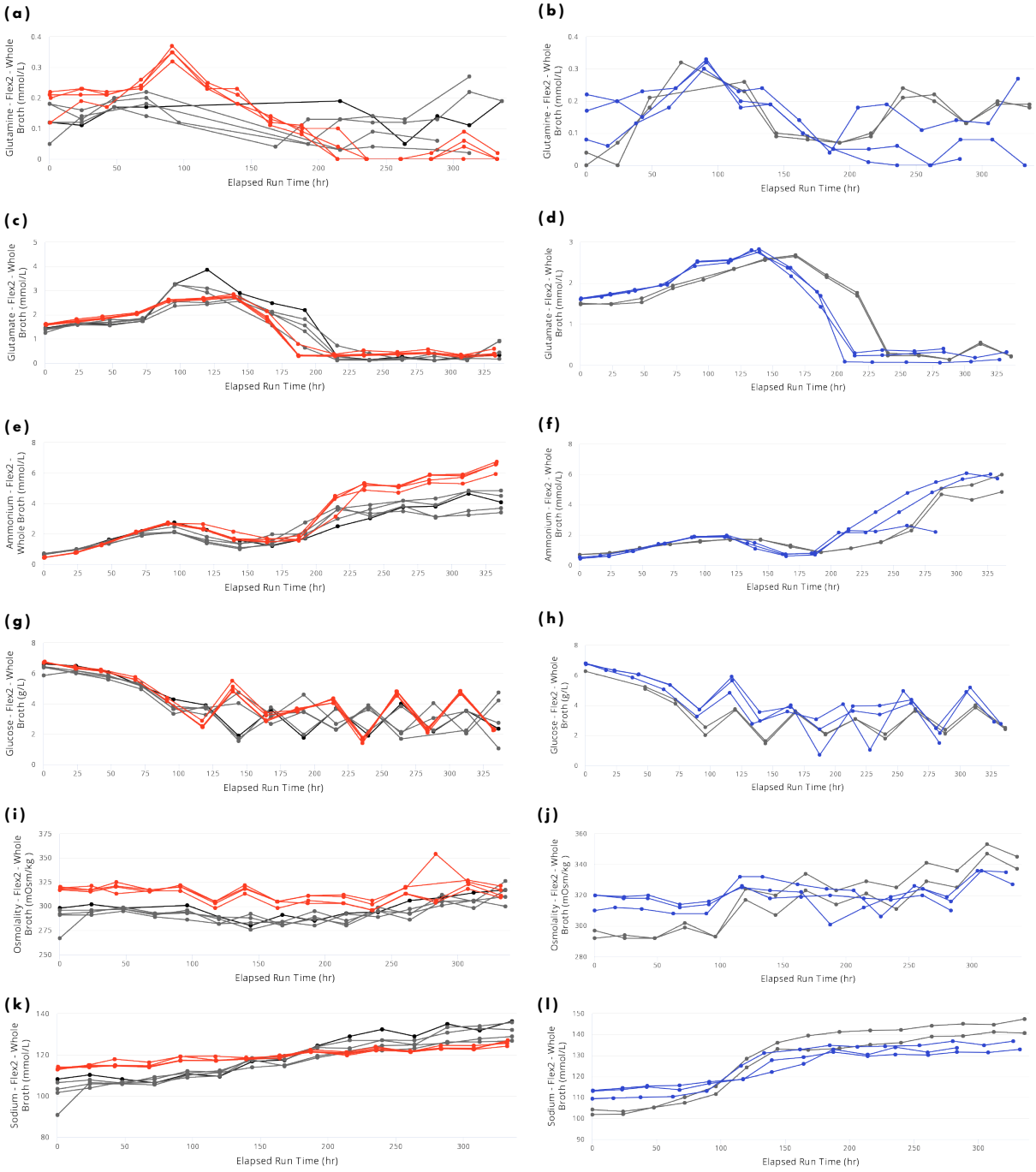
Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
B	Cloud 2.1 & 2.2	Achieved 7.2 target	Within deadbands throughout
B	Benchtop B-1	pH high 7.3	Drifted high in second half of run

Figure 5. Offline pH, Lactate, and pCO_2 trends from cell lines A and B evaluated in Process 2 in either Culture Biosciences' 250mL cloud bioreactor or the customer's 1L glass benchtop bioreactor

Additional Process Indicators: Metabolites and Chemistry

Measurements of offline metabolites, ions, and osmolality for cultures grown in Culture Biosciences' 250mL cloud bioreactors were similar to those for cultures grown in the customer's 1L glass benchtop bioreactors.

Given that media were delivered ready-to-use, analytical equipment was identical across sites, and split ratios from seed train were aligned; any day-zero differences in chemistry or metabolites were a reflection of analytical instrument or off-site media preparation variability.



Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
A	Cloud 2.2	Achieved 7.3 target	Within deadbands throughout
A	Benchtop A-1	pH high 7.4	Drifted high in second half of run
A	Benchtop A-2	pH low 7.0	Drifted high in second half of run

Cell Line	Process	Pre-Shift pH Control	Pre-Shift pH Control
B	Cloud 2.1 & 2.2	Achieved 7.2 target	Within deadbands throughout
B	Benchtop B-1	pH high 7.3	Drifted high in second half of run

Figure 6. Glutamine, Glutamate, Ammonium, Glucose, Osmolality, and Sodium trends from cell lines A and B evaluated in Process 2 in either Culture Biosciences' 250mL cloud bioreactor or the customer's 1L glass benchtop bioreactor

CONCLUSION

Culture Biosciences' high-throughput bioreactor infrastructure can reproducibly execute CHO cell culture experiments with scalable results. The data presented here should give confidence to upstream bioprocess engineers that Culture's Cloud Lab can be an effective resource for generating high quality data to develop, optimize, and scale their cell culture processes.

With access to more bioreactor capacity than ever before and advanced analytical software to generate insights faster, teams working with Culture Biosciences can spend more of their time focusing on what matters most: delivering quality therapeutics to the patients who need them.

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