SCALE-UP OF A S. CEREVISAE FERMENTATION FROM 10L BENCH TO 1000L SINGLE USE FOR RECOMBINANT GM-CSF PRODUCTION.

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CSR® Custom Single Run

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Introduction

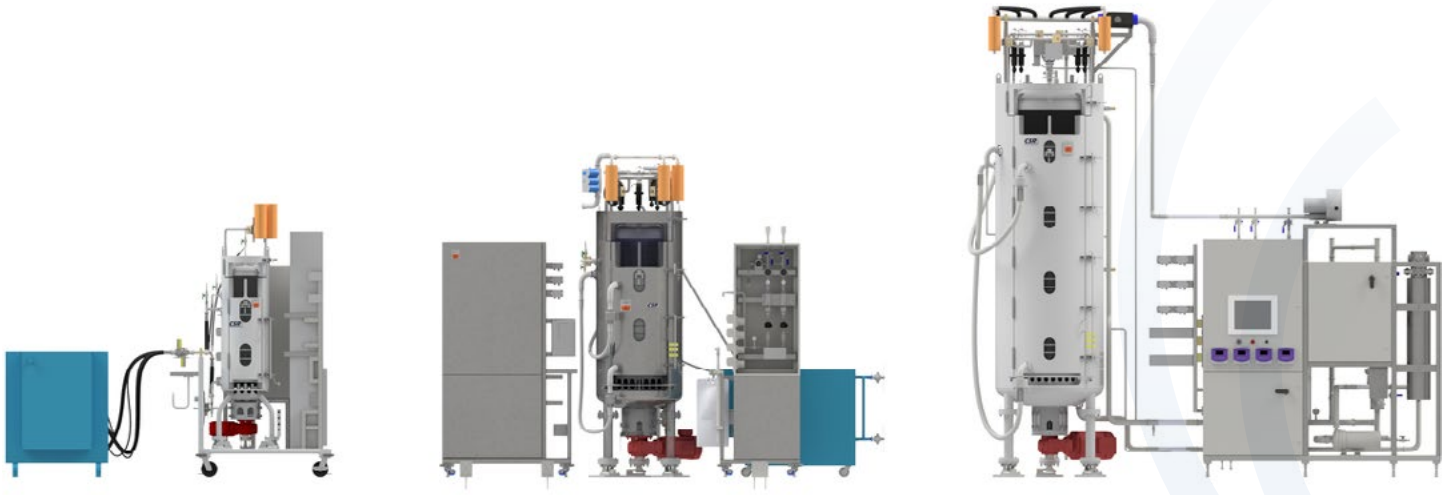
Microbial cultures, such as *E. coli* or yeast cultures, mammalian cells (cell culture), plant cells, or insect cells, can be used to produce biopharmaceuticals (biologics). *Saccharomyces cerevisiae* are used to produce r-hu-GM-CSF (sargramostim) for which current market demand is rising. Demand for Sargramostim may soon exceed manufacturing capacity due to both increased consumption of existing therapeutics and the potential for new indications.

Traditionally, microbial processing has been carried out in stainless steel fermenters due to demanding process requirements. Single Use Technology (SUT) is widely used in biopharmaceutical applications, mostly in mammalian cell platforms, due to its rabid implementation, removal of cleaning processes, and superior flexibility. SUTs have also helped pharmaceutical companies and CDMOs to streamline and intensify their operations. However, in the case of microbial applications, SUT still faces additional challenges in mass transfer, temperature control, and scale limitations. All of these factors have historically delayed the implementation of SUT for microbial applications.

In a conventional expansion strategy, cultured cells are grown and then transferred to increasingly larger vessels until the desired production volume and cell density are reached. Ongoing commercial sargramostim production requires two expansion steps (shake flask and seed fermenter) followed by production fermentation.

Multiple stainless-steel fermenters are utilized for the seed and production fermentation steps. Alternatively, dual-volume expansion can be performed in a single fermenter, eliminating the need for an additional seed fermenter. This improvement can lead to a reduction in CAPEX and OPEX.

In the work presented, we demonstrate the successful expansion of high-density yeast in a dual-volume expansion utilizing ABEC's 1000L Vw single-use Custom Single Run (CSR®) Fermenter. The results obtained at both seed and production scales showed comparable process attributes to the 10L reference culture, demonstrating seamless scale-up and tech transfer performance.



