Strategies for Measuring Viable Biomass

Novel Technique Aims to Extend Application of RF Impedance to Single-Use Bioreactors

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Of the available on-line biomass assays, the radio-frequency impedance method (often referred to as capacitance) is generally regarded as the most robust and reliable method to monitor viable biomass during fermentation and cell culture.

The first paper to show that capacitance could be used to estimate microbial biomass dates back over 20 years, and today the technology is routinely used for monitoring and controlling mammalian cell culture processes and high-density yeast and bacterial fermentations in research, process development, and manufacturing applications.

The capacitance method has been shown to be robust, easy to scale up, and insensitive to gas bubbles or debris with cells in suspension or attached to inert carriers.

In this article, we show how capacitance measurements for live-cell concentration can be more widely applied to disposable bioreactors as a result of the availability of a dedicated disposable biomass probe. These days an increasing number of disposable bioreactors can be used with conventional reusable biomass probes. We also show how major improvements have been made to installation/operation qualification procedures so that conventional reusable probes can also be utilized within a cGMP production environment.

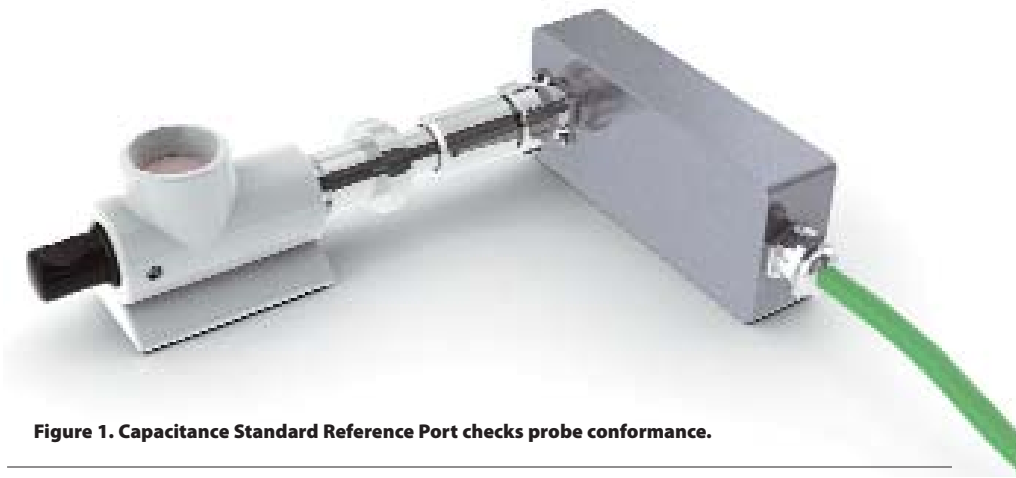

Figure 1. Capacitance Standard Reference Port checks probe conformance.

Process Validation

Aber Instruments' (www.aberinstruments.com) Biomass Monitor, which is based on RF impedance, is widely used for monitoring and controlling the viable biomass in cell culture manufacturing processes. It measures the live cell biovolume in a bioreactor, which is often expressed as live cells/mL or g/L of live biomass. These readings are derived from the real-time capacitance value in pF/cm. The concept is quite similar to an on-line dissolved oxygen probe where the value is derived from the sensor current measured in nA and normalized to a set temperature.

In a cGMP cell culture process, it is essential to verify the performance of capacitance-based viable biomass probes if they are used on-line for critical decisions or process control. Capacitance probes, however, cannot be simply verified against known standards of viable biomass. There are also no suitable known national standard capacitance solutions or suspensions.

Readily available standard liquids such as water or ethanol have specific capacitance values of 7 pF/cm or less and are not representative of the often much larger capacitance values recorded during a cell culture process. In some high density processes where the cells are grown on carriers or in a fixed bed at over 10^8 cells/mL, values in excess of 100 pF/cm have been recorded.

A device for validating the Aber Biomass Monitor probe has now been developed and is set for launch later this year. The Capacitance Standard Reference Port (patent pending) is an electrical method used to generate capacitance that does not require the handling of biological material. When the biomass probe is installed within this new device (*Figure 1*) and connected to the head amplifier, a capacitance (in pF/cm) within the same range as encountered during the cell culture or fermentation process is generated and recorded by the Biomass Monitor. This method will allow a rigid protocol to be developed for thoroughly testing the Biomass Monitor probe prior to use in a cGMP process.

Figure 2. Flow-through viable biomass probe is used with silicone tubing.

Probes for Disposable Bioreactors

Many biopharma companies are now switching from conventional glass or stainless steel vessels to disposable, single-use bioreactors. On-line probes are provided in some disposable bioreactors but typically provide only limited options—just pH, temperature, and pO_2. The majority of these bioreactors are used in cell culture for production of seed or for low cell densities using batch production processes where accurate measurement and control is not so critical and the systems often do not require validation.

New processes requiring cGMP production are now being developed using disposable bioreactors and consequently there will be the increasing demand for the same range of sensors (including viable biomass) used with conventional bioreactors.

The Biomass Monitor with conventional reusable probes can be used with most of the larger, single-use, disposable bioreactors. For example, with the Hyclone Single-Use Bioreactor (SUB) from **Thermo Fisher Scientific**, it is possible to use existing half-inch ports on the bags for insertion of a reusable 12 mm biomass probe designed to the correct insertion length. The Hyclone

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SUB uses a **Pall** Kleenpak connector to allow the presterilized probe to be inserted into the bioreactor, and there is a special tray to support the array of conventional stainless steel probes including pH, pO_2, and biomass.

This approach has the advantage that the protocols and validation processes developed for using biomass probes with conventional bioreactors can be immediately transferred to the disposable bioreactor. The main disadvantage is that the insertion of any probe into the bioreactor can compromise the sterility of the system.

An alternative solution for measuring viable biomass in disposable (and conventional) bioreactors is to use a reusable, small diameter flow-through probe. The probe (*Figure 2*) has four annular electrodes on the inside making it ideal for insertion into a silicone tubing, recirculation loop from a disposable (or conventional) bioreactor.

The flow-through probe can also be used for measuring the bleed off of biomass in a continuous or perfused bioreactor system or for confirming the transfer of the correct amount of seed to a production bioreactor. The latter application is similar to the process used in conventional brewery processes where the viable biomass probe is used to accurately dose the correct amount of live seed (in this case, yeast) into a production fermentor.

A disposable biomass probe is also available from Aber Instruments. It has been designed to be welded into most single-use bioreactors and is suitable for bags with agitators (e.g., the Hyclone SUB) or those using the rocker type platform (e.g., the Appliflex single-use bioreactor from **Applikon**).

The disposable biomass probe has pure platinum electrodes with the same dimensions as the existing reusable production biomass probes with flush electrodes that are often used in cGMP manufacture. As both the Aber disposable and reusable biomass probes will produce the same radio-frequency field, this will allow users to directly compare viable cell density data between conventional and disposable bioreactors.

The electrode support material is HDPE, which meets FDA and USP Class VI requirements, and the probe can withstand gamma sterilization and be stored for prolonged periods before use. The disposable probe is easily connected to a mini-lightweight preamplifier (*Figure 3*) so that the weight load or torque on the bag is minimal and the bulk of the electronics is then located well away from the bag. Trials of the single-use biomass probe are under way.

The use of RF impedance to monitor and control cell culture processes is well established in conventional stainless steel or glass bioreactors, and it is crucial that RF impedance can also be applied to single-use bioreactors. Recent developments addressing the validation of the reusable probe and a complimentary range of disposable probes that can be used in bags will allow this technology to be used in confidence with most types of bioreactors from process development through to cGMP production. GEN

Figure 3. Disposable biomass probe is used with single-use bioreactors.