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On-line measurements and control of viable cell density in cell culture manufacturing processes using radio-frequency impedance

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Abstract

In this work, radio-frequency (RF) impedance is reviewed as a method for monitoring and controlling cell culture manufacturing processes. It is clear from the many publications cited that RF Impedance is regarded as an accurate and reliable method for measuring the live cell bio-volume both on-line and off-line and the technology is also sutable for animal cells in suspension, attached to micro-carriers or immobilized in fixed beds. In cGMP production, RF Impedance is being used in three main areas. Firstly, it is being used as a control instrument for maintaining consistent perfusion culture allowing the bioreactor to operate under optimum conditions for maximum production of recombinant proteins. In the second application it has not replaced traditional off-line live cell counting techniques but it is being used as an additional monitoring tool to check product conformance. Finally, RF Impedance is being used to monitor the concentration of live cells immobilized on micro-carriers or packed beds in cGMP processes where traditional off-line live cell counting methods are inaccurate or impossible to perform.

Introduction

There is an increased focus on in-line techniques for bioprocess monitoring driven primarily by the initiative of the FDA regarding 'Process Analytical Technology' (PAT) www.fda.gov/cder/OPS/PAT.htm. Traditionally, parameters such as pH, dissolved oxygen and temperature are routinely monitored in a bioreactor however to gain better process knowledge and control, it is important to measure biological or biochemical parameters. One of the most important, yet also the most challenging components to monitor on-line is biomass (Kell et al. 1990; Konstantinov et al. 1994). Most of the cGMP processes at the production scale using mammalian cells lack appropriate probes to evaluate on-line, in real time, non-invasively and reliably, the biomass content of the bioreactor.

On-line biomass has been measured directly with *in situ* probes using a diverse range of principles that includes acoustics, laser light, fluorescence and radio-frequency impedance (RFI). Merten et al. (1987) described an infra red sensor for determination of the cell number during continuous hybridoma fermentations but the range of cell densities tested and length of operation was limited. Laser turbidity probes have been used in batch culture (Konstantinov et al. 1992; Zho and Hu 1994; Wu et al. 1995) and the probe performance in general is acceptable for high viability cultures. The light based probes measure the total

number of cells and during process operation upsets the optical measurements have been shown to deviate from the cell density determined by trypan blue exclusion (Wu et al. 1995).

Of the available on-line biomass assays, the radio-frequency impedance (RFI) method has a clear advantage for process development and manufacturing because it is an unambiguous reflection of viable cell biovolume rather than the total number of cells. The viable cell concentration is of prime importance in metabolic studies and those relating to the efficiency of target protein production. RF impedance is also suitable for measuring the live cell density in bioreactors when the cells are attached to micro-carriers and to inert discs.

In this work, we review how RF Impedance has been used on-line to monitor or control the viable cell concentration during cell culture in a range of applications. The recent trend has been to use the technology for process control and an example of maintaining a constant level of live biomass will be shown. The article will also discuss how the introduction of a new range of probes has allowed the technology to be implemented successfully into cGMP production facilities. For the purpose of the review, we will concentrate on the Biomass Monitor (BM) from Aber Instruments Ltd., Aberystwyth, UK as this has a large publication base and is the only commercial system reported to have been used in cell culture based manufacturing processes. The other more laboratory based systems under development will be considered but unless otherwise specified, the work being reviewed will be studies using the Biomass Monitor or its derivative, the Viable Cell Monitor 520.

Theory of RF Impedance and the β-dispersion

For modelling purposes, a suspension of cells can be regarded as being composed of three separate parts: the cytoplasm, the outer plasma membrane and the suspension medium. The cytoplasm is a highly complicated and structured mixture of salts, proteins, nucleic acids and smaller molecules (Clegg 1984). In addition, in eukaryotes various internal, membrane-bound structures are also present which can affect the cell's dielectric properties (Foster and Schwan 1989; Asami et al. 1996). Surrounding the cell's conducting core is the plasma membrane, which is essentially non-conducting (Takashima et al. 1988). The suspension medium is generally aqueous and ionic. Thus, electrically a cell suspension can be regarded as a suspension of spherical capacitors each containing a conducting matrix (cytoplasm) and all surrounded by a conducting suspension medium.

When an electric field is applied to a suspension of cells in an aqueous ionic solution, the ions in that solution are forced to move. The positively charged ions are pushed in the direction of the field, whilst the negatively charged ones are pushed in the opposite direction (Figure 1a). The ions both inside and outside the cells can only move so far before they encounter the plasma membranes which act as an insulating physical barrier, preventing further movement. This results in the development of a charge separation or polarization at the poles of the cells (see Figure 1a). The magnitude of the suspension's field induced separations is measured by its capacitance (C) in Farads (F). However, as a Farad is a very large capacitance, one normally sees its values expressed in pico-Farads (pF). Thus by measuring the capacitance of the suspension at one or more appropriate frequencies, its biomass can be estimated (Harris et al. 1987; Kell et al. 1990; Davey et al. 1993a, b; Kell and Todd 1998) because, as the volume fraction of the cells increases, there are more polarized membranes which, in turn, gives a higher measured capacitance. Dead cells (operationally defined – Barer et al. 1998; Kell et al. 1998) and non-biomass solids do not possess intact plasma membranes and so do not polarise significantly, therefore they do not contribute significantly to the capacitance of the cell suspension (Harris et al. 1987; Stoicheva et al. 1989). Similarly, if dead cells, oil droplets, debris or gas bubbles are present in the medium, they do not contribute directly to the measured capacitance, as they are also not membrane enclosed particles. However, if they are present at high concentration they will reduce the net suspension capacitance by virtue of the fact that they are reducing the cellular volume fraction. In microcarrier culture, this is also the case and so it may be necessary to carry out the calibrations in the presence of the normal concentration of microcarriers.

