Culture

GROWTH AND SITE TO SITE COMPARISON OF A HEK FED-BATCH PROCESS IN STRATYX™ 250

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ABSTRACT:

INTRODUCTION:

As new bioprocessing equipment is developed and tested, ensuring consistency across multiple sites is critical. Demonstrating seamless reproducibility across locations is essential for building trust in new technologies and ensuring their adoption. To assess how well a new platform performed in different environments, we conducted a multi-site evaluation to compare its reliability and consistency.

Here we evaluated the Stratyx™ 250 bioreactor platform at three different sites: Culture Biosciences (CB), Bioprocess Site 1, and Bioprocess Site 2, where Sites 1 and 2 were independent, non-CB affiliated bioprocess development sites. To assess the robustness of the bioreactors during early development testing, the same process was run at all three locations, each of which had different operators, different in-process analytical equipment and different standard operating procedures. Additionally, to increase the rigor of this assessment, we elected to run a fed-batch process using human embryonic kidney (HEK) cells, which are sensitive to minor changes in protocol and equipment and are therefore reliable indicators of system robustness. In-process analytics such as viable cell density, cell viability, and lactate production were used to compare data across sites. This work demonstrates that our Stratyx 250 bioreactor platform is capable of replicating cell growth and other critical process parameter data across sites.

MATERIALS & METHODS

VIAL THAW AND PASSAGING

Vials from a Research Cell Bank (HEK293 VPC 2.0 cell line sourced from ThermoFisher,

A49784) were thawed by submerging the vial in a 37 °C water bath with the water level below the cap. Vial contents (~1mL) were transferred to 9mL of pre-warmed thaw medium (Dynamis + 6mM L-Gln) and mixed by slowly pipetting up and down with a serological pipette. The resuspended cell solution (~10mL) was then transferred to 20mL of additional thaw media in a 125mL non-baffled shake flask.

Seed train passages performed after thaw targeted an initial viable cell density (VCD) and a working volume as described previously. Commonly, we follow a 3-4-3 passaging routine targeting VCDs of $0.3x10^6$ cells/mL over a total seed train length of 21 days.

MEDIA

Thaw media (Dynamis + 6mM L-Gln)
Seed train & batch/production media (Dynamis + 6mM L-Gln + 1%ACA)
Nutrient Feed (CD EfficientFeed C AGT Nutrient Supplement)
Glucose feed (360 g/L)
Nutrient feed (EfficientFeed C+ 2X supplement)
Antifoam (FoamAway)

BIOREACTOR SET UP AND PROCESS PARAMETERS

Bioreactors were set up as follows: one day prior to inoculation, media, appropriate feeds and additional solutions were prepared in a biosafety cabinet (BSC). Sterile production media was transferred into each bioreactor vessel using a serological pipette at the defined batch volume of 200mL. Syringe packs were loaded with appropriate fill volumes of nutrient feed, glucose feed, and antifoam as defined in the Experimental Plan and executed on the Autofiller. The filled Vessel and Syringe Pack were connected via the fluid manifold in the BSC, and the filled single use items were removed from the BSC and loaded onto the bays to start the experiment. Bays automatically progressed through reaching the temperature setpoint and completing the DO calibration, and the system was allowed to remain equilibrated overnight prior to inoculation. The next day, bioreactors were inoculated with HEK cells at a VCD of $0.3x10^6$ viable cells/mL. The same process parameters were used across all experiments (Table 1).

These experiments used a sample size of n=6 (CB), n=4 (Site 1) & n=2 (Site 2). The variation in sample size was due to the number of bays available.

TABLE 1: PROCESS PARAMETERS	
Agitation (RPM)	**509**
Sparge Aeration (sccm)	**Nitrogen sparge - 3sccm**
Overlay Aeration (sccm)	**4**
DO Setpoint (%)	**40**
pH Setpoint	**7**
pH Deadband	**+ 0.05**
pH control	**CO2**
Temperature (°C)	**37**
Temperature Shift (°C)	**N/A**
Fill Volume (mL)	**200**
Glucose Feed	**Bolus added on Days 3-13 when concentration was <3g/L to reach a target concentration of 6g/L**
Nutrient Feed	**Added daily on Days 3-13 at a concentration of 1% of the vessel working volume**
Antifoam	**Added as needed**

ANALYTICAL EQUIPMENT

Viable cell density and % viability were assayed on Vi-Cell Blu and Vi-CELL XR (Beckman Coulter) at all sites. Glucose and lactate were assayed on CEDEX (Roche Diagnostics Corporation) at Culture Biosciences and Bioprocess Site 1, and on a YSI Analyzer (YSI Life Sciences) at Bioprocess Site 2.

