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Use of Small-Scale, Single-Use Bioreactors for Streamlining Upstream Process Development

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Single-use processing solutions spanning both upstream and downstream applications are being embraced by the biopharmaceutical industry. The advantages of the single-use approach to industrial cell culture versus traditional stainless steel and/or glass bioreactors has resulted in the recent commercialization of several single-use bioreactors at small (3–15L), intermediate (50–500L) and large scales (>1000L).

Recent innovations are combining the features of conventional bioreactors with the ease-of-use benefits associated with single-use technology for the optimization of mammalian cell growth and recombinant protein expression. This article will provide a detailed characterization of the single-use Mobius® CellReady 3L Bioreactor capabilities, as compared to a glass bioreactor, in terms of CHO cell growth, mixing, and volumetric mass transfer coefficients (k_La) for oxygen.

Introduction

Upstream process development can be effectively streamlined using the Mobius CellReady 3L single-use bioreactor to scale-up inoculum (*i.e.*, seed-train) for large-scale bioreactor experiments. By pooling the cultures from 2–3 small-scale single-use bioreactors, sufficient biomass was readily achieved to inoculate directly into the 200L bioreactor at a 40L working volume. Subsequently, the 3L single-use bioreactor was used to monitor the performance of the 200L large-scale cell culture experiments as a satellite bioreactor. In addition to its use in process characterization and development work, this study demonstrated that the predictable performance of the 3L single-use bioreactor makes it an effective and convenient platform for both seed-train and satellite applications.

Comparison to Glass

In this study, performance of the Mobius CellReady single-use 3L bioreactor was compared to a 3L glass bioreactor. Table 1 summarizes the specifications of both. The k_La of each bioreactor was assessed via the static gassing-out method.

Each experiment was performed with a pH probe installed to simulate the baffling that occurs during a cell culture process. The dissolved oxygen probe was initially calibrated to

TABLE 1. Comparison of 3L single-use and glass bioreactors.

	Mobius CellReady 3L (DBR)	Glass 3L (GBR)
Impeller	• Larger diameter • Marine scoping low shear	• Smaller diameter • Steeper–angled flat blades
Microsparger	• 15 – 30 micron polyethylene • Bottom of vessel	• 15 micron sintered steel • Centered below the impeller
Open Pipe Sparger	• Single–hole • Bottom of vessel	• 7–hole L sparger • Centered below the impeller

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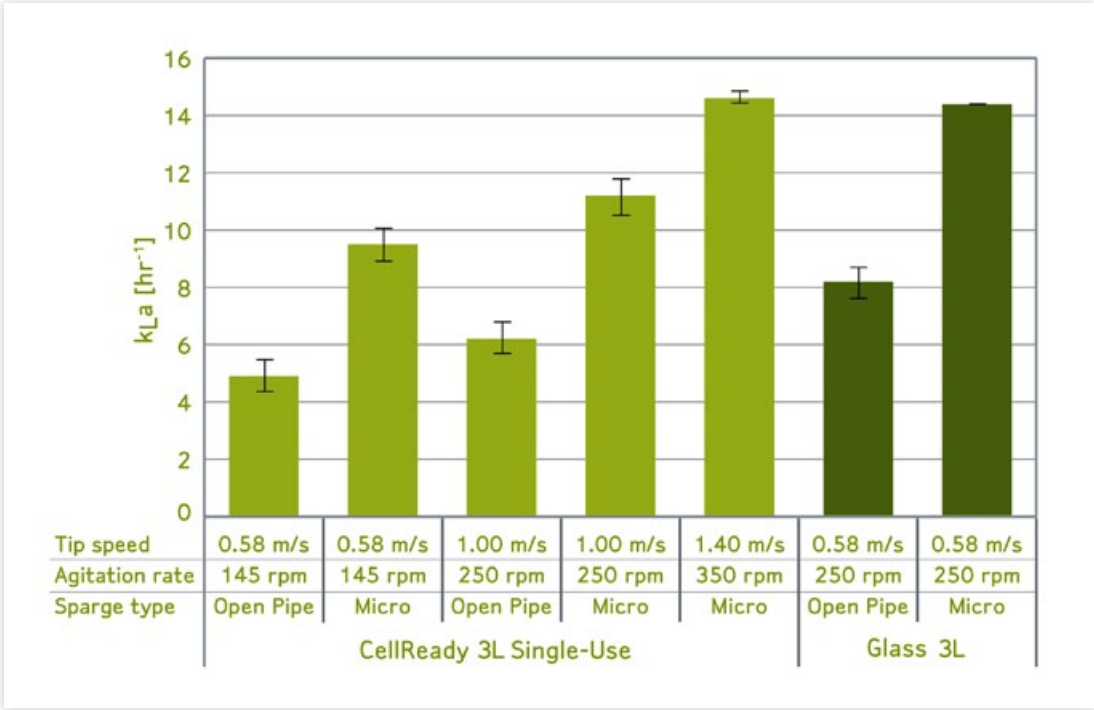

FIGURE 1. Comparison of bioreactor k_La values using water as the buffer.

100% for air saturation and was brought down to < 2% with nitrogen before each experiment commenced. The sparge rate was set at 0.2 vvm with air, and no overlay was applied. Each experiment was performed in 37°C water and repeated in triplicate (Figure 1).

Bubble size, sparge rate, impeller design, and agitation rate are key operating variables that impact k_La. The Mobius CellReady bioreactor axial flow impeller has larger diameter, curved blades versus the glass bioreactor's standard axial flow impeller. The glass bioreactor standard configuration includes a 15 micron microsparger and/or a seven-hole open pipe sparger, both made of stainless steel and placed centrally below the impeller. The CellReady bioreactor comes standard with a 15–30 micron polyethylene microsparger and a single-hole open pipe built into the bottom of the vessel.

To further characterize k_La performance between the single-use and glass bioreactors, vessels were assembled by mixing and matching impellers, spargers, and vessels. A variety of gas flow and agitation rates were then used to explore how the specific components of the single-use bioreactor (plastic impeller, polyethylene microsparger) compare to the specific components of the traditional glass bioreactor (steel impeller and sintered microsparger).

As expected, increasing agitation and gas flow rates had significantly positive impacts on k_La (Figure 2). Despite the design differences, impeller and sparger types had no

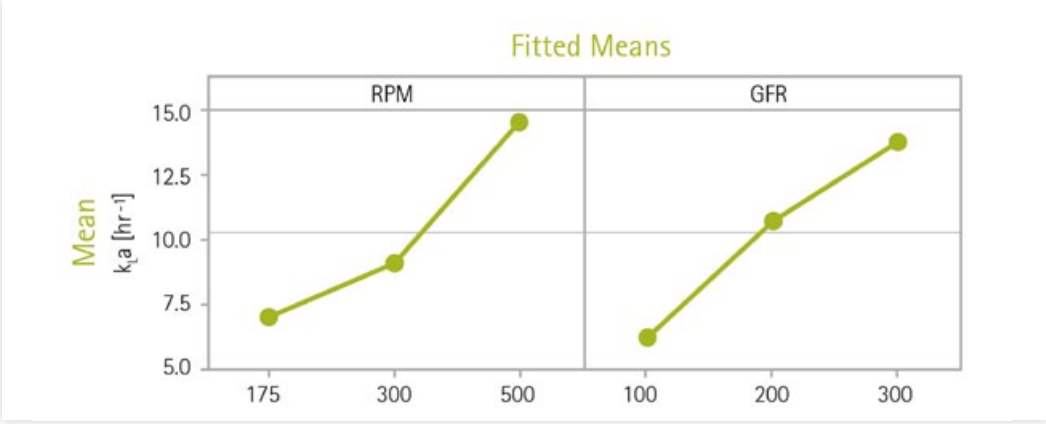

FIGURE 2. Increase in k_La values with increasing impeller speed (RPM) and gas flow rate (GFR) using microspargers and water as a buffer.

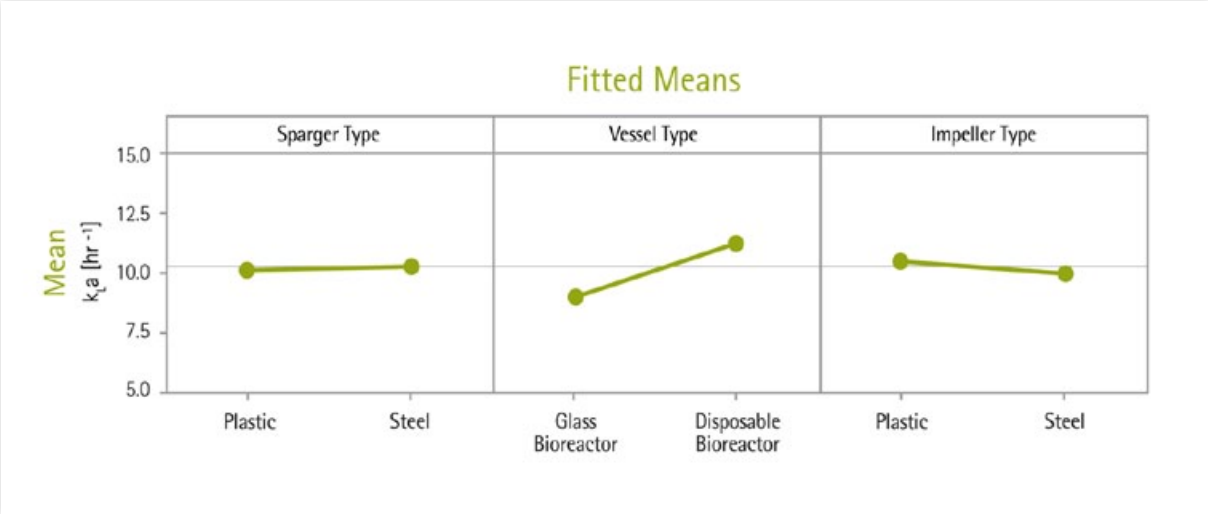

FIGURE 3. A comparison of spargers, vessel types, and impellers using microspargers and water as a buffer.

significant impact on k_La performance. In this study however, vessel type was determined to have a significant effect on k_La. The results of higher k_La performance with the single-use vessel may be due to the slight differences in the placement and positioning of the sparger, impeller, and dissolved oxygen probe in each vessel when executing these experiments (Figure 3).

A comparison of buffer effects on k_La values for the two bioreactors was also conducted using the same methodology as described above (Figure 4). While results indicate similar k_La values were obtained between the glass and the Mobius CellReady bioreactor with water, PBS, or PBS/Pluronic® F68 surfactant (BASF), the k_La values were significantly dependant upon the buffer used during the gassing-out methodology.

Mixing characterization was also assessed employing the measurement of changes in conductivity. Agitation rate was set to 200 rpm for each vessel tested. Conductivity range was 10–50 mS/cm and was measured for both bioreactors at five second intervals. Although results indicate that impeller designs contribute subtle differences in conductivity profiles between vessels, the T95 mixing time was less than 15 seconds

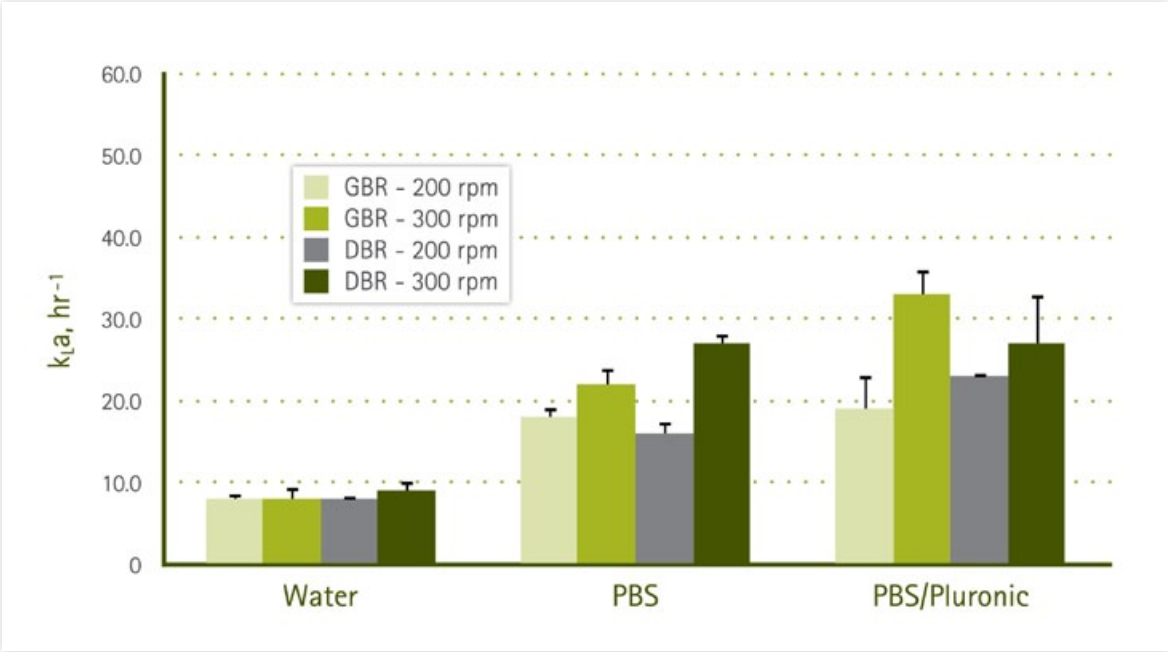

FIGURE 4. Comparison of buffer effects on k_La values using the microsparger.

in both vessels, and indicated negligible differences in mixing properties (Figure 5).

Cell Culture Performance

The Mobius CellReady 3L Bioreactor can be used in conjunction with a Mobius CellReady 200L Bioreactor to create an "all single-use" workflow (Figure 6). In this workflow, the 3L disposable bioreactor is used in the seed train and then as a satellite control. This simplified workflow from seed train through production significantly reduces the time and energy used for bioreactor cleaning, preparation, and sterilization.

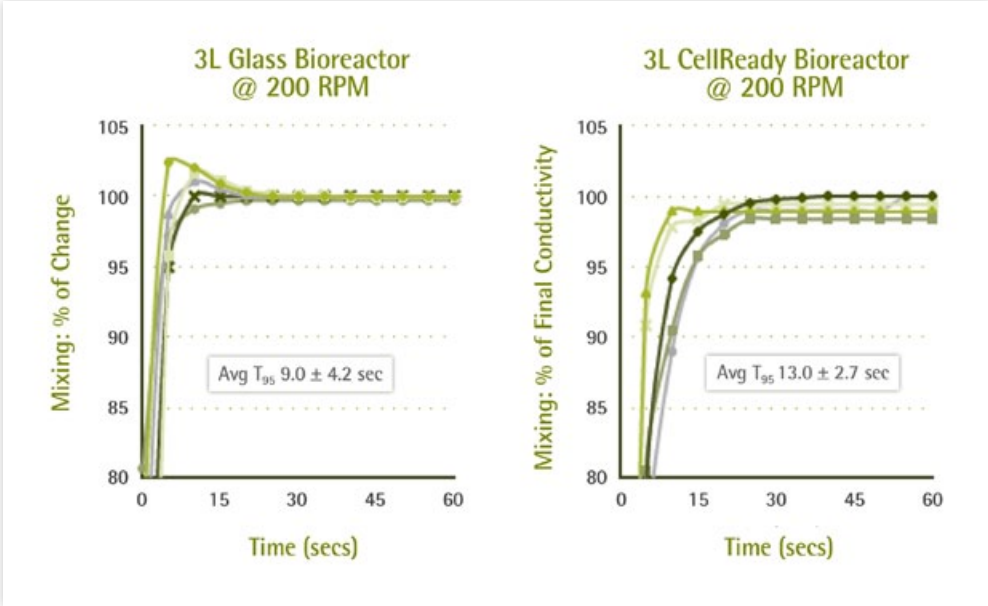

FIGURE 5. Comparison of bioreactor mixing characterizations using conductivity-based mixing time.

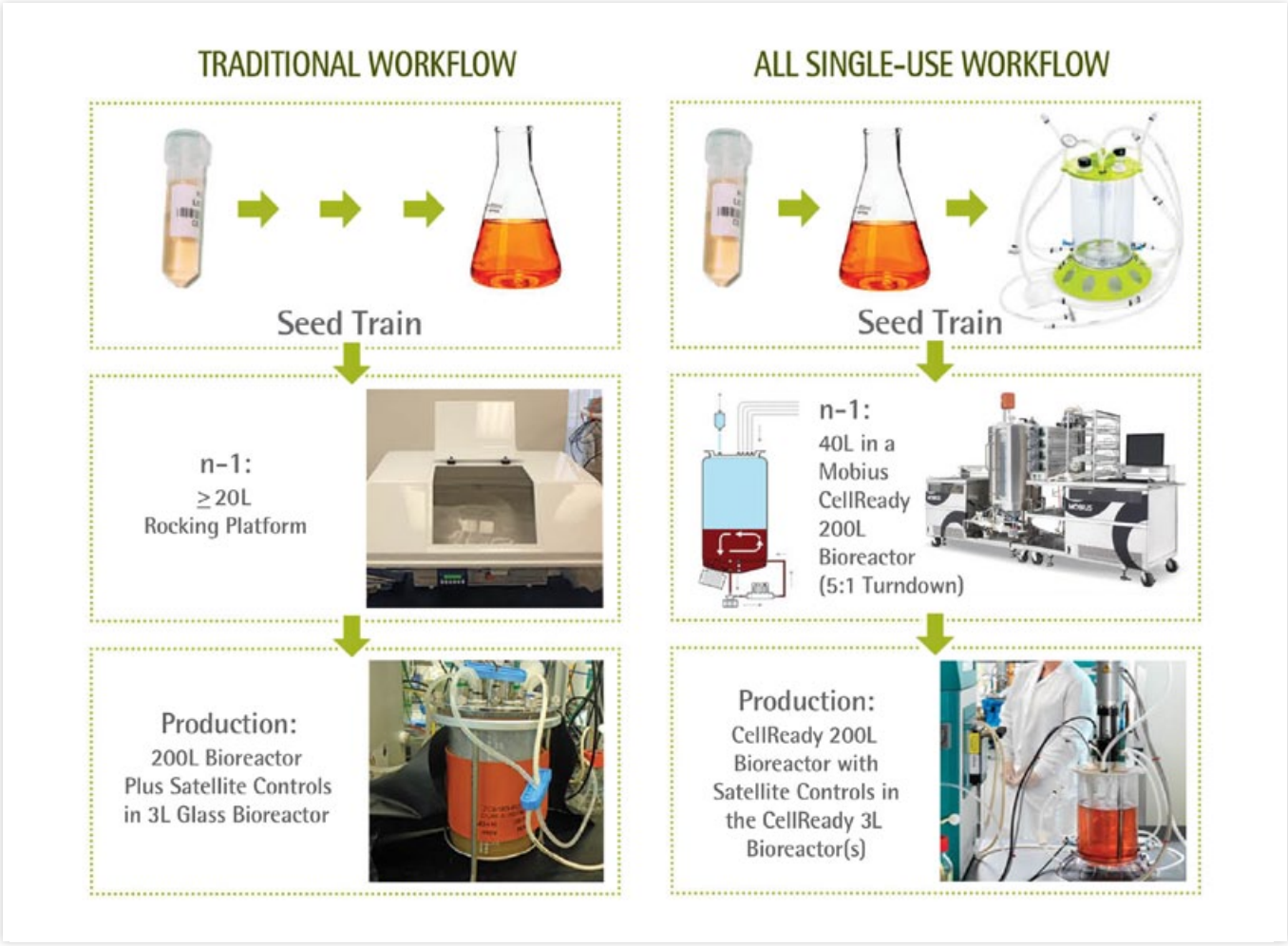

FIGURE 6. Comparison of a traditional workflow for a 200L cell culture with an all single-use workflow using the Mobius CellReady 3L Bioreactor.

TABLE 2. Process parameters for seed train runs through production.

Operating Parameters	3L Set Point Values	200L Set Point Values
Agitation Rate	200 rpm	83 rpm
Power/Volume	14 W/m³	14 W/m³
Sparger Type	Polyethylene Microsparger	Polyethylene Membrane Sparger
Temperature	37°C	37°C
DO	30%	30%
pH	6.95	7.00
Flow Rate of Mobius SensorReady Pump	NA	3L/min
Seeding Density	2 x 10^5 cells/mL	2 x 10^5 cells/mL

The process parameters for executing the seed train and production are summarized in Table 2. The 3L single-use bioreactors were controlled with Applikon Biotechnology ez-Controllers, and the CellReady 200L bioreactor was controlled with a Finesse TruViu™ RDPD control system.

Figures 7 and 8 summarize the viable cell density and cell viabilities for the seed train experiments (n-2 and n-1). Bioreactors were run in batch mode and sampled daily.

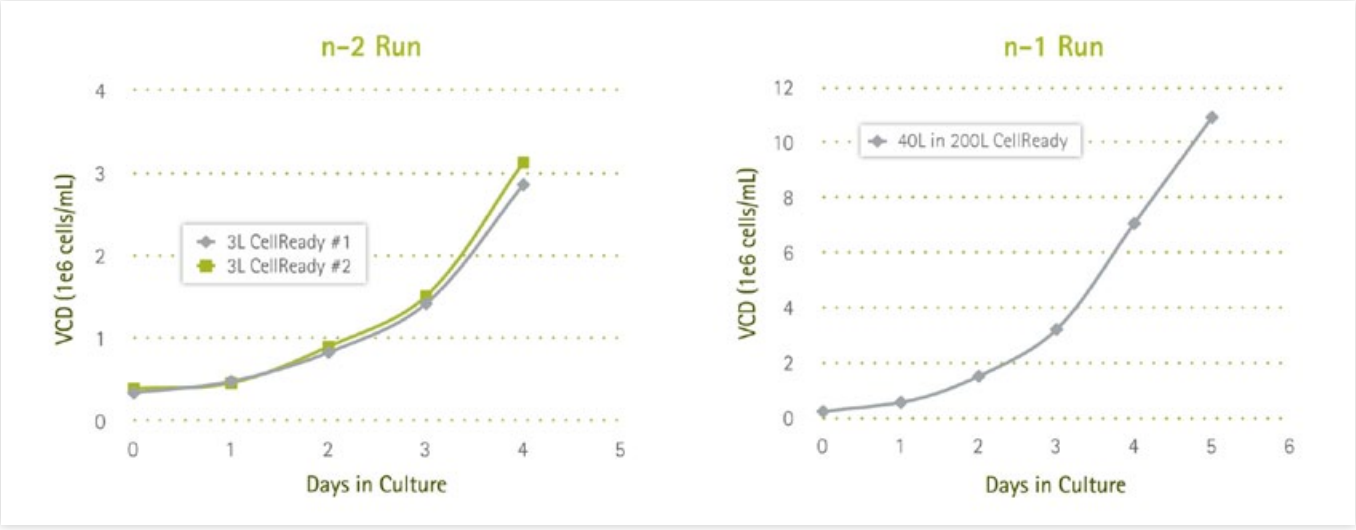

FIGURE 7. Viable cell density for n-2 and n-1 runs.

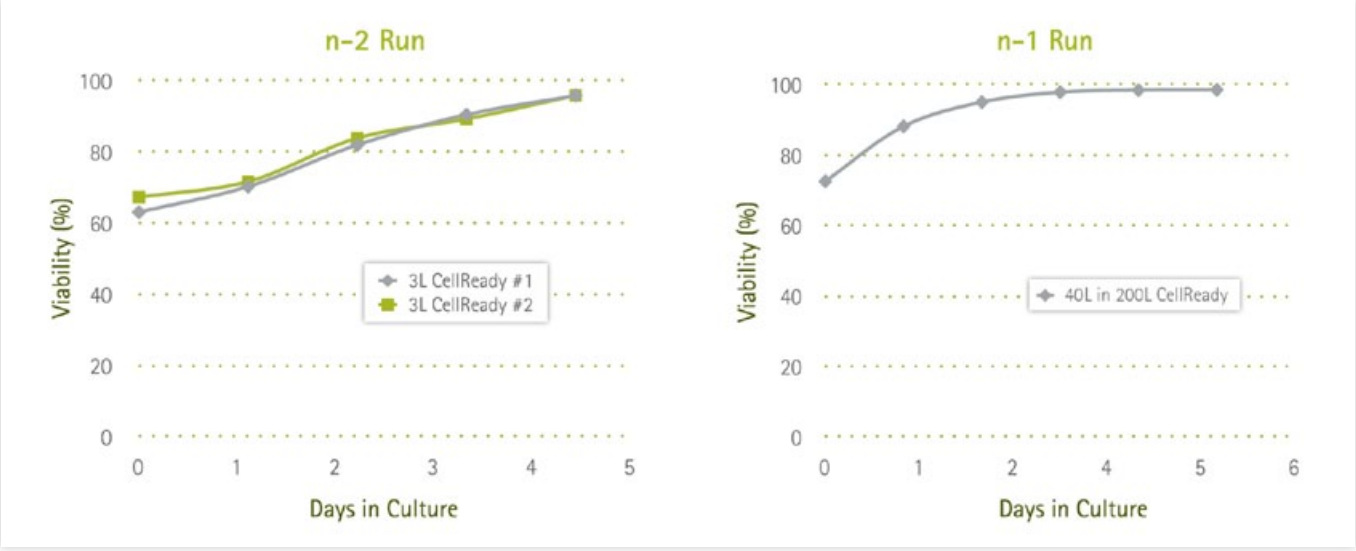

FIGURE 8. Cell viability for n-2 and n-1 runs.

Cell number was determined using a Vi-CELL® XR Analyzer (Beckman Coulter). After four days in culture, the two 3L disposable bioreactors used for the n-2 step yielded enough biomass to inoculate a 40L n-1 culture in the 200L bioreactor. After five days the volume was increased to 200L with the addition of fresh media, reducing the cell density to 2×10^5 cells/mL to commence day 0 of the production batch. Once a production bioreactor was seeded, a 3L single-use bioreactor's addition line was welded to the 200L single-use bioreactor's weldable sample line in order to transfer 2L of cell culture for satellite bioreactor operation in parallel to the full-scale cell culture experiment.

At each step, cell viabilities remained consistently high and growth rates met expectations. The low cell viabilities on day 1 of each run can be attributed to the presence of antifoam in the media, which was determined to impair the measurement of viability in a separate experiment (data not shown).

For each 200L production batch, the 3L satellite condition demonstrated comparable cell culture performance. Table 3 summarizes culture growth rates, peak viable cell densities, viabilities, and peak residual lactate as measured daily by the Vi-CELL XR Analyzer and BioProfile® FLEX Analyzer (Nova Biomedical).

TABLE 3. Production culture performance summary of the 200L and 3L CellReady satellite bioreactors.

		Growth Rate [1/hr]	Peak VCD [e6 cells/mL]	Day 7 %V [% viable]	Peak Lactate [g/L]
Exp A	200L	0.029	23	90	3.4
	2L	0.025	22	95	3.3
Exp B	200L	0.028	22	95	2.7
	2L	0.026	22	97	2.0

Conclusion

The Mobius CellReady 3L single-use bioreactor evaluated in this study represents a convenient alternative to traditional methods used to seed large-scale production runs. The same 3L single-use bioreactor can also be used as a satellite bioreactor to monitor the 200L bioreactor process. The 3L single-use bioreactor, coupled with the low operating volume requirements within the 200L Mobius CellReady Bioreactor, can effectively reduce the amount of media required for the seed train. Through use of the same platform for seed-train and satellites, it is possible to simplify the equipment requirements as the same controllers and process parameters can be used to meet the needs of both functions.

For further information about this study and the Mobius CellReady line of single-use bioreactors, contact the corresponding author and visit the Mobius website today.

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