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Application Note

Mobius® SensorReady Technology

A Flexible Approach for Monitoring Mobius® Single-Use Bioreactors

Abstract

The Mobius® SensorReady technology, which employs a continuous flow loop, is used with Mobius® Single-Use Bioreactors to provide users with unparalleled sensor configurability. The data presented herein show that the data collected for critical process parameters, such as pH and dissolved oxygen (DO), from sensors mounted in the Mobius® SensorReady assemblies are accurate and representative of the bulk process. Cell growth and viability profiles for a variety of cell types (e.g., CHO, hMSCs, Sf9 and EB66® cells) grown in bioreactors employing the Mobius® SensorReady assembly do not differ from their satellite controls, demonstrating the utility of the Mobius® SensorReady loop's low shear pump. The flexibility of the Mobius® SensorReady assembly is likewise demonstrated with a Mobius® Single-Use Bioreactor that was configured with dissolved CO_2 and viable cell density sensors, in addition to standard pH and DO sensors.

Introduction

Industrial-scale mammalian cell culture processes are optimized to produce large amounts of high-quality protein. Over the years, cell culture processing, cell lines, media development and the equipment used in production have advanced significantly to enable increased cell densities (≥ 20 x 10^6 cells/mL) and titers on the order of ~ 10 g/L (Li et al., 2010).

Precise process monitoring and control is essential for achieving these cell densities, maximizing specific productivity, and for the proper folding, glycosylation, and the reliable production of biopharmaceuticals. Traditionally, this has been achieved through monitoring of temperature, pH and DO concentration coupled with continuous feedback control systems developed to maintain these parameters at specified set points. As our understanding of the factors impacting cell culture processes increases, implementation of the principles of Quality by Design into cell culture performance is also expanding. Therefore, the need to develop and implement new sensor technologies that enable feedback control based on the measurement of additional parameters, such as viable cell density (VCD), dissolved carbon dioxide or glucose concentrations, is apparent. As these new sensor technologies are developed, users are challenged with their implementation in the context of fixed bioreactor designs.

Our platform of pilot- and clinical-scale single-use bioreactors addresses this challenge through its Mobius® SensorReady assembly, an external circulation loop that is connected to the Flexware® assembly via Lynx® sterile-to-sterile connectors. The Mobius® SensorReady technology is a modular assembly that enables users to customize the number and type of sensors that can be attached to their bioreactors in order to meet specific process monitoring requirements. Its implementation does not require customization of the Flexware® assembly, thereby providing the users with unparalleled flexibility for sensor evaluation, optimization and implementation.

Design

The Mobius® SensorReady assembly consists of a continuous flow loop, which is connected to the Flexware® assembly using Lynx® sterile-to-sterile connectors. For suspension cultures, a fixed flow rate of 3 LPM is maintained in the loop using a Levitronix® bearingless, magnetically coupled, centrifugal pump that has been designed specifically for low shear cell culture applications (Blaschczok et al., 2013). Flow through the loop is continuously monitored using a non-invasive flow meter that will signal any flow disruptions to the control software. Each Mobius® SensorReady assembly offers two options for sampling: a closed loop sampling option fitted with weldable C-Flex® tubing or ports fitted with needle-free, swabable valves, as shown in Figure 1B. The Mobius® SensorReady assemblies can be configured to enable from two to eight individual probes, provided that they are 12 mm in diameter, employ standard PG-13.5 threads and are 100 – 120 mm in length. All probes are mounted vertically, providing a consistent configuration that adheres to the manufacturer's guidelines for optimal operation of pH sensors. After calibration, the probes are installed into the desired Mobius® SensorReady assembly, sterilized by autoclave and connected to the Flexware® assembly during its installation into the hardware. The Mobius® SensorReady assemblies may be used singly or in pairs that can be connected in tandem, providing the ultimate flexibility in sampling and control. Each Mobius® SensorReady assembly is compatible with all of the Mobius® Single-Use Bioreactors from a pilot-scale (i.e., 50 L) to a clinical-scale (i.e., 2000 L) bioreactor.

Mobius® SensorReady Assembly Configured on a Mobius® 50 L Single-Use Bioreactor

Figure 1A.