RESULTS

A fed-batch HEK process was performed at Culture Biosciences, Bioprocess Site 1 and Bioprocess Site 2 to evaluate the Stratyx 250 system for robustness and cross-site repeatability. Cultures were grown in reactors for 14 days and sampled daily for cell density, viability, and metabolites. Similar trends are observed for VCD (Figure 1), percent viability (Figure 2), and Lactate (Figure 3) across all sites. After 14 days of culturing, the average VCD of all 12 reactors was 16.10^6 cells/mL with a coefficient of variation (%CV) of 10.8%, with no significant difference detected between sites. Lactate (g/L) curves of the three different experiments across sites demonstrated similar profiles during the lactate shift. Differences in lactate between sites were likely due to the minor differences in starting VCD due to different analytical equipment used at each site.

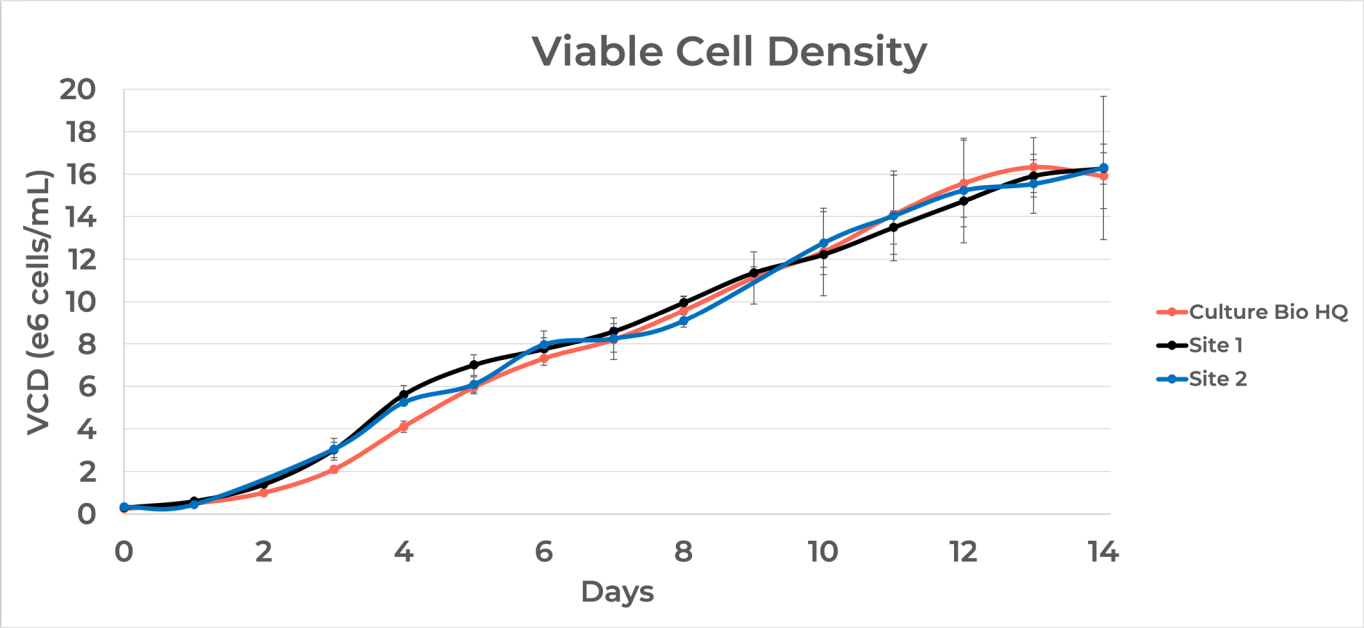


Figure 1: Viable cell density trends were very similar across the three sites (CB in red, Bioprocess Site 1 in black, Bioprocess Site 2 in blue).