The rate at which the electric field changes direction can also be varied. The number of times the field changes direction per second is measured by its frequency in Hertz (Hz): The greater the rate

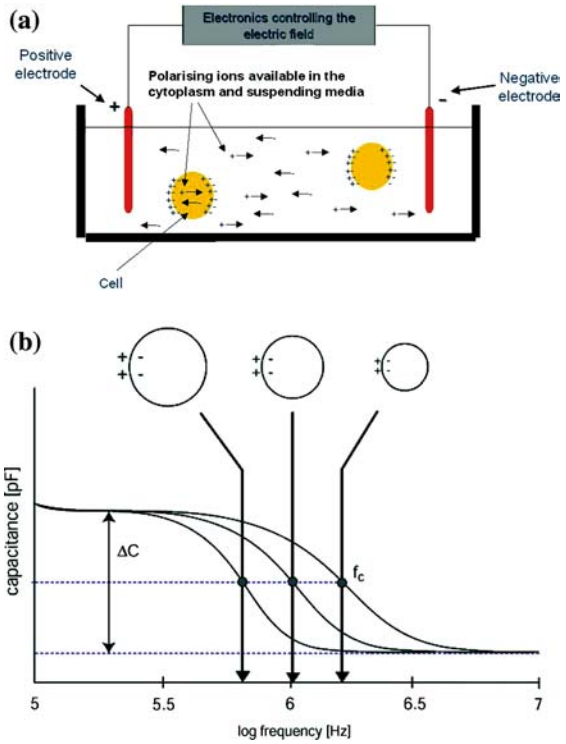

Figure 1. (a) Principle of radio-frequency impedance. When the electric field is applied to a suspension of the cells in an aqueous ionic solution, the positive ions are pushed in the direction of the field and the negative ions in the counter direction. The ions can only move so far until they encounter the cells' plasma membranes, which prevent further movement. This results in a charge separation or polarization at the poles of the cells. (b) The capacitance of a cell suspension as a function of frequency shown along with the equivalent polarizations of the cell. At approximately 10^5 Hz many ions have time to reach the cells' plasma membrane before the electric filed is reversed. At very high frequencies typically over 10^7 Hz, few ions have time to polarize the cell membranes before the field is reversed, this results in a negligible contribution towards the capacitance. When the fall in capacitance is half completed, we have the characteristic (critical) frequency (f_c).

of change, the higher the frequency. The frequency has a marked effect upon the capacitance of a cell suspension as the ions moving up to, and polarizing the plasma membranes, take a finite time to reach them and cause the polarizations (Pethig 1979; Foster and Schwan 1986; Pethig and Kell 1987).

Figure 1b illustrates the polarizations induced across the cells within a suspension as the frequency of the electric field is increased. At low frequencies, below approximately 0.1 MHz many ions have time to reach the cells' plasma membranes before the field is reversed, driving the ions in the opposite direction. In this case, the induced polarizations are large, and hence the capacitance of the cell suspension is high. As the frequency is increased over 1 MHz, fewer ions have time to reach the plasma membranes before the field is reversed and, therefore, the extent of the transmembrane polarization is less, and hence the capacitance of the suspension is lower. At very high frequencies, typically 10 MHz and above, even fewer ions have time to polarize the membranes and so the resulting membrane polarization is small, giving a negligible contribution to the overall measured capacitance. What remains is a background capacitance due largely to the dipoles of the water in the suspending medium.

From Figure 1b one can also see that, as the frequency is increased, the capacitance of the suspension falls from a high low-frequency capacitance plateau (maximal cell polarization) to a low high-frequency plateau (minimal cell polarization). This fall in capacitance, due to the loss of induced charging of the cells' plasma membranes as frequency is increased, is called ***β***-dispersion, and for most cells it is centered between 0.5 and 3 MHz

(Pethig 1979; Foster and Schwan 1986; Pethig and Kell 1987; Davey and Kell 1995), which is the frequency range used for the radio-frequency impedance based on-line measurement of viable cell density.

Probe developments

The original on-line probes for estimating viable biomass by RF Impedance used a high performance polymer body, e.g. the Aber Biomass Monitor and the Hewlett-Packard E5050A Colloid Dielectic probe (Siano 1997). The resin has been shown to perform well after repeated steam sterilizations or 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 and 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 the inert and dielectrically stable 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 (Carvell 2003). Probes with the 4-pin electrode arrangements are used in cGMP manufacturing processes but an alternative probe design incorporating four annular ring electrodes arrangement is now available (see Figure 2). The annular ring design has been shown to be more sensitive in applications with highly aerated mycelial bacterial processes (Ferreira et al. 2005) but 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 RF Impedance to monitor and control cell culture processes

The application of the Biomass Monitor (BM) to animal cell culture monitoring has only occurred in earnest in the last few years (Cerkel et al. 1993; Degouys et al. 1993; Beving et al. 1994; Davey et al. 1997a; Guan and Kemp 1997; Guan et al. 1998; Zeiser et al. 1999; Ducommun et al. 2002a, b; Dowd et al. 2003) for studying the growth of a wide variety of animal cells, whether in suspension or in an immobilized state.