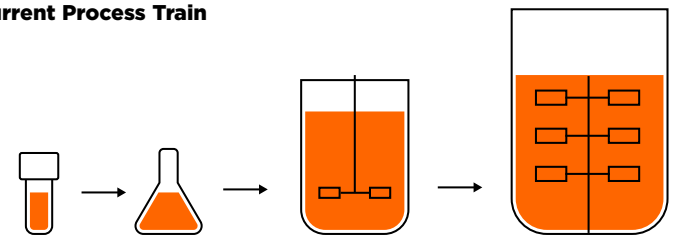
Example microbial scale-up with ABEC's (left to right) 50L, 300L, and 1000L CSR® Fermenters.

Goals

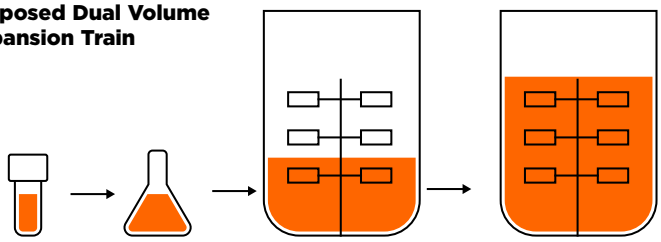
This evaluation was performed with three goals in mind: to demonstrate the scalability of the High-Yield Yeast Process from 10L to 1000L, to determine the feasibility of using a single-use fermenter in place of a stainless-steel fermenter, and finally to evaluate the dual-volume expansion capability of a 1000L ABEC CSR Fermenter, eliminating the need for two vessels in the production train.

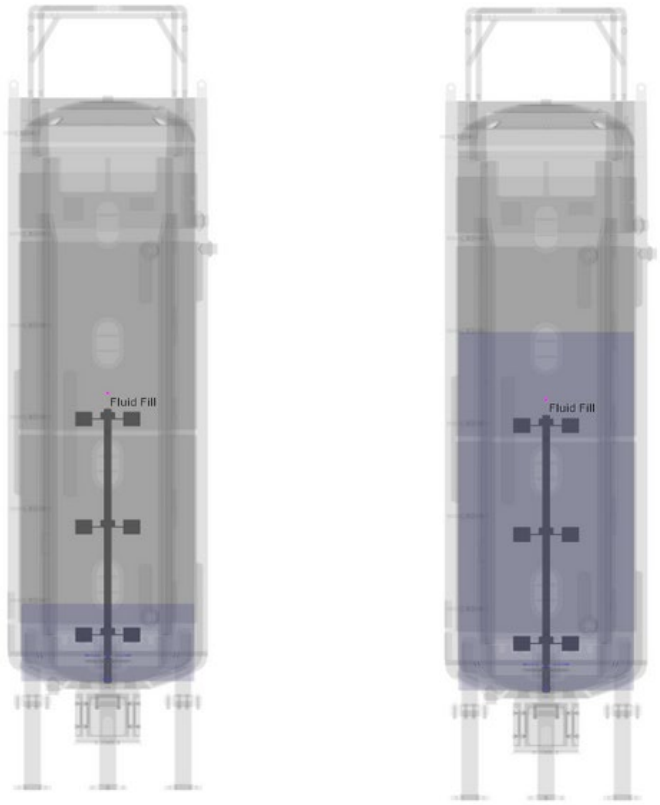
The evaluation of the culture performance and ABEC system performance would successfully establish a “proof of concept” for the suitability of the ABEC system with regards to r-hu-GM-CSF manufacturing.

Current Process Train



Proposed Dual Volume Expansion Train





CFD model of ABEC’s 1000L CSR Fermenter at 200L (left) and 1000L (right) working volume

Materials and Methods

1000L Run was Performed at ABEC Process Sciences Laboratory, Bethlehem, PA

Shake Flask Inoculation: Four 5L Corning Erlenmeyer flasks were seeded under laminar flow hoods with thawed, 1.0mL vials from a working cell bank. Flasks were incubated for 24 hours at 30°C and an agitation speed of 250 RPM. Single use flasks were used in order to maintain sterility when inoculating the seed fermenter. To minimize the split ratio during inoculation, each of the four flasks contained an additional 0.75L of flask media compared to the existing process.

Seed Fermentation: Seed fermentation was performed in the 1000L CSR fermenter, operated at 199L working volume. The CSR fermenter is a fully customizable, single-use fermenter consisting of a holder and a Disposable Container (DC). The DC was designed with three Rushton impellers, a thermowell, dual pH probes, dual DO probes, and pressure control. Impeller spacing was customized for this process so the lowest impeller was submerged for optimal seed performance.