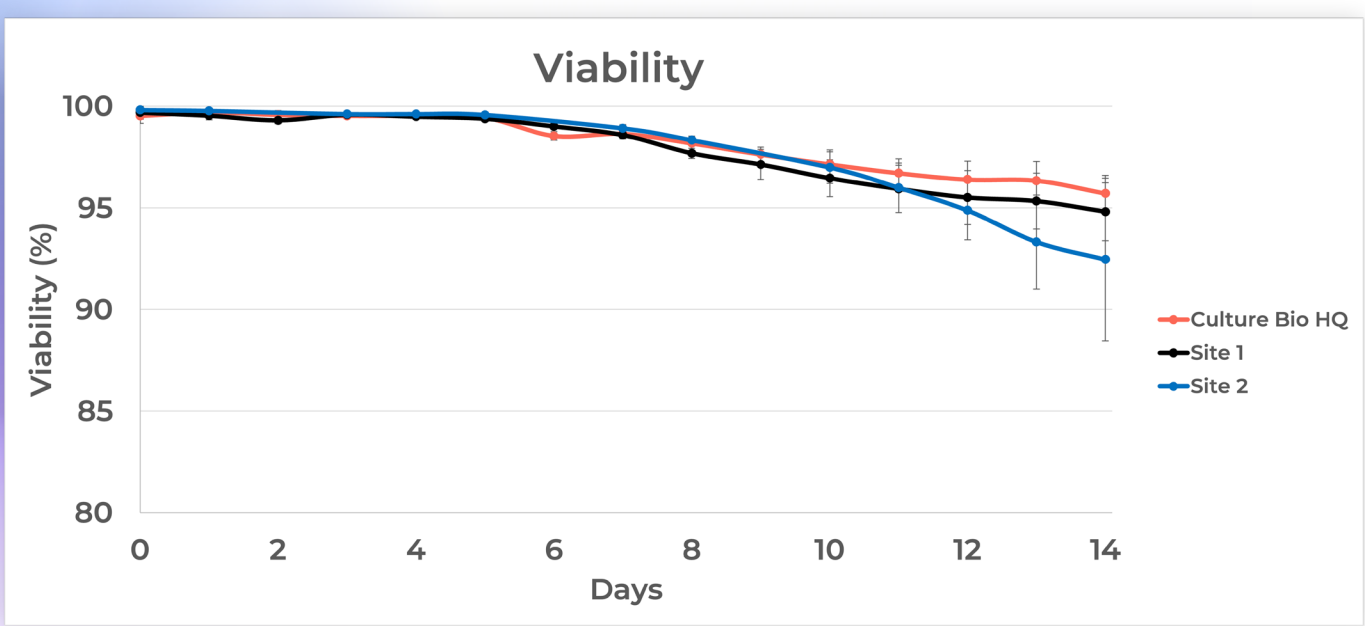


Figure 2: Percent viability trends were similar across all three sites, with values all falling in the mid-90% range on Day 14 (CB in red, Bioprocessing Site 1 in black, Bioprocessing Site 2 in blue).