Cerkel et al. (1993) investigated the dielectric properties of Chinese Hamster Ovary (CHO 320) cells and HeLa cells grown in suspension culture at a concentration of $0.5 - 3 \times 10^6$ cells/ml and scanned at frequencies between 0.2 and 10 MHz using a BM. Cell numbers were determined using a Coulter Counter model Z_b and a linear relationship between capacitance and cell number was observed. It was found that using the 0.5 MHz as

Figure 2. The Biomass Monitor with the variety of probes used in manufacturing processes.

the measuring frequency gave the best compromise in terms of loss of sensitivity vs. quality of biomass evaluation.

A bioreactor culture of Sf-9 cells infected with an AcNPV expressing β-galactosidase was used to evaluate the ability of the Biomass Monitor to monitor the progress of a baculoviral infection under different infection conditions (Zeiser et al. 1999; Elias et al. 2000, 2003; Zeiser et al. 2000). These authors used relative permittivity measurements (ε') that can be calculated from the capacitance and an example (adapted from Zeiser et al. 2000) showing the value of monitoring ε' in insect cell culture is given in Figure 3(a, b) at two different MOI values (multiplicity of infection). Prior to baculovirus addition, growth proceeded as expected and the ε' profile matched the off-line viable cell density. The addition of baculovirus successfully arrested cell division at a MOI value of 10. The near doubling in ε' during the first 48 h post-infection (hpi) paralleled an increase in the cell diameter. The peak in the ε' was observed at about 24 hpi followed by a plateau extending to about 48 hpi beyond which the ε' decreased continuously, paralleling trends in viable cell density and cell diameter corresponding to the time of onset of cell lysis. At 72 hpi, the decrease in ε' accelerated, appearing to reflect a more rapid decline in cell diameter. The ß-galactosidase concentration reached a maximum at the same time (results not shown here). The results for the infection at a low MOI (0.001) are shown in Figure 3(b). The growth of cells is not arrested at infection and continues for 48 hpi. The peak in the ε' profile was seen to be delayed with respect to the earlier results and occurs at about 66 hpi. However, the peak still occurs at the time of onset of virus and recombinant protein production and cell lysis. The capacitance probe has also been used successfully to monitor the biomass and infection process at higher Sf-9 cell densities of up to 5.2×10^7 cells/ml using a fed batch process (Elias et al. 2000).

Macroporous carriers are a useful means of increasing the numbers of cells in a culture which can be low, particularly in batch cultures using CHO 320 cells (Guan and Kemp 1997). One of the problems in using macroporous carriers to cultivate animal cells in culture has been to assess cell viability on-line; this is because many of the cells inhabit the macroporous infrastructure of the bead. Guan and Kemp (1997) measured the cell concentration of CHO 320 cells grown on Cytopore 1 microcarrier beads using off-line protein estimations and compared them to dielectric measurements using a BM. The results indicated that the dielectric estimations for biomasss in the microcarriers were more accurate than the protein estimations, and were also able to give a viable cell-count.

Degouys et al. (1993) used the Biomass Monitor to evaluate the concentration of anchorage dependant HTC cells grown on Cytodex microcarriers in spinner vessels. Capacitance values measured at 0.8 MHz on the Biomass Monitor were compared to measurements from a Coulter Counter Z_b. It was found that the cellular biomass estimations made from the Biomass Monitor were extremely accurate when concentrations of Cytodex of 5 g/l and higher were used. These microcarrier concentrations are those commonly used in cGMP processes for the mass production of recombinant anchorage-dependant cells.

Davey et al. (1997a) used suspensions of immobilized Chinese Hamster Ovary (CHO 320) cells that had been genetically adapted to produce interferon-γ to evaluate the relationship between capacitance and the concentration of viable cells. Dielectric data were compared with the data from a Coulter Counter (Model D) and from flow cytometry; comparisons were also made with the traditional microscope counts (haemocytometer) and the fluorescein diacetate and ethidium bromide viability assay. An excellent relationship was again observed between capacitance and viable cell number. This is important, as the conventional means of assessing biomass are not possible with immobilized cells.

Guan et al. (1998) combined on-line BM and microcalorimetric measurements to control a stirred aerobic batch culture of CHO 320 cells that had been genetically modified to produce human interferon-γ. This approach was chosen as cell growth is associated with an enthalpy change which is a direct reflection of metabolic rate. Noll and Biselli (1998) evaluated the Biomass Monitor using immobilized hybridoma cells grown in continuous suspension in a fluidized bed reactor batch culture. The capacitance data reflected changes in cell physiology and could be correlated directly to the cell metabolic activity which enabled a closed loop control of the glutamine feed rate according to the metabolic needs of the cells.