The seed fermentation had a higher split ratio (0.04) than the existing process (0.11) resulting in a lower amount of inoculum per liter. Seed media was compounded using an ABEC 300L single-use General Mixer (GenMix) CSR system. Once compounded, the media was sterile-filtered into the 1000L ABEC CSR. Seed fermentation occurred for 15 hours and samples were taken to determine when the required production seeding density was met.

Production Fermentation: Complex batch media was used and was supplemented with glucose and ethanol feeds. To achieve target seeding density at the production scale, 80L of culture was removed from the fermenter and 881L of fresh media was added. Media was compounded using a 1000L CSR GenMix and sterile filtered into the fermenter. Process conditions were matched from the existing process, with slight modifications made due to equipment differences (e.g. overlay pressure).

Table 1. Process Conditions

	Temperature (°C)	pH	DO (%)	Agitation (RPM)
Set Point	**28.6 → 27.6**	**5.45**	**25**	**Set by DO control loop but capped @ 260**

Data Analysis

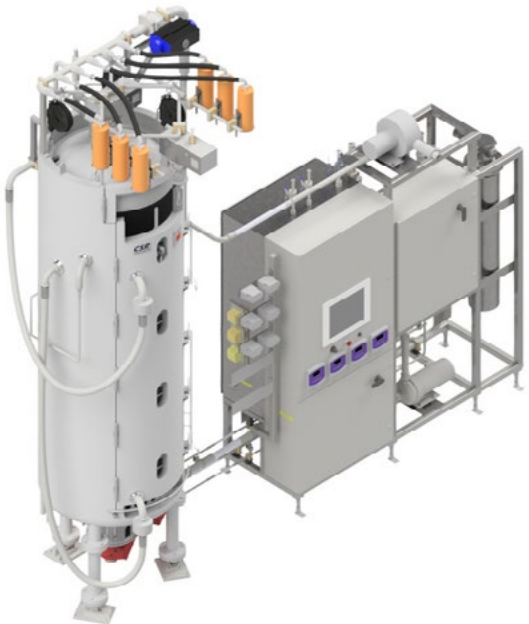
Two main process attributes were taken into account in this evaluation: Wet Cell Weight (WCW) and Optical Density (OD). The main reason for selecting these two process attributes was that any differences in mass transfer and temperature control performance would be reflected in growth rate and cell mass differences. Dissolved Oxygen (DO) profiles were also analyzed as indicators of metabolic flux changes within the cells. Differences between DO trends could indicate changes in the expected carbon source metabolism.

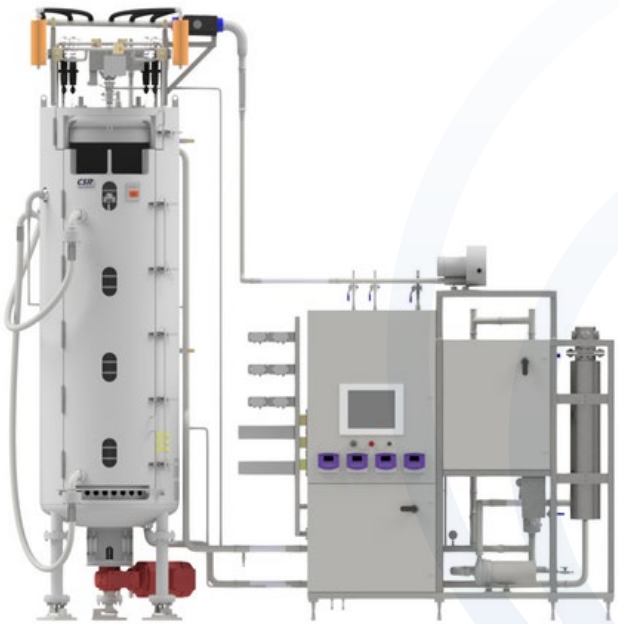
The following analytical methods were used to characterize the data from the scale-up study. Measurements of OD and WCW were used to gain insight into cell growth; used along with profiles of pH, temperature, and dissolved oxygen, a comparison can be made between the 10L and 1000L fermentations.

10L Fermentation data was provided by Partner Therapeutics Inc.

Table 2. Description of Analytical Methods to Characterize Data

Analytical Method	Description
Optical Density (OD)	Culture optical density was measured at 550nm wavelength using a Shimadzu UV-mini 1240V spectrophotometer.
Wet Cell Weight (WCW)	Culture WCW was determined by centrifugation of 1 mL of cell broth in a pre-weighed 1.8 mL centrifuge tube. Supernatant was aspirated off, and the tube was weighed again to calculate the WCW.