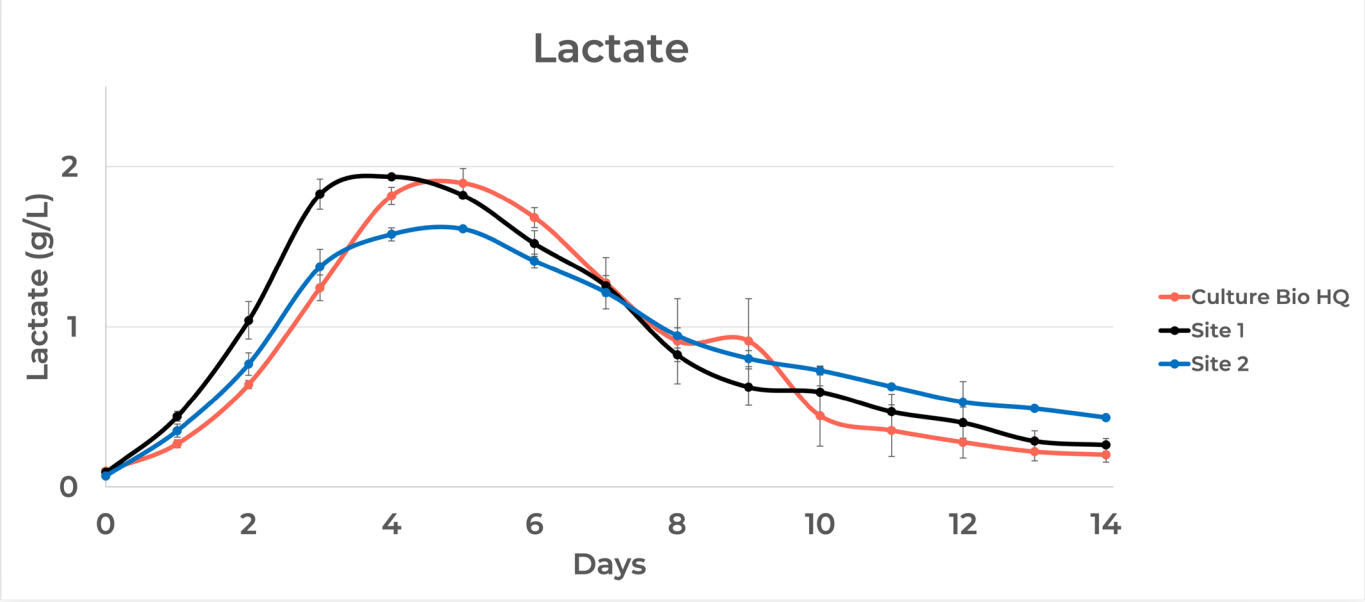


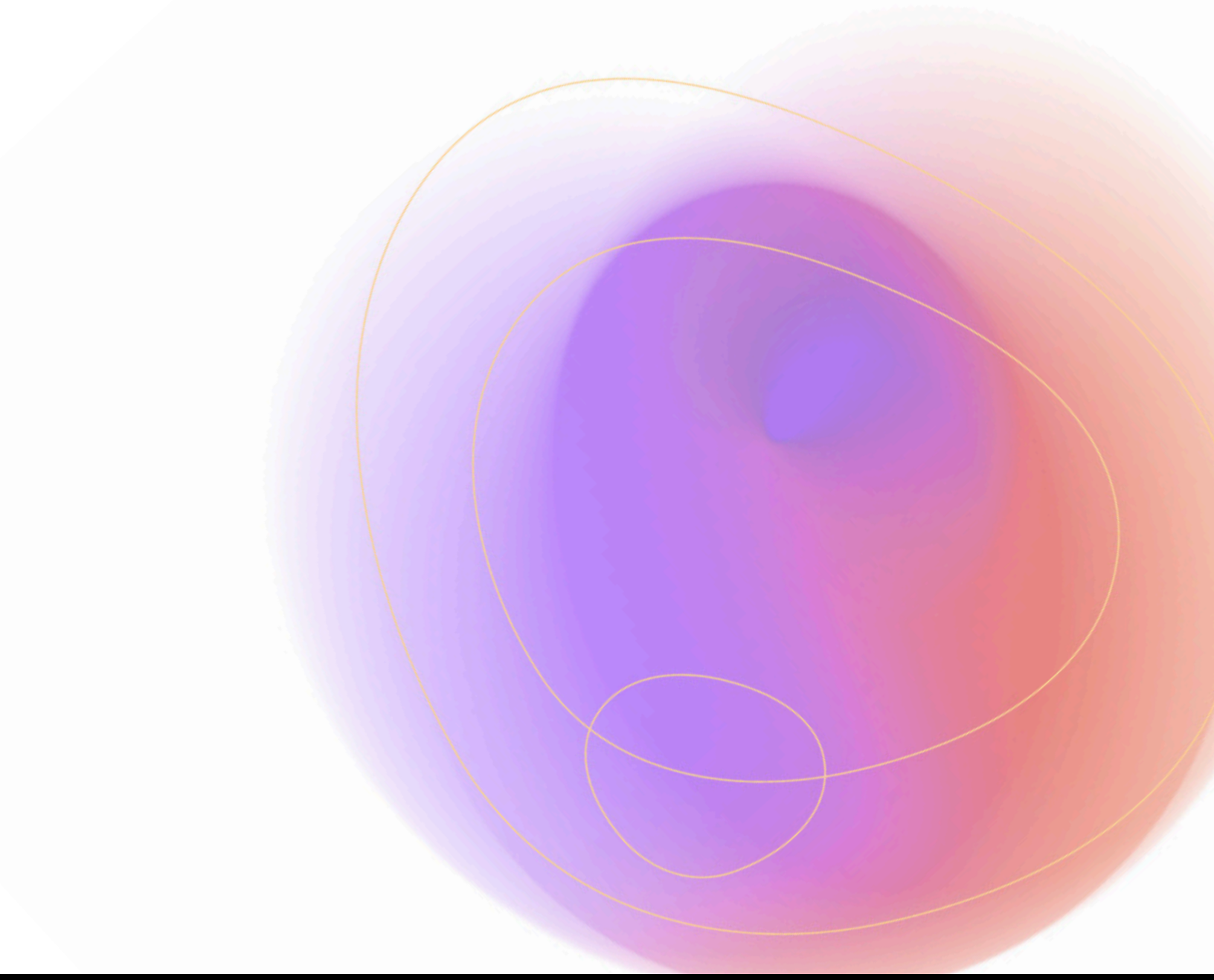
Figure 3: ~~The~~ Lactate (g/L) curves across the three sites demonstrated similar profiles before and during the lactate shift (CB in red (CEDEX), Bioprocess Site 1 in black (CEDEX), Bioprocess Site 2 in blue (YSI)).

CONCLUSIONS

Here we ran the same process on Stratyx 250 bioreactors at three different sites to evaluate site to site reproducibility with a HEK fed-batch process. There is inherent variability within processes, and that variability was further exacerbated by the different analytical tools used across sites. However, while there were some small differences in lactate and viable cell density early on, they did not last the entire experiment. Overall, the HEK fed-batch process performed similarly across all sites, as determined by viable

cell density, cell viability, and lactate production. Furthermore, for all parameters tested, there were no statistically significant differences between the sites, indicating that the system's performance was reproducible.

These results demonstrate that the Stratyx 250 bioreactor platform enables reliable bioprocess development across multiple sites while generating robust and consistent process data. With its user-friendly design, seamless cloud connectivity, and proven reproducibility, the Stratyx 250 provides a dependable solution for bioprocess development at any location.



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