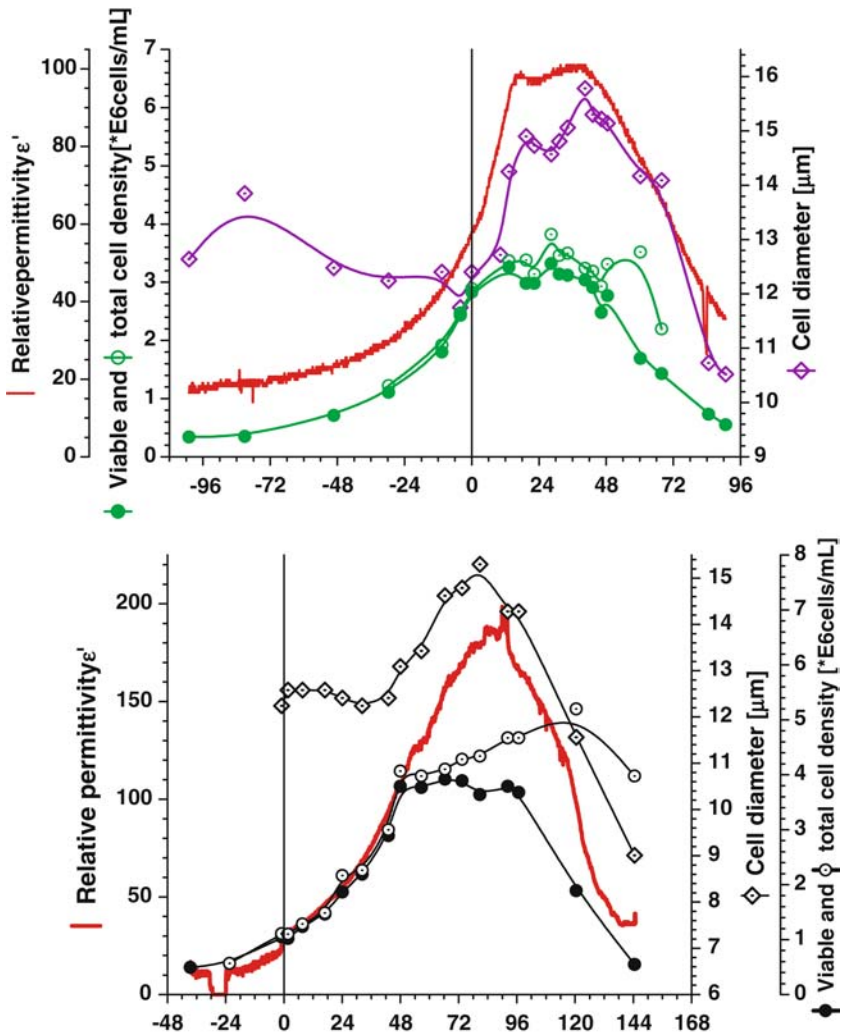

Figure 3. (a) The progress of a Sf-9-baculovirus infection process under synchronous infection (MOI = 10) (Top panel). (b) Infection under asynchronous conditions (MOI = 0.001)(Bottom panel). (Data courtesy of Cynthia Elias, CNRC, Canada).

The Biomass Monitor has been applied to industrial high density cell culture processes with CHO cells immobilized in a packed bed of Fibra-Cel disc carriers (Ducommun et al. 2002a). The Biomass Monitor was used as a tool to characterise the packed bed process. It was possible to determine both the maximum cell concentration that could be reached in the system, 2×10^{11} cells/kg of carrier and to quantify the increase of the specific protein productivity induced by the production phase from 5.14×10^{-8} µg cell^{-1} h^{-1}. RF Impedance has also been utilized to evaluate the effect of temperature on specific metabolic rates during 3 months of continuous culture of CHO cells in a 40L industrial process (Ducommun et al. 2002b).

Dowd et al. (2003) used RF Impedance to optimize and control perfusion culture for recombinant protein production (tissue-type plasminogen activator) from a CHO cell line. Perfusion feeding was automatically adjusted based on the live cell concentration from the on-line RF impedance probe. The probe allowed cell specific perfusion rate selection for optimal process operation. The exponential and death phases of CHO cells have been described by Schmid and Zacher (2004) with a Biomass Monitoring System. During fed batch cultivation the RF Impedance signal indicated changes in the physiological state of the cells.

The utility of collecting the 'Capacitance Spectrum' using scanning RF impedance has been

studied by Cannizzaro (unpublished results). He showed that differences in the measured capacitance at various frequencies can be exploited to provide valuable information on the metabolic state of the culture, to improve models for viable and non-viable cell number prediction, and even to provide an on-line estimate of cell size. In Figure 4, a phase plot is shown, whereby the capacitance at 1.05 MHz was plotted vs. the capacitance at 0.35 MHz. The end of feeding, the end of lactate utilization, and the point of zero viability are clearly elucidated. Graphical representation of the capacitance data in this way provides an online and intuitive representation of culture state, without the need for sampling. During the batch and perfusion phases, the capacitance at 0.35 MHz was linearly correlated with that at 1.05 MHz. A linear calibration model for biomass established during this period would be relatively independent of chosen excitation frequency. However, it is quite clear from the phase plots that for the latter stages of cultivation, the capacitance is dependent upon excitation frequency.

Using RF Impedance in cGMP cell culture

RF Impedance is now used in production scale processes for monitoring and controlling animal cell processes. The Biomass Monitor Models 214 and 220 and the Viable Cell Monitor 520, a RF Impedance instrument dedicated to cell culture applications, all from Aber Instruments Ltd., UK, are presently being used in cGMP production scale facilities.

The benefits from having the probe installed in a cGMP plant fall into 3 distinct categories:

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 due to high and fluctuating cell concentrations (Vits and Hu 1992) that can rapidly change environmental conditions. With infrequent manual daily sampling based and trypan blue exclusion haemocytomer cell counting, the control system can have too little information on which to base an appropriate decision to manipulate the process.

Tight control of the perfusion or concentrate addition rate allows the bioreactor to be operated under the optimum conditions for maximum recombinant protein production. In the fed-batch system, concentrate additions based on cell numbers is a straightforward control scheme. In a manner analogous to perfusion feed rate control, concentrate additions can be based on a 'per cell per day' rate.

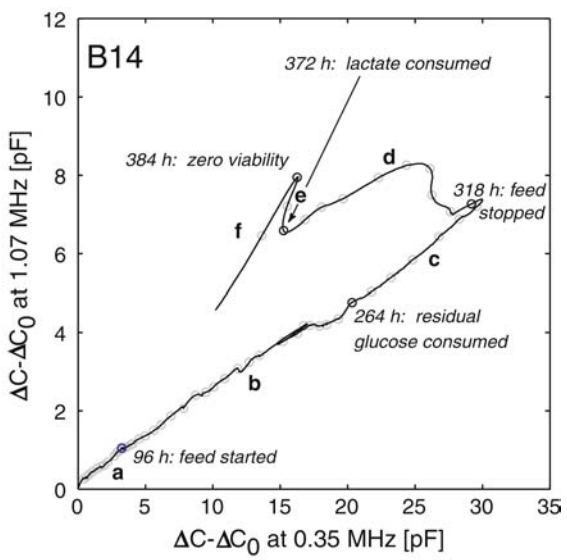

Figure 4. Capacitance at 1.05 MHz vs. the capacitance at 0.35 MHz for a CHO perfusion culture (from Cannizzaro (unpublished results), with permission).

Simple control of perfusion bioreactors, based on infrequent daily sampling and estimation of the live cell concentration, will lead to large process deviations. In the initial growth phase, prior to steady-state, the cell numbers may be doubling every day. A robust automatic perfusion rate control system based on the on-line Biomass Monitor probe is now being used in cell culture manufacturing processes. The system operates in a completely closed loop i.e. no samples need to be taken to obtain process information. In the control algorithm, a cell specific perfusion rate is specified and the Biomass Monitor cell density signal is converted into a perfusion flow rate through calculation and implementation with a variable speed controlled pump (Figure 5). An example of the actual time-dependent capacitance and conductivity trace of a perfused Hela cell culture evolving from batch (preset volume, increasing concentration) to fed-batch (increasing volume, preset cell concentration) growth conditions is shown in Figure 6 (data courtesy of Cilbiotech S.A., Belgium). The stable capacitance value can be seen when the culture is operated in a fed-batch mode with a preset cell concentration of 10^7 cells/ml. Small fluctuations of the culture medium conductivity are observed during the fed-batch culture phase. The peak in capacitance observed after operating in a fed-batch mode for 4 days represents an increased cell concentration due to an insufficient fresh medium supply to maintain a stable capacitance.

On-line optical sensors have been used in some cases to maintain the process (Merten et al. 1987; Konstaninov et al. 1992; Zho and Hu 1994; Wu et al. 1995). In general, probe performance is acceptable for high viability culture but the light based probes do not distinguish between the viable and non-viable cells and these will also detect debris that can collect during the process. Moreover the optical sensors are prone to fouling over the extensive production runs. The Biomass Monitor only measures the viable cell mass and is therefore ideal for this application and 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.

Control for perfusion bioreactors needs to consider the following three control loops: perfusion feed rate, cell purge rate and vessel level (or weight). Perfusion cultures are actively growing cultures, and some associated cellular debris can

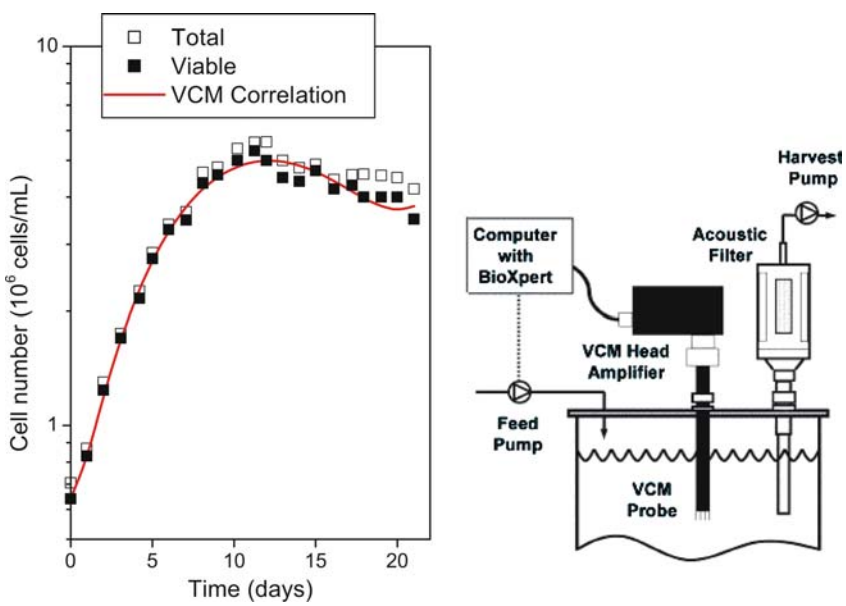

Figure 5. Schematic of a perfusion bioreactor with an Aber Viable Cell Monitor (VCM) and acoustic filter. In the control algorithm, a cell specific perfusion rate is specified and the biomass density signal is averaged and converted to a perfusion flow rate. (from Dowd and Carvell 2005, with permission).

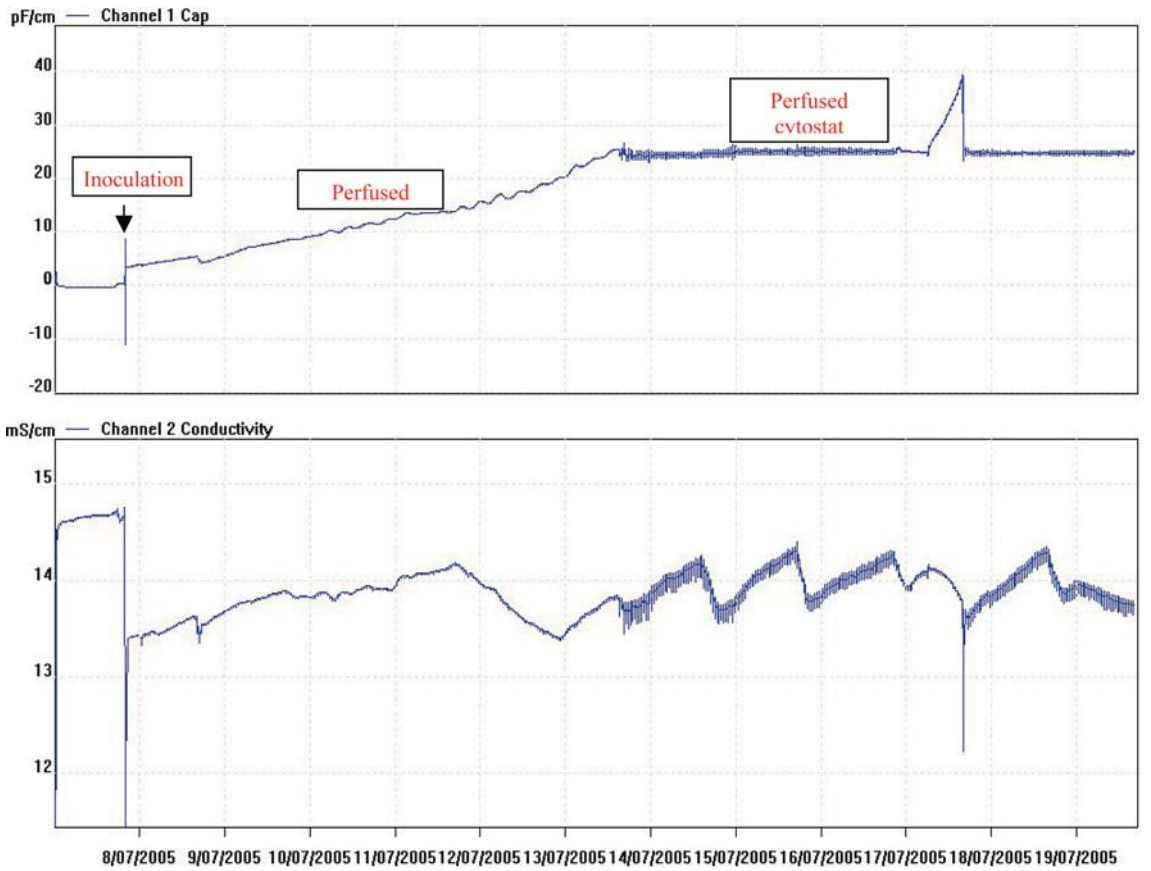

Figure 6. Time-dependent capacitance measurement with an Aber Viable Cell Monitor of a perfused Hela cell culture evolving from batch (preset volume, increasing concentration) to fedbatch (increasing volume, preset cell concentration) growth conditions. The bottom panel shows the conductivity of the medium in mS/cm tracked by the Viable Cell Monitor; significant fluctuations of conductivity are seen during the fed batch phase.(Courtesy of Cilbiotech s.a., Belgium.)

accumulate with time. Often, a cell purge is utilized, to retain a high cell growth rate and ensure that cellular debris is maintained at a low level. The vessel level (or weight) is generally a simple control loop, triggering the cell supernatant outflow to harvest. The Biomass Monitor can be used in either perfusion feed rate or cell purge rate control loops or both. The Biomass Monitor provides the process information, as summarized in Table 1.

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

Table 1. Process information provided by the biomass monitor.

Control loop	Set point	Input (process information)	Output (manipulated variable)
Perfusion feed rate	Cell specific value, obtained through optimization studies (e.g. 0.3 nL/cell/day as in Dowd et al. 2003)	Aber live cell concentration	Medium pump speed
Cell purge rate	Cell concentration	Aber live cell concentration	Purge pump speed (or on/off)

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 and so a number of companies realize 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, i.e. change of feed rates, or concentrate addition based on a cell count. These changes to feed rates or concentrate additions 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 additional significant costs to investigate non-conformance events. The Aber Biomass Monitor, in this case, is not used in any process control decisions or validation of the process, but it provides real-time information back up for the bioprocess. Used to its full potential the Biomass Monitor would supply additional information to operators and supervisors, helping to eliminate any process production losses due to operator errors in cell counting, and provide a ready means to complete non-conformance event reporting. An example is shown in Figure 7 comparing the live cell count against RF impedance derived capacitance at a Biopharmaceutical Company 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 2×10^5 cells/ml at seeding increasing to 17×10^6 cells/ml at the end of the process run (Figure 8). 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, 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' (Kell et al. 1990). 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 (Cannizaro, unpublished results). The loss of correlation between capacitance and live cell number have been reported by Zeiser et al. (1999) for insect cells once they have been infected with baculovirus and swell in size.

Monitoring microcarrier culture. Measuring the cell density of animal cells grown on microcarriers (macroporous) can only be achieved by sampling

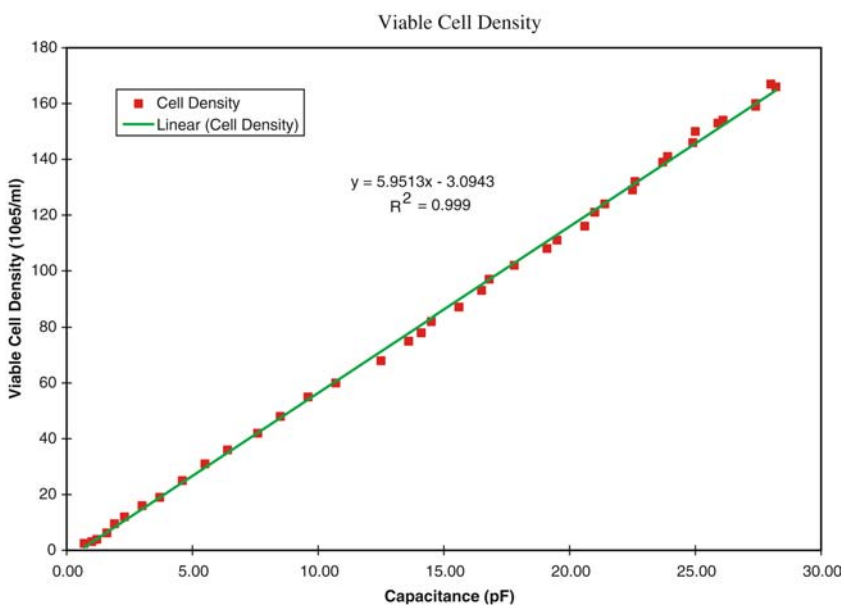

Figure 7. Live cell count vs. capacitance. Data courtesy of a Biopharmaceutical Company producing a therapeutic protein by genetically engineered SP2/0 cells using a high cell density perfusion technology.

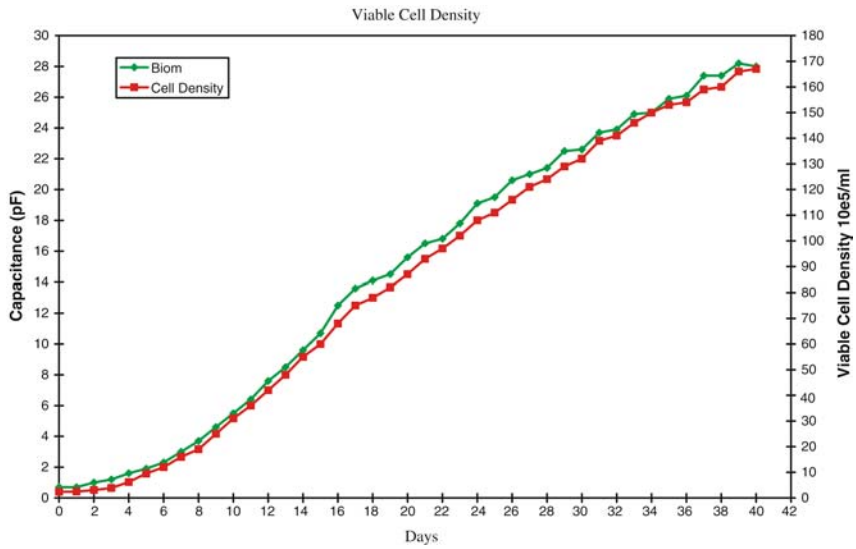

Figure 8. Monitoring live cell count and capacitance during a manufacturing process for a therapeutic protein by genetically engineered SP2/0 cells using a high cell density perfusion technology.

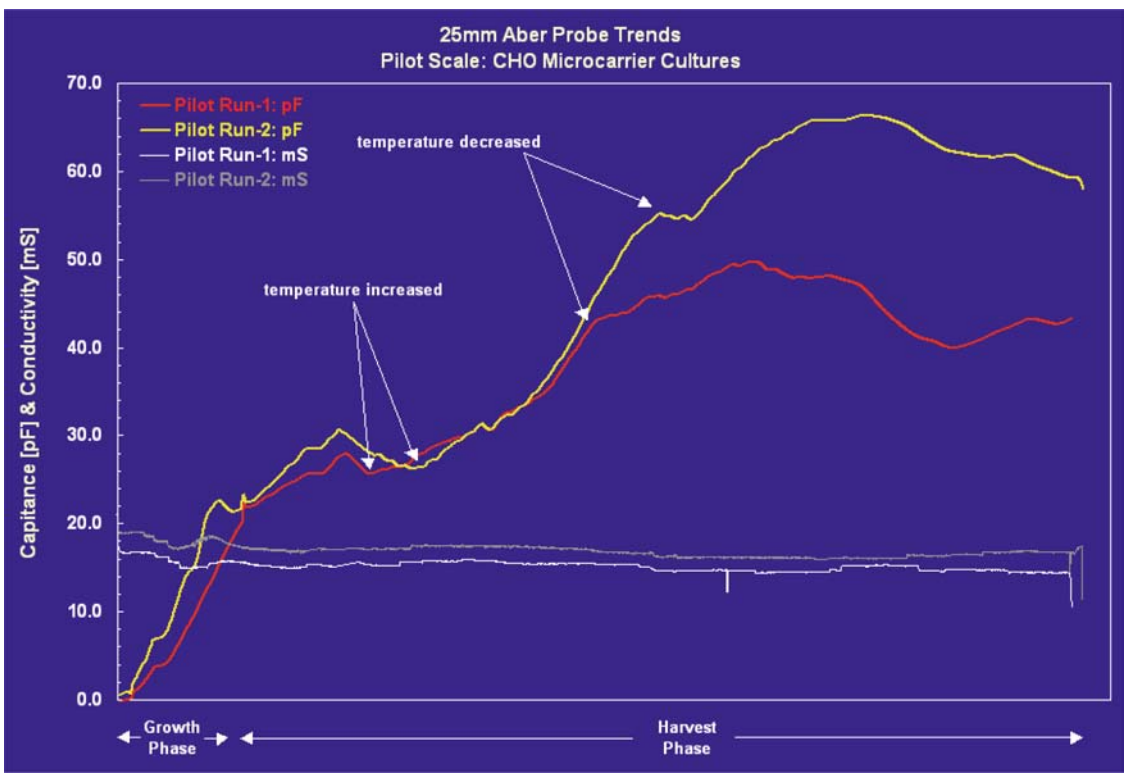

Figure 9. The application of RFI for monitoring the viable cell density of CHO cells grown on macroporous microcarriers during long term culture runs for both the growth and harvesting phases. The trace shows two runs in a pilot plant bioreactor with the viable cell concentration expressed in pF/cm and the media conductivity expressed in mS/cm. The viable cell concentration can be converted to live cells/ml using a calibration graph (data not shown). Changes in temperature can be seen to increase or decrease the rate of cell growth (data courtesy of Genzyme Inc., USA).

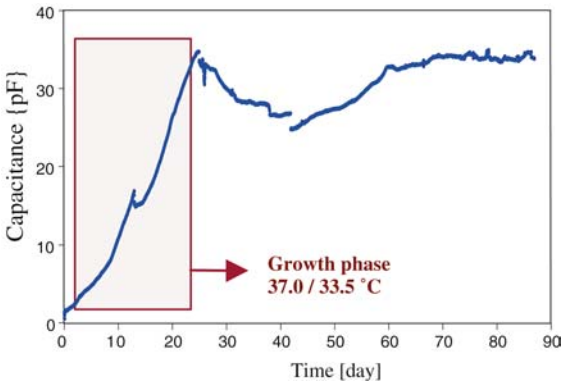

Figure 10. Monitoring CHO cells in a 40 L, industrial scale, packed bed reactor with the Biomass Monitor. The instrument was used to characterise the packed bed in terms of growth kinetics (data courtesy of Serono).

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 based on the packed bed system, 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 9). In packed bed systems, the Biomass Monitor is also 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 (Ducommun et al. 2002a, b). Figure 10 shows how the Biomass Monitor has been used at the industrial scale to monitor the live cell concentration in a packed bed reactor through the initial growth phase (up to 4×10^7 cells/ml of packed bed) followed by a stationary production phase with a high specific protein production rate.

Conclusions

The PAT initiative by the FDA will result in an increasing number of cGMP cell culture processes incorporating live cell mass monitoring probes. RF impedance based biomass measurements such as the Biomass Monitor have proven to be an accurate and reliable method of determining viable cellular biomass on-line. For manufacturing processes the instrument shown to be robust and has the advantage that it measures the live cell concentration and that it can be used in packed bed and microcarrier (surface and porous) systems.

Many companies producing under cGMP conditions are using the 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 to keep the bioreactor under optimum conditions for maximum production of recombinant proteins. In other cases the instrument has not replaced traditional off-line live cell counting techniques but it is being used as an additional monitoring tool to check production conformance.

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