Mobius® SensorReady Assembly

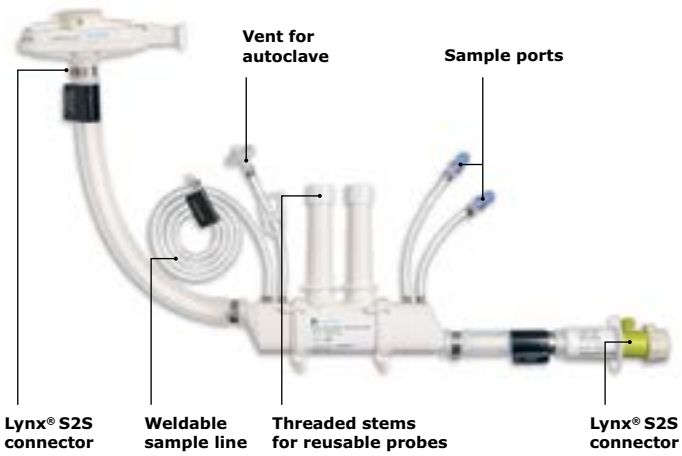

Figure 1B.

Data Accuracy

It is essential that any data obtained from probes positioned in the external circulation loop of the Mobius® SensorReady assembly accurately reflect the bulk cell culture to ensure effective process control. This was demonstrated by comparing conductivity probe response curves measured either in the bulk or within a Mobius® 2000 L Single-Use Bioreactor containing a Mobius® SensorReady assembly. The 2000 L bioreactor was chosen for this analysis, as it represents the most extreme configuration of the platform when operated at full volume. The T_{95} mixing time at the two probe locations was determined as the time required for conductivity profiles to reach 95% of their final value with the impeller operating at a non-gassed power input of 10 W/m^3 (70 RPM). One Mettler Toledo InPro 7100i Conductivity probe was installed in a Mobius® SensorReady Quad RS assembly; a second probe, used to measure conductivity directly in the bulk, was placed at the bottom of the bioreactor near the impeller. The response curves shown in Figure 2 represent mixing trials in which 600 mL of aqueous salt solution (1.25 M NaCl) was introduced into the 2000 L bulk volume as a bolus at the liquid surface.

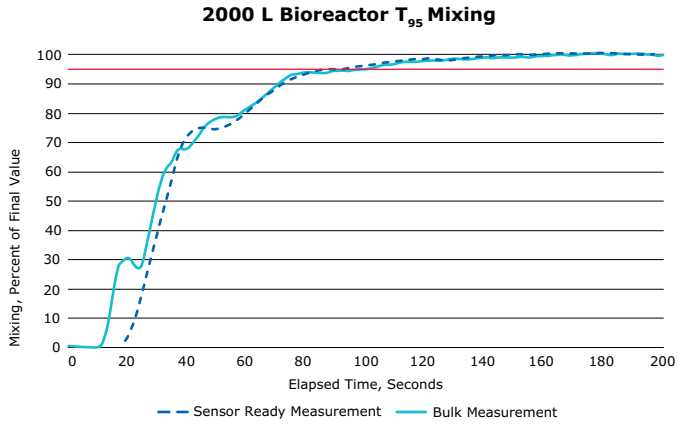

Figure 2.

Mixing in a Mobius® 2000 L Single-Use Bioreactor. Comparison of T95 mixing times measured in the Mobius® SensorReady assembly versus the bulk using conductivity probes in a Mobius® 2000 L Bioreactor.

Data were collected once every second from each probe, normalized and compensated for known sensor lag. The T_{95} mixing time measured using the probe in the Mobius® SensorReady assembly was in good agreement with that measured from the probe in the bulk (99 versus 94 seconds), as shown in the response curves in Figure 2 (red line). Mixing times measured at impeller speeds corresponding to 1 and 20 W/m^3 were likewise similar between the two probes (data not shown). Process data collected using the Mobius® SensorReady assembly are representative of the bulk, and suitable for cell culture process monitoring and control.

To demonstrate the suitability of the sampling port located on the Mobius® SensorReady assembly, an experiment was conducted to show that the contents in the loop are representative of the contents in the bulk bioreactor. A sampling port was fitted on the bioreactor to enable samples to be drawn directly from the bulk to allow for comparison to samples drawn from the sample lines located on the Mobius® SensorReady assembly. An eight-day, CHO-S batch cell culture process was performed in a 200 L bioreactor; samples were drawn daily from both sample port locations.

Data Accuracy (continued)

Figure 3 shows that the samples drawn from the Mobius® SensorReady assembly accurately reflect the bulk contents for cell counts (viable cell density, Figure 3A), media composition (glucose concentration, Figure 3B), and dissolved gas concentrations (carbon dioxide, Figure 3C). The cell culture environment within the loop is therefore comparable to the cell culture environment in the bulk in terms of cell density, metabolites, and dissolved gas concentrations throughout the entire process.

Cell culture sampling, process monitoring and process control are accurate and effective through the use of the Mobius® SensorReady assembly. Sampling from the Mobius® SensorReady assembly requires minimal purging and eliminates the tendency for cells to settle near the sample location. In contrast, it was difficult to get an accurate cell density count from the sample port in the bulk due to cell debris that persistently collected in the line.

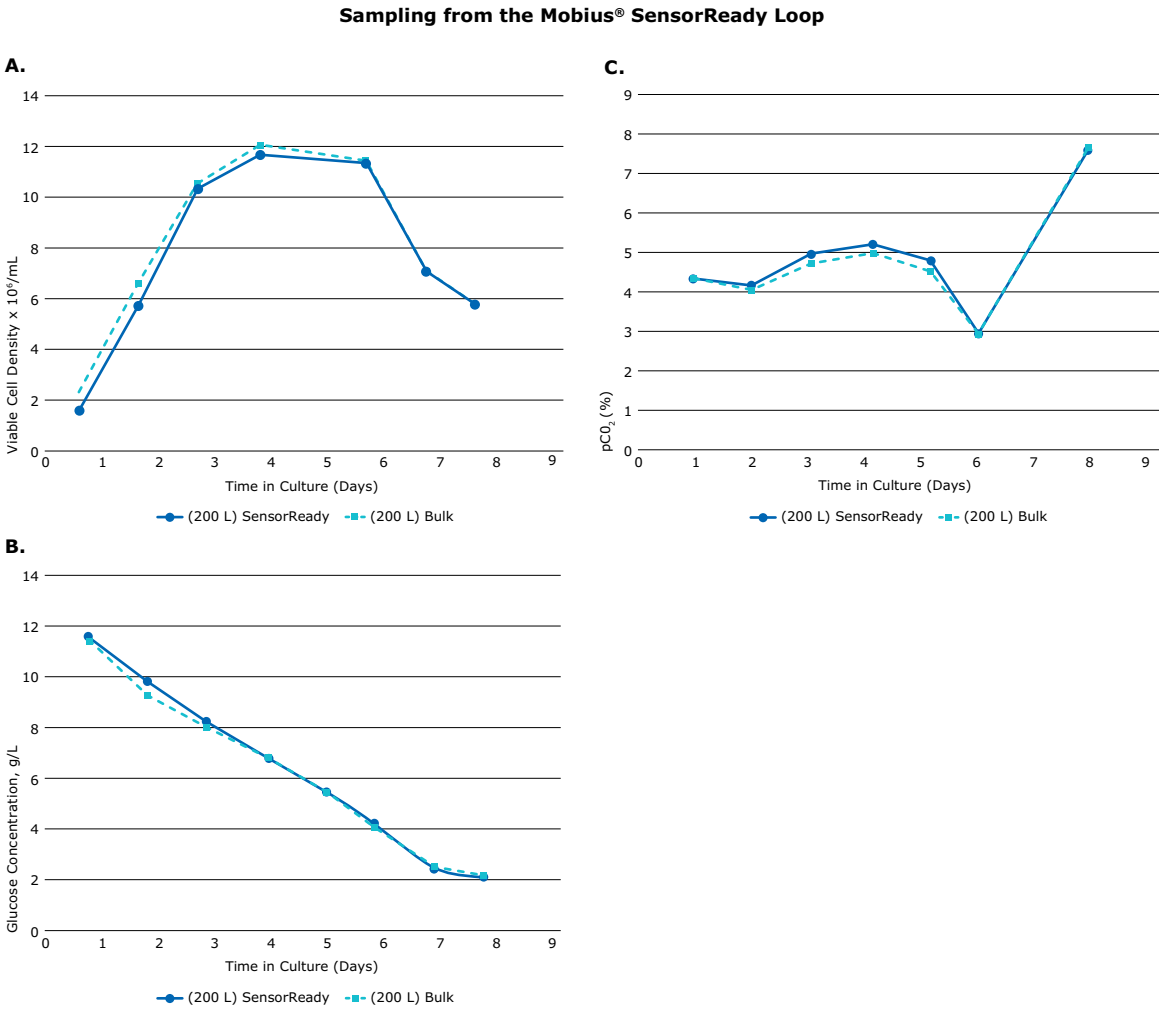

Figure 3.

Comparison of daily viable cell density measurements (Panel A), residual glucose concentrations (Panel B), and dissolved carbon dioxide profiles (Panel C) for samples drawn from the Mobius® SensorReady loop and a bulk sample port. Samples were analyzed on a Vi-Cell™ XR (Beckman Coulter), Bioprofile® Flex system (Nova Biomedical), and Siemens Rapidlab® 248.

Cell Culture Performance

Any equipment employed in biomanufacturing processes has the potential to cause cell damage due to hydrodynamic shear. The inclusion of a continuously operating external circulation loop during cell cultivation could conceivably impart potentially damaging shear. The sources of additional shear are varied and include wall shear forces generated as the cells flow through the loop, shear generated due to turbulent jets formed as the culture media returns back into the bioreactor, as well as from shear generated within the pump itself (Chisti, 2001). Estimation of cell sensitivity to hydrodynamic shear has been provided in the literature and has been shown to be dependent on several factors, including cell type, media formulation and processing conditions (Godoy-Silva et al., 2008). Computational fluid dynamic studies of magnetically coupled pumps, along with experiments using bovine blood, have confirmed that these types of pumps are highly efficient and impart minimal shear stress on cells (Zhang et al., 2006). Additionally, shear protectants such as Pluronic® F-68 are effective in mitigating the impact of hydrodynamic stress on cells in culture (Tharmalingam and Goudar 2015; Goldblum et al, 1990). To demonstrate that the external circulation loop and pump used with the Mobius® SensorReady technology is compatible with cell culture, a series of experiments was performed using a variety of cell types that were grown in the Mobius® 50 L Bioreactor containing the SensorReady loop. The 50 L bioreactor was chosen, as it represents an extreme case where the entire contents of the bioreactor will pass through the loop approximately four times per hour throughout the course of the culture. Results were then compared to cells grown in satellite Mobius® 3 L Bioreactors.

Figure 4A shows results that were obtained with CHO-S cells grown in a Mobius® 50 L as compared to 3 L Bioreactors during a 10-day batch culture. Each process was run at 37°C using a non-gassed power input of 14 W/m^3, and with pH and DO maintained at 6.95 ± 0.05 and 30% air saturation, respectively. The cells were seeded at 2 x 10^5 cells/mL and monitored daily by sampling via the needle-free port located on the Mobius® SensorReady assembly. The data show that the cells grown in the 50 L bioreactor displayed a similar growth profile, reached the same maximum cell density (20 x 10^6 cells/mL, solid lines), and maintained similar viability (dotted lines) throughout the run, as compared to the 3 L satellite control; metabolite profiles similarly did not differ between the two cultures (data not shown). The data presented in Figure 4B show growth curves obtained using human mesenchymal stem cells (hMSCs) grown on SoloHill® collagen-coated microcarriers in a fed-batch process. In this experiment, trypsinized hMSCs (0.7 – 1.1 x 10^4 cells/mL) were inoculated into Mobius® 3 L and 50 L Bioreactors with 2.4 L and 10 L, respectively, of cell culture media (DMEM supplemented with 10% EmbroMax® ES fetal bovine serum, 2 mM glutamine and 8 ng/mL basic Fibroblast Growth Factor) and 7.0 g/L microcarriers. The SensorReady speed was adjusted to 1 LPM, which corresponds to the hMSCs passing through the SensorReady loop an estimated once every 10 minutes. Samples were taken daily and assayed for viable cell density using a NucleoCounter® NC-200™. Figure 4B shows that the growth profiles and cell densities obtained with hMSCs grown in the presence (50 L Bioreactor) or absence (3 L Bioreactor) of the Mobius® SensorReady loop, as measured directly by cell counts or via visual inspection of the microcarriers under a light microscope (inset), were similar. Therefore, cell growth on microcarriers is supported in the bioreactors containing the Mobius® SensorReady assembly.

Cell Culture Performance (continued)

An evaluation of the Mobius® 50 L Bioreactor for vaccine production was performed by researchers at Valneva, SE, wherein an avian (i.e., duck) embryonic stem cell-derived cell line was used to produce measles virus in culture (Madeline et al., 2015). The data presented in Figures 4C1 and 4C2 were reproduced from Madeline et al. (2015), and show the results of a fed-batch process where the EB66® cells (inoculated at 4 x 10^5 cells/mL) were cultured in either Mobius® 3 L or 50 L Bioreactors at one-third of the final operating volume for three days (pH 7.2, 50% DO, and 37°C). The bioreactors were then filled to their final operating volumes (2.4 L and 50 L, respectively) with a cell culture media that supports viral proliferation, and the operating temperature was lowered to 33°C prior to infection with measles virus. Viable cell density (Figure 4C1) and virus titer (Figure 4C2) were monitored daily. Both the VCD (up to Day 6) and measles virus production were similar in the Mobius® 50 L Bioreactor containing a Mobius® SensorReady assembly, as compared to the Mobius® 3 L Bioreactor. Additionally, these data were similar to historical data obtained by the Valneva researchers using glass bioreactors (Madeline et al., 2015). It should be pointed out that after the sixth day of growth, the observed VCD in the glass bioreactor was highly variable (CV of approximately 38%), likely due to the impact of virus infection of the EB66® cells (see Figure 4 in Madeline et al., 2015).

The data presented in Figure 4D show growth profiles generated with *Spodoptera frugiperda* Sf9 cells used for the production of Hepatitis C virus-like particles in a study performed by researchers at Instituto de Biologia Experimental e Tecnológica (iBET). Mobius® 3 L and 50 L Bioreactors were inoculated with Sf9 cells (0.5 x 10^6 cells/mL) and allowed to grow for 24 hours (30% DO, 27°C and with a non-gassed power input of 6 W/m^3) in SF900™ II SFM serum-free, protein-free insect cell culture medium prior to infection with baculovirus using a multiplicity of infection of 2 infectious particles/mL (arrow). The culture continued for four additional days prior to harvest and purification of the virus-like particles. Both the viable cell densities and viabilities were similar between the two cultures. Virus-like particle productivity in these cultures were within 10% of historical results (data not shown).

The data presented in Figure 4 collectively demonstrate the utility of the Mobius® Bioreactors with a variety of cell types, grown in either suspension or micro-carrier based cultures and used in several different applications. In all cases, cell growth in the Mobius® 50 L Bioreactor, where the Mobius® SensorReady assembly and pump were included, was similar to that observed in satellite 3 L bioreactors that did not have a SensorReady loop. These data support previously published work wherein it was shown that bearingless pumps of this type are suitable for cell culture (Blaschczok et al., 2013, Zhang et al., 2006). Furthermore, estimations of the level of hydrodynamic shear generated by the bearingless pump has been shown to be well below the threshold where cell damage is thought to occur (manuscript in preparation). In this study, the CHO-S cells that were passed through the loop more than 60 times per hour displayed similar growth and metabolite profiles while maintaining protein production that matched controls (manuscript in preparation).

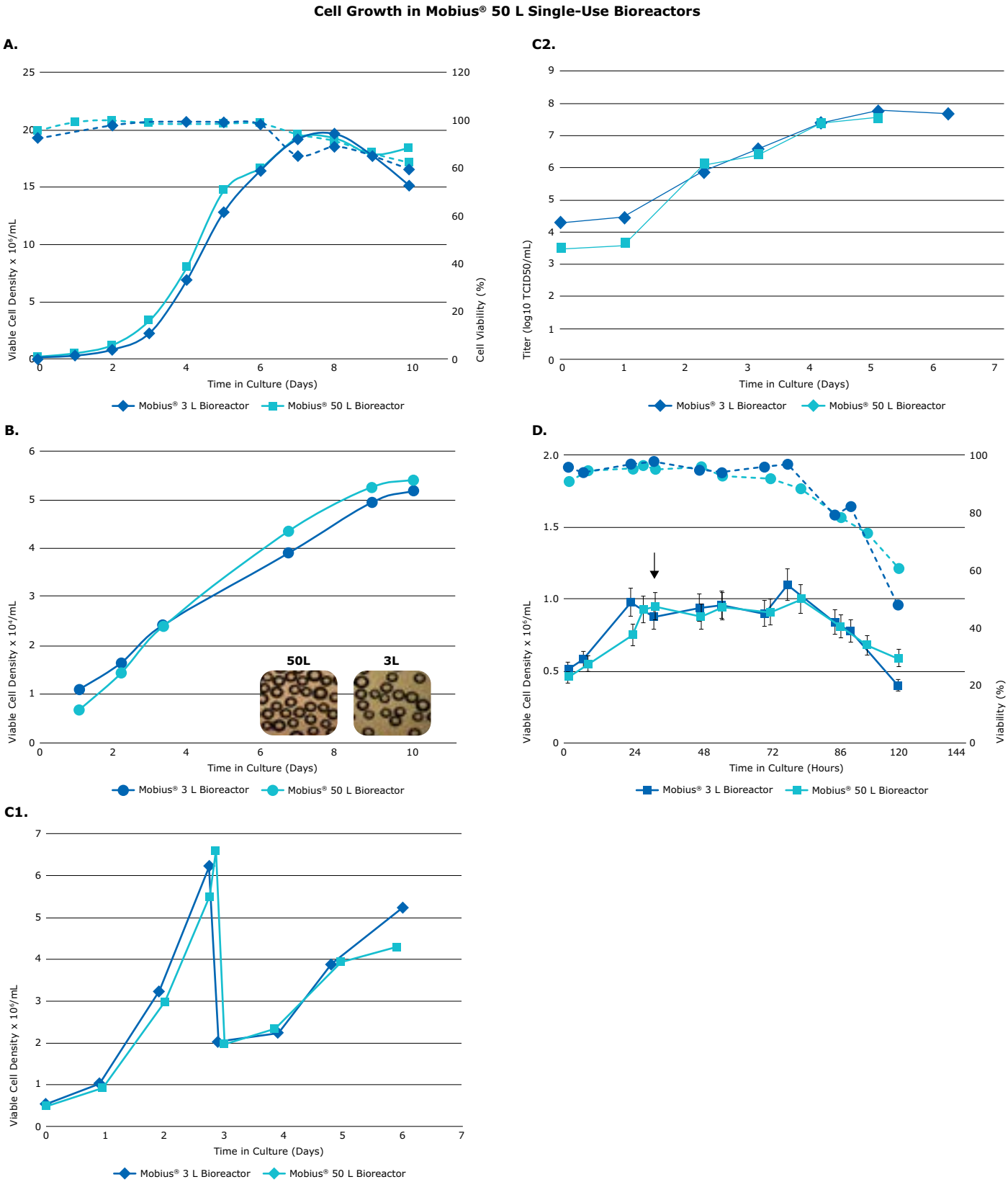

Figure 4.

Comparison of growth profiles obtained in 50 L and 3 L single use bioreactors with CHO-S cells (Panel A), hMSCs (Panel B), avian EB66® cells (Panels C1 and C2) and Sf9 insect cells used for the production of Hepatitis-C virus-like particles (Panel D).

Demonstration of Flexibility

The drive to further our understanding of cell culture processes through the implementation of new sensor technologies is facilitated by the flexibility of the Mobius® SensorReady assembly technology. This is highlighted in Figure 5, where sensors to measure four different parameters were used during a Mobius® 2000 L Single-Use Bioreactor run. A Mobius® SensorReady Quad RS assembly, configured with redundant pH (Mettler Toledo 405-DPAS low maintenance pH probe) and DO (Mettler Toledo polarographic oxygen probe) sensors, was connected to the bioreactor in tandem with a Mobius® SensorReady Dual RS assembly containing a Hamilton® Incyte Cell Density sensor and a YSI 8500 Biovision™ Dissolved CO_2 probe. The latter probes were included to enable the additional real-time measurement of viable cell density and carbon dioxide levels in the culture, respectively. Note that the Flexware® assembly did not require any modification in order to accommodate the additional sensors; all desired customization was achieved solely via the Mobius® SensorReady assemblies.

Each of the sensors were calibrated per the Manufacturers' recommendations and installed into their respective Mobius® SensorReady assembly. After sterilization and assembly via the Lynx® sterile-to-sterile connectors, as described below, the sensors were connected to their respective transmitters and the CHO-K1 cell culture process was initiated. Samples were taken for daily process monitoring and/or recalibration of probes over the course of the 11-day fed-batch process. Figure 5A shows the pH profile obtained from the pH probe; these results are typical for this cell line and process, and the data correlate well with daily off-line measurements (data not shown). Figure 5B and 5C show the DO profile of the culture, which was maintained at 50% air saturation, and the data from the YSI 8500 Biovision™ Dissolved CO_2 sensor, respectively. The data generated from the Hamilton® Incyte Cell Density sensor (Arc View 265) that measures capacitance in real time is shown in Figure 5D. Following the manufacturer's instructions, a correlation was developed previously between capacitance units, measured in picoFarads/cm, and viable cell density for the process employed during this culture. The results confirm that the correlation is maintained when the cell density sensor was used with the Mobius® SensorReady assembly and operated under constant flow conditions. The capacitance measurements thereby provide a method for estimating viable cell density in this system and, if desired, can be used with the system software to control process additions that are based on cell density. By using the Mobius® SensorReady assembly in this fashion, real-time monitoring of process data from a variety of sensors is facilitated.

Implementation of Multiple Sensors on a Mobius® 2000 L Single-Use Bioreactor

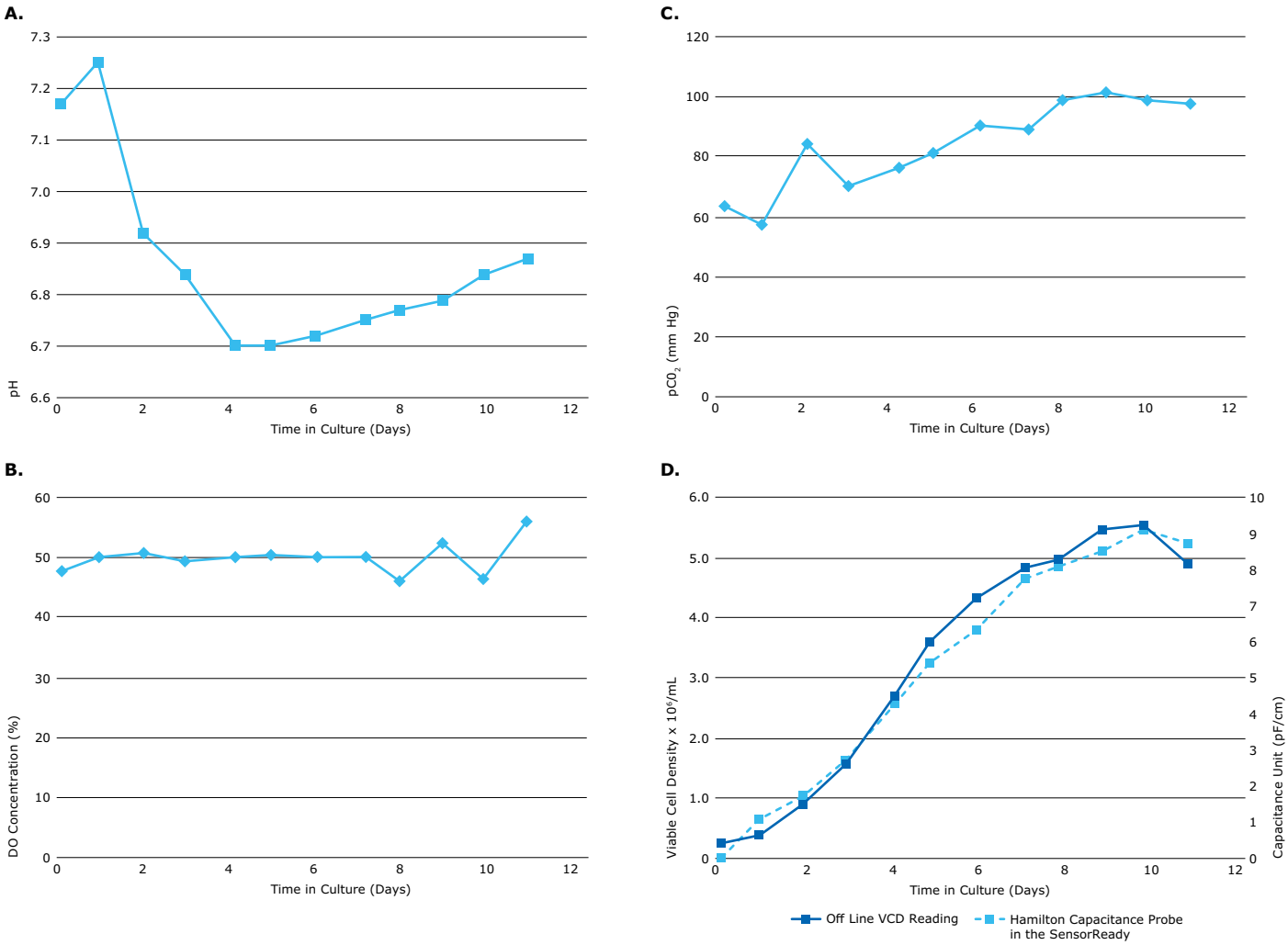

Figure 5.

A Mobius® SensorReady Quad RS assembly with redundant pH (Panel A) and DO (Panel B) probes was connected in tandem with a Mobius® SensorReady Dual RS Assembly containing CO_2 (Panel C) and VCD (Panel D) probes.

Autoclaving the Mobius® SensorReady Assemblies

Sterilization of the Mobius® SensorReady assembly can be accomplished by autoclave. Studies have been conducted that demonstrate sterilization effectiveness using a Getinge autoclave (Model 191945) operated with a liquid media cycle and programmed with a pre-vacuum ramp (1.2 PSIA) and slow exhaust cycle. The experiments utilized threads impregnated with 1.9 x 10^6 *Geobacillus stearothermophilus* spores, which were placed at several locations within a Mobius® SensorReady Quad RS assembly (Figure 6, A – E), to confirm sterilization. Since the Mobius® SensorReady assemblies needed to be disassembled for spore threads insertion, the assemblies were tested by pressure decay to ensure integrity after reassembly and insertion of pH and DO sensors. After autoclaving at 121°C, the Mobius® SensorReady assemblies were disassembled and the spore threads recovered. All spore threads and controls were aseptically placed in Tryptic Soy Broth growth medium, incubated at 60°C, and observed for growth after seven days. The results (n=6) demonstrated that the minimum autoclaving time required for sterilization was 30 minutes, as growth of *Geobacillus stearothermophilus* was only observed in positive control samples. Similar results were obtained when the samples were autoclaved for 45 minutes. Therefore, the recommended autoclave cycle for the Mobius® SensorReady assemblies is 30 minutes at 121°C using a liquid cycle. Higher autoclave temperatures or longer times were not evaluated.

Sterility Assurance

Once configured with the calibrated probes and sterilized by autoclave, the Mobius® SensorReady assemblies must be attached to the bioreactor process container in a manner that maintains a sterile flow path. This is achieved using single-use Lynx® sterile-to-sterile connectors, which are preconfigured onto both the Mobius® Single-Use Bioreactor and Mobius® SensorReady assemblies. Through a series of simple steps, the two independently sealed fluid paths (one on the bioreactor and one on the Mobius® SensorReady assembly) are joined, creating one continuous path that has never been exposed to outside contamination. This path is considered to be sterile once the Lynx® connection between the gamma-irradiated bioreactor and a properly autoclaved Mobius® SensorReady assembly has been made. The connection has been demonstrated to provide a sterile connection in a non-classified environment using an aerosolized bacterial challenge and a direct bacterial soiling method with greater than 10^6 CFU of *Brevundimonas diminuta*.

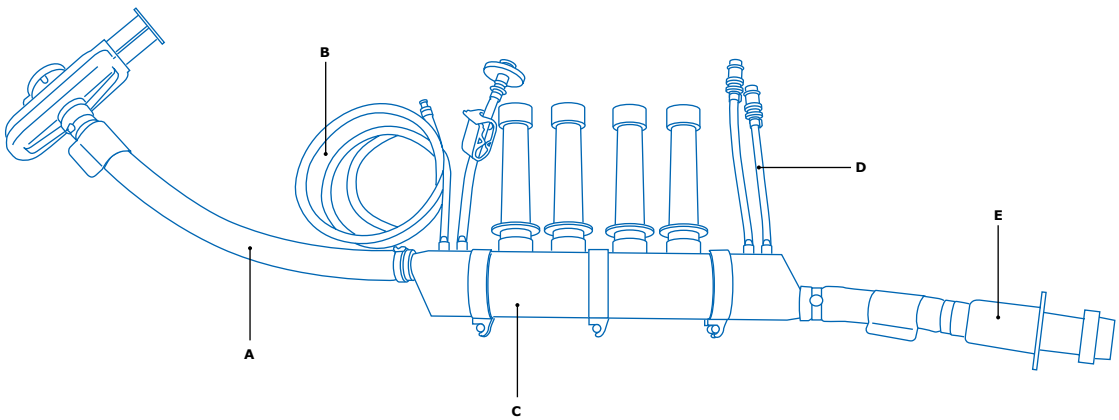

Figure 6.

Location of Spore Threads for Sterilization Study with Mobius® SensorReady Quad RS Assembly.

Conclusions

- The Mobius® SensorReady technology employed with Mobius® Single-Use Bioreactors provides a flexible and convenient way to configure the sensors used during a cell culture process.
- The low shear, bearingless pump has been characterized extensively to demonstrate its compatibility with a variety of cell types and culture systems.
- This continuous flow loop approach provides flexibility, effective measurement capability reflective of the bulk process, and both the open- and closed-loop sampling options commonly employed in Single-Use Bioreactors.
- The Lynx® sterile-to-sterile connectors provides confidence in the installation of sensors to Mobius® Single-Use Bioreactors.

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