Measurement and Control of Viable Cell Density in cGMP Manufacturing Processes

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Of the available on-line biomass assays, the radio-frequency (RF) impedance method has a clear advantage for current good manufacturing process (cGMP) because it is an unambiguous reflection of viable cell bio-volume rather than the total number of cells. Although other more approximate methods are available for cells in suspension, RF impedance is practically the only on-line method available for cells in suspension, attached to microcarriers and immobilized cells at high cell densities.

Data are presented to show how live cell concentrations derived from an RF impedance-derived instrument, the Aber™ Biomass Monitor, have been used in a cGMP environment. The recent trend has been to use the Aber Biomass Monitor for process control, and an example of maintaining a constant level of live biomass will be shown.

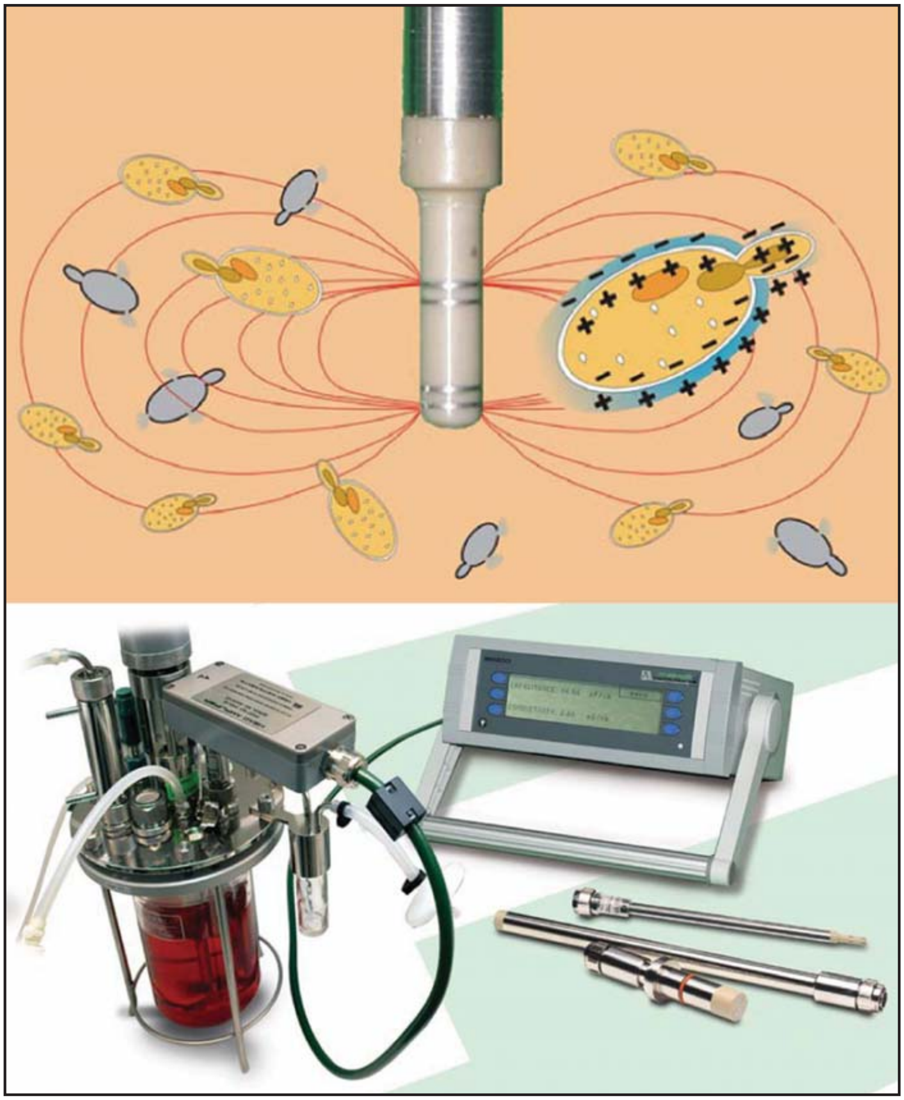

Figure 1. Typical RF impedance probe field for the Aber Annular Probe together with the new Biomass Monitor 200 (to be launched this year).

Biomass Monitor — Principle of Measurement

A probe incorporating four electrodes is fitted into a standard fermenter port where it can be steam sterilized *in situ*. When fermentation or cell culture commences, the probe applies a low current RF field to the biomass passing within 20 to 25 mm of the electrodes. The probe (see Figure 1) uses four electrodes to produce a radio-frequency electrical field that polarizes the viable cells. The nonviable and leaking cells are effectively invisible to the detection system. The Biomass Monitor processes the signal from the probe to produce an output that is a highly accurate measure of the viable cell concentration. The system is insensitive to cells with leaky membranes, evolved gas bubbles, cell debris, and

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other media components. Consequently, it detects only viable cells.

Probe Developments

The original on-line probes for estimating viable biomass by RF impedance used a high-performance polymer body. The resin probe has been shown to perform well after repeated steam sterilizations or clean-in-place (CIP) and is suitable for most laboratory and pilot plant applications. However, for manufacturing in a cGMP environment, all wetted materials are required to conform to FDA regulations—so a probe was specifically developed for use in this crucial application area. The probes used with the latest RF impedance instruments have the main body made from 316L stainless steel and the end holding the electrodes made from inert and dielectrically stable polyetheretherketone (PEEK). The polymer PEEK is commonly used in the biopharmaceutical industry and has USP class VI and FDA 21 CFR177.2415 accreditation for repeated food contact. Side entry probes in diameters of 25 mm (to fit standard Ingold-type housings) and top entry probes of 12 mm and 19 mm, in lengths of up to 600 mm, are available.

Tests have shown that the probes can withstand in excess of 100 SIP (steam-in-place) or autoclave cycles. Probes with the 4-pin electrode arrangements are used in cGMP manufacturing processes but an alternative probe design incorporating an annular ring electrode arrangement is now available (see Figure 1). The annular ring design has been shown to be more sensitive in applications with highly aerated mycelial bacterial processes.[1] The main advantage in cell culture is that the ring design can be used with the 12 mm diameter probes commonly used on small bioreactors.

Using the Aber Biomass Monitor in cGMP Cell Culture

The Aber Biomass Monitor is now utilized extensively on a wide range of processes using animal cells. In this application, the probe provides an accurate and reproducible count of viable cells in real time. The original Biomass Monitor (Model 214M) has been mainly used in process development. With this instrument, variable- and two-frequency measurements can be made, which allows for its use in either highly aerated microbial fermentations or in cell culture. The system can be expanded with the addition of a controller and multiplexers to monitor and control up to 32 bioreactors.

The Viable Cell Monitor 520, an RF impedance instrument dedicated to cell culture applications operating at a fixed frequency of 0.5 MHz, was introduced in 1997. This system converts the capacitance signal into the traditional "viable cells/ml" and stores the conversion factor. The model 520 is still being manufactured and has been used in a variety of cGMP production scale facilities; primarily in control applications. Although the Viable Cell Monitor can be expanded for up to 16 bioreactors (with the addition of two multiplexers), the single channel system dedicated to each bioreactor has been favored at cGMP sites.

In 2003, the model 214M was replaced by the Biomass Monitor 220 that has improved sensitivity, and an integral multiplexer, allowing up to four bioreactors to be monitored or controlled. A new Biomass Monitor (the model 200) is planned for release into the market this year. This latest version will be compact, simple to set up, GAMP4-compliant, and designed for operation with just one bioreactor, and it will also be significantly lower in cost than the previous versions. The benefits from having the biomass probe installed in a cGMP plant fall into three distinct categories:

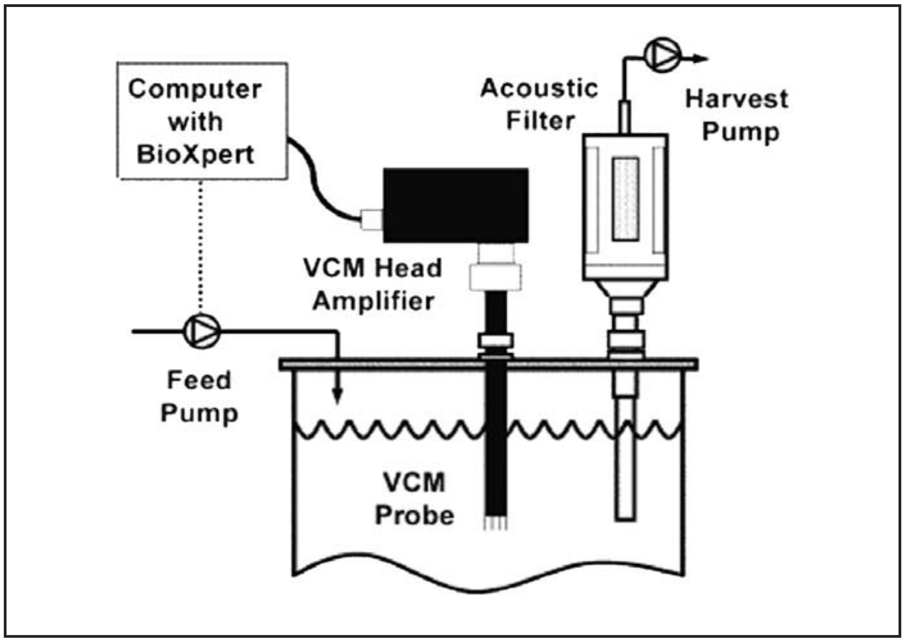

Figure 2. Schematic of a perfusion bioreactor with an Aber Viable Cell Monitor (VCM) and acoustic filter. In the control algorithm, a cell-specific perfusion rate is specified, and the biomass density signal is averaged and converted to a perfusion flow rate. (Courtesy of Dowd, *et al* 2003[1]).

On-line Process Control

Many cGMP cell culture processes are based on a perfusion or fed-batch bioreactor system. Control of the feed or addition rates to maintain pseudo-steady-state conditions in these bioreactors can be especially challenging. A robust, automatic perfusion rate control system based on the on-line Aber RF impedance probe is now being used in cell culture manufacturing processes to maintain a constant level of biomass within the bioreactor. In the control algorithm, a cell-specific perfusion rate is specified and the RF impedance cell density signal is converted into a perfusion flow rate through calculation and implementation with a variable speed controlled pump (see Figure 2).

On-line optical sensors have been used in some cases to maintain the process but these will only measure the combination of total cells and debris that can collect during the process. Moreover, the optical sensors are prone to fouling over extensive production runs. The Biomass Monitor only measures the viable cell mass and is therefore, ideal for this application. It has been applied for process control in sono-perfused cytostats, spin-filter perfused bioreactors, and for maintaining steady-state, continuous culture of bioreactors with external loop filters for monoclonal antibody and recombinant protein production.

An example of the control of the concentration of HeLa cells by the Aber Viable Cell Monitor in a sono-perfused cytostat at Cilbiotech, in Belgium, is illustrated in Figure 3. The time-dependent capacitance trace of a perfused HeLa cell culture is shown evolving from batch (preset volume, increasing concentration) to fedbatch (increasing volume, preset cell concentration) growth. The stable capacitance value can be seen when the culture is operated in a fedbatch mode with a preset cell concentration of 10^7 cells/ml.

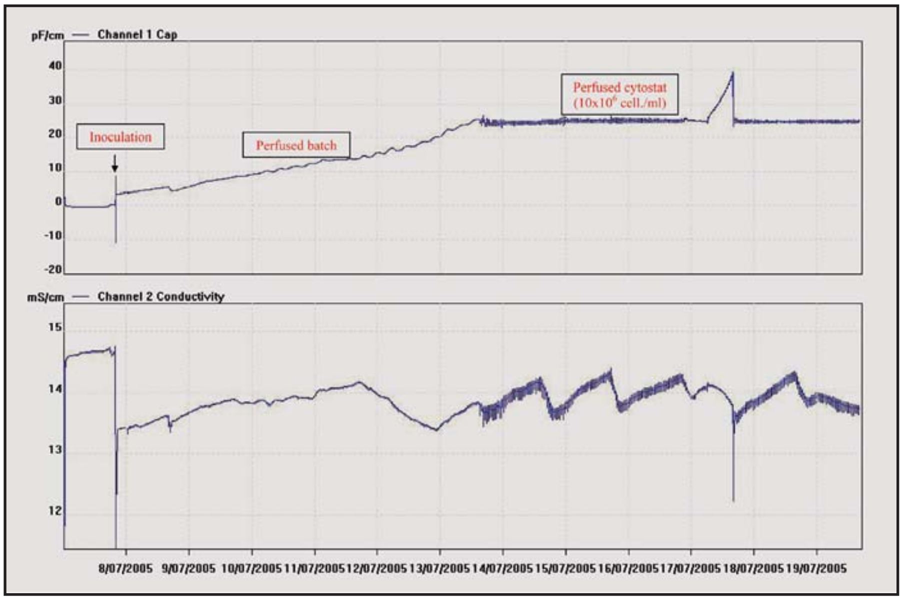

Figure 3. Time-dependent capacitance measurement with an Aber VCM of a perfused Hela cell culture evolving from batch (preset volume, increasing concentration) to fedbatch (increasing volume, preset cell concentration) growth conditions. (Courtesy of Cilbiotech s.a., Belgium.)

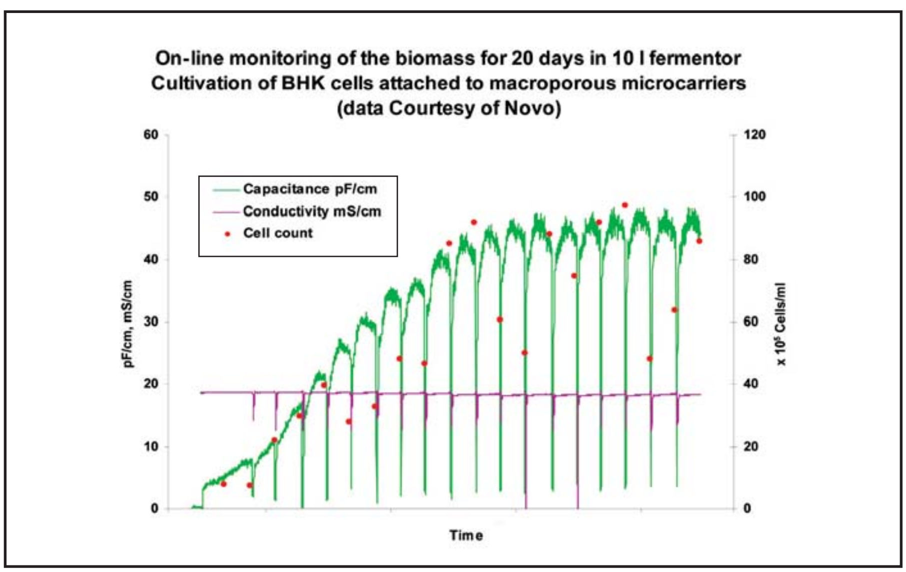

Figure 4. The application of RF impedance for monitoring the viable cell density of BHK cells grown on macroporous microcarriers. The traditional cell counts are unable to show the trend in cell growth. (Data courtesy of Novo Nordisk, Denmark.)

Monitoring Microcarrier Culture

Measuring the cell density of animal cells grown on microcarriers (macroporous) can only be achieved by sampling and then estimating a total cell count based on nuclear counts. Apart from the errors of sampling microcarrier culture, the off-line method only provides a total cell count. For cell culture systems grown in packed beds, it is very difficult to have access to a sample of the carriers in a sterile way and biomass can only be estimated by indirect methods such as oxygen uptake. In both cases, the Biomass Monitor provides a unique (orthogonal) on-line method for estimating the live cell mass (see Figure 4). In packed bed systems, the Biomass Monitor can also be used to characterize the packed bed process in terms of growth kinetics and to calculate the change of specific productivity as the process moves from a growth to a production phase.

Checking Conformance

Many of the existing cGMP licensed processes have a qualified off-line method for estimating viable cell density. The off-line method is normally based on microscopic counting after the addition of the trypan blue stain and there are a variety of automated cell counting devices that can replace the laborious manual cell count (*e.g.*, CEDEX, Vi-Cell). Operator errors can be made during the sampling, analysis, and data processing so a number of companies have realized that there are significant cost implications with potential errors. For example, an error in the cell count can lead to incorrect changes to the process by changing feed rates, or concentrate additions based on a cell count. Adjustments like these may cause protein titres or other process indices to be out of specification and harvested material to be discarded. The cost implications for batch failure are obvious and there are the significant additional costs when investigating non-conformance events.

The Aber Biomass Monitor, in this case, is not being used in any process control decisions or validation of the process, but it provides real-time information backup for the bioprocess. When used to its full potential, the

Biomass Monitor supplies additional information to operators and supervisors, helping to eliminate any process production losses due to operator errors in cell counting, and provides a ready means to complete non-conformance event reporting. An example is shown in Figure 5 comparing the live cell count against RF impedance-derived capacitance at a biopharmaceutical facility producing a therapeutic protein by genetically engineered SP2/0 cells using a high cell density perfusion technology. There is an excellent correlation in the process that extends for 40 days with cell concentrations varying from $2x10^5$ cells/ml at seeding, increasing to $17x10^6$ cells/ml at the end of the process run (Figure 6).

The correlation between the capacitance and the live cell number derived from cell counting techniques can be lost when there is a change in the dielectric properties of the cell. For example, changes in membrane conductivity and cell internal conductivity can all have an effect on the dielectric properties of a cell. RF impedance also measures the biovolume, (i.e., the total volume that is enclosed by the cytoplasmic membranes of the cells in suspension). Experimentally, it is known that the mean size of population changes in response to numerous factors such as growth conditions, substrate limitation, and osmotic stress.[2] The loss of correlation between capacitance and live cell number has been reported for insect cells once they have been infected with baculovirus, swelling in size.[3,4]

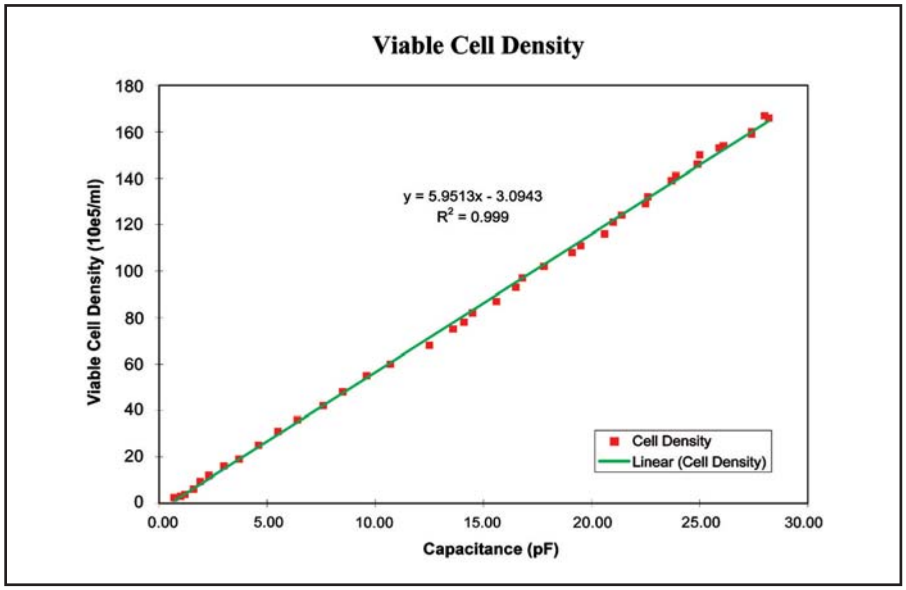

Figure 5. Live cell count (based on a microscope count) versus capacitance. (Data courtesy of a biopharmaceutical company producing a therapeutic protein by genetically engineered SP2/0 cells using a high cell density perfusion technology.)

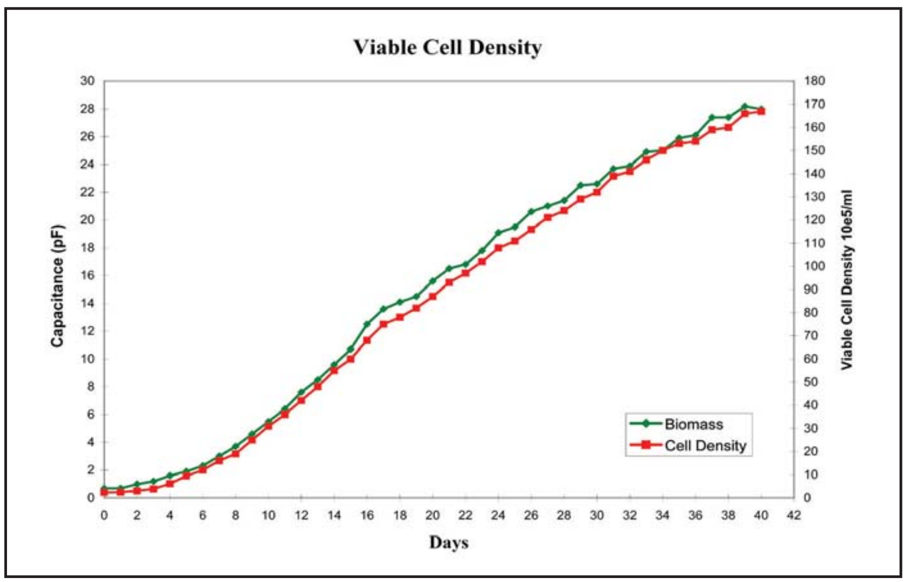

Figure 6. Monitoring live cell count (based on a microscope count) and capacitance (shown as biomass) during a manufacturing process for a therapeutic protein by genetically engineered SP2/0 cells using a high cell density perfusion technology.

Conclusions

RF impedance based biomass measurements are an accurate and reliable method of determining cellular biomass on-line and are being used in cGMP processes in biotechnology. The Aber Biomass Monitor has been shown to be robust and has the advantage that it measures the live cell concentration and can be used in packed bed and microcarrier (surface and porous) systems.

Many companies in cGMP are using the Aber Biomass Monitor for control purposes in perfusion bioreactors. A cell-specific perfusion rate is controlled by the input of live cell mass concentration from the Biomass Monitor, allowing the bioreactor to operate under optimum conditions for maximum production of recombinant proteins.[5]

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