REAL-TIME MONITORING OF THE BIOMASS CONTENT OF ANIMAL CELL CULTURES USING DIELECTRIC SPECTROSCOPY.

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1. Introduction.

The monitoring of the biomass content of "fermentors" is a problem common both to microbial and animal cell culture systems. The Aber Instruments "Biomass Monitor" (Science Park, Aberystwyth, Dyfed, SY23 3AH, Wales, U.K.) was developed to allow on-line and real-time biomass measurements to be made in a wide variety of culture systems from the laboratory scale up to full industrial plant [1]. Biomass measurements have been made on bacteria and fungi (including filamentous ones, [2]), and plant cells [3]. Measurements have been successful in both liquid and solid substrate fermentations [4] as well as in immobilised cell systems [5]. Applications in yeast biomass estimation have been particularly successful with dielectric measurements now routinely used for controlling the inoculation of brewery fermentors and monitoring and control of yeast fermentations [6], [7].

The application of the Biomass Monitor to animal cell culture monitoring has only occurred in earnest in the last few years [8], [9],[10]. Earlier studies had shown that the dielectric method could in principle be used to measure animal cell biomass but these measurements (for practical reasons) were not made in bioreactors and were usually done under non-physiological conditions. The Biomass Monitor has overcome many of the problems (especially high medium conductivities and electrode polarisation) that had previously prevented the application of this technology to actual animal cell cultures *in situ*.

In this paper the basic theory of measuring biomass using dielectric spectroscopy will be introduced in a highly simplified manner. Reviews of the full theory can be found in [11] and [12]. After this some of our recent animal cell culture data will be described.

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2. Theory.

The application of an electric field to a suspension of cells in an aqueous ionic solution is illustrated in Figure 1. The electric field can be considered as a force field that pushes electrical charges along, in this case the ions in the solution. It pushes the positive ions in the direction of the field and the negative ions in the counter direction. As can be seen from the figure, many of the ions both inside and outside the cells can only move so far before they bump into the cell's plasma membranes and are prevented from moving any further. The result of this is that there develops a charge separation or polarisation at the poles of the cells. The capacitance of the suspension (measured in Farads, F) gives a measure of the extent of these field induced polarisations. As the volume fraction of cells increases the amount of membranes polarised increases and so the capacitance of the suspension increases further. Dead cells and non-biomass solids do not have intact plasma membranes and so do not polarise, i.e. do not give a significant contribution to the capacitance of the cell suspension [1], [13]. It is by the measurement of a suspension's capacitance that one monitors its biomass content [1].