ABEC 1000L CSR Fermenter

Results

199L Expansion Stage: The DO profile shown in Figure 1 shows that the small-scale and the ABEC seed fermentation followed the same general metabolic pattern,but the ABEC run had higher DO levels. The temperature profiles in **Figure 2** show that the ABEC system was able to remain close to set-point during the duration of the seed fermentation. The pH profile shown in **Figure 3** shows adequate control since the pH remained within the control range (10L Green, 200L Blue).

Figure 1.

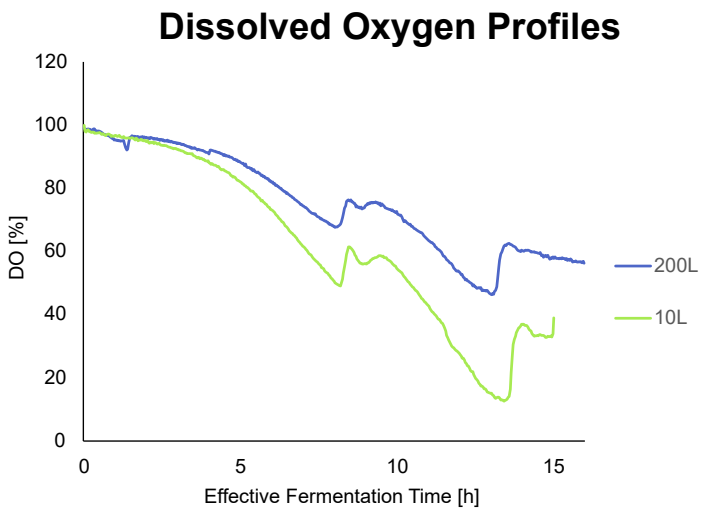


Figure 2.

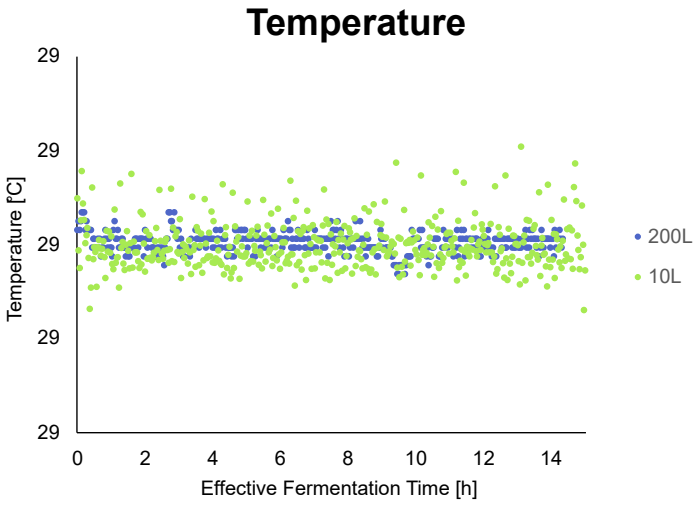
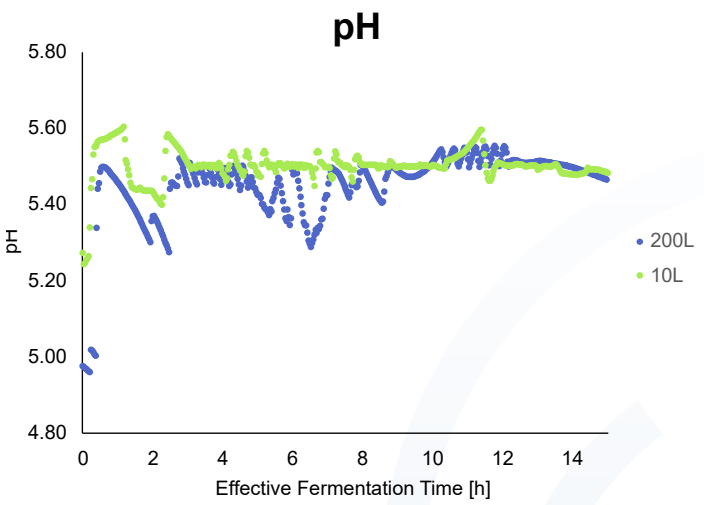


Figure 3.



1000L Production Stage: Biomass data from the ABEC production fermentation had higher values compared to the small-scale production fermentation **(Figures 4, 5).** Profiles for DO were similar between the small-scale and ABEC fermentations **(Figure 6)** (10L Blue, 1000L Orange).

Figure 4.

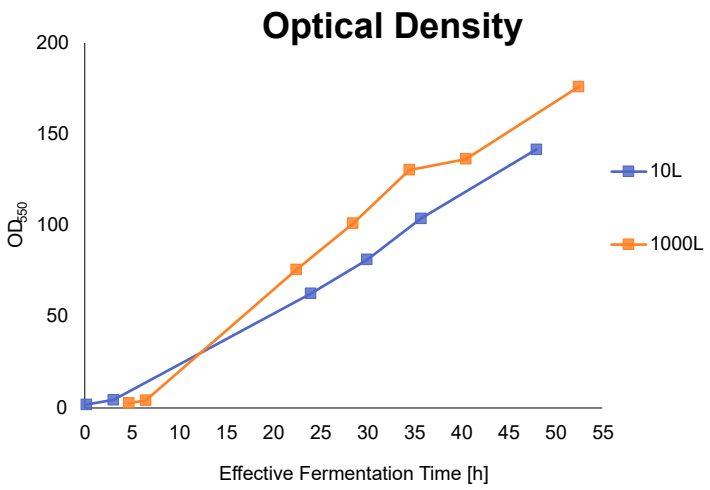


Figure 5.

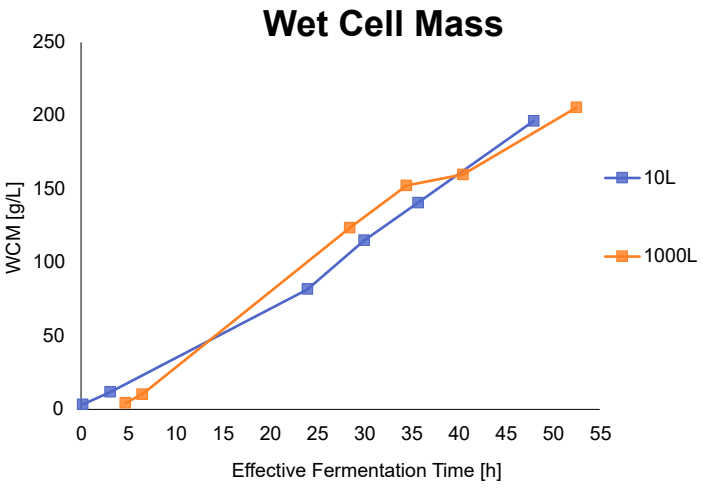
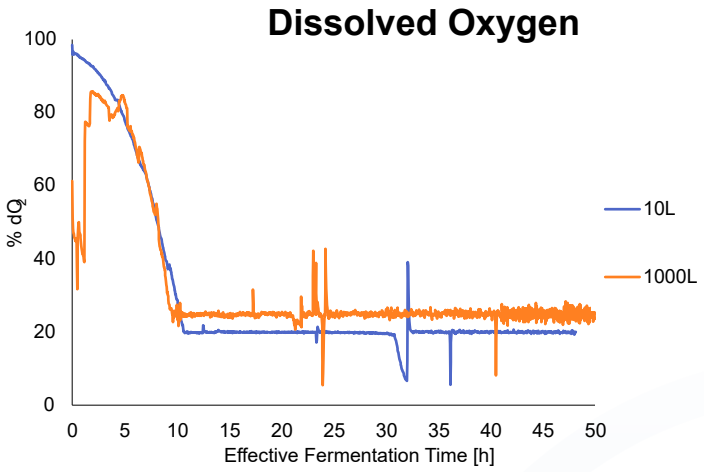


Figure 6.



1000L Production Stage (cont.): Several differences between the small-scale and ABEC fermentation temperature profiles are shown in **Figure 7.** Profiles for pH control show differences between the small scale and ABEC fermentations in **Figure 8.** Difference in agitation profiles **(Figure 9)** can be attributed to unique DO control strategies, culture metabolism and vessel related oxygen transfer capabilities between the small-scale and ABEC fermentations (10L Blue, 1000L Orange).

Figure 7.

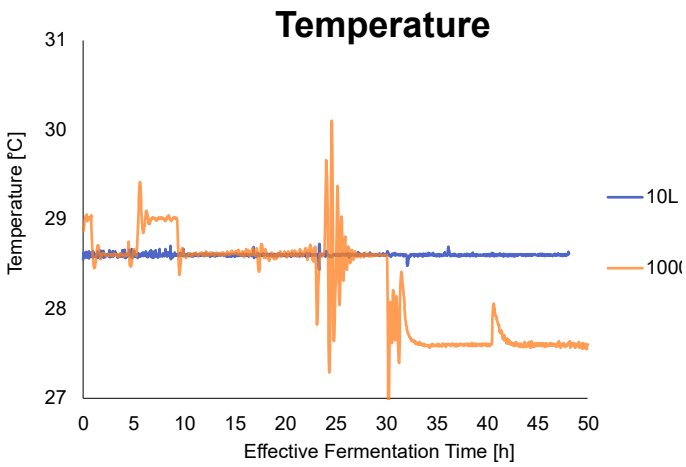


Figure 8.

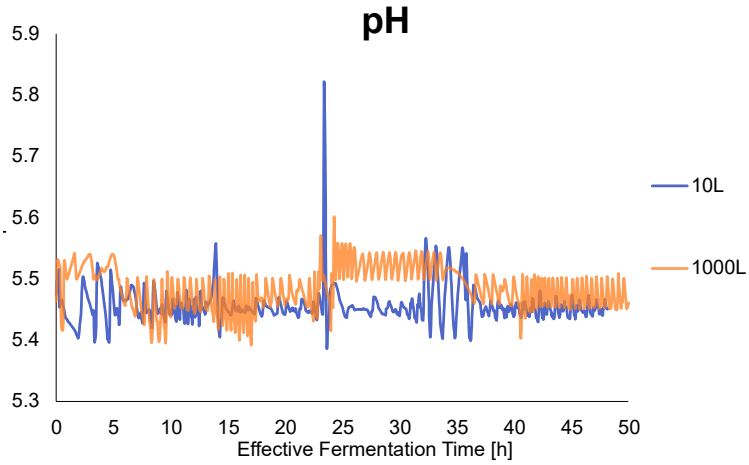


Figure 9.

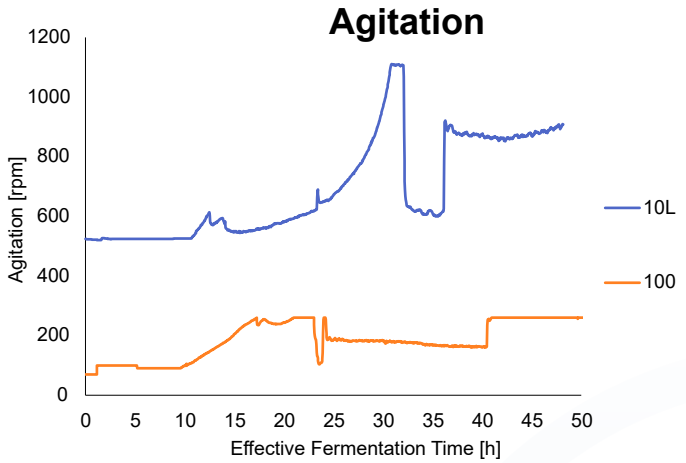


Table 3. Summary of Fermentation Performance Outputs

Run ID	WCM (g/L)	Final OD	Total Process Time (h)
10L	196.3	176	48
1000L	205.5	176	52.5

Discussion

The study encountered a process deviation due to insufficient filtration area and mechanical limitations with the media addition pump. This resulted in the production culture inadvertently being exposed to a glucose-deficient environment and extended the total run time by approximately 4 hours.

Further refinements of process operations, media filtration requirements, and automation tuning can be performed to further improve the reliability of the system and comparability of the 1000L and 10L processes.

Conclusion

The results of this evaluation demonstrate that the ABEC 1000L CSR Fermentation System can support the demands of a high-density *S.cerevisiae* process by meeting the cell density requirements. Scalable performance was demonstrated through a comparison of the growth curves and metabolic shifts (DO trends). Additionally, the potential of the "dual-volume" concept was confirmed with a successful seed and production fermentation performed in the same single-use CSR fermenter system.

In conclusion, the use of large-scale SUFs, custom consumable designs, and dual-volume expansion strategies can improve manufacturing flexibility, reduce turn-around times, reduce validation efforts and reduce CAPEX/OPEX by eliminating CIP, SIP, and intermediate process steps.

References

Evaluation of the ABEC CSR 1000 L Single-Use Fermenter in Sargramostim Seed and Production Fermentation Ireland, J. (2021).

G.Miller., T. Zawistowski. 2011. GM-CSF Production Fermenter Characterization Report.

S. Lillie. 2021. Development of the Sargramostim High-Yield Fermentation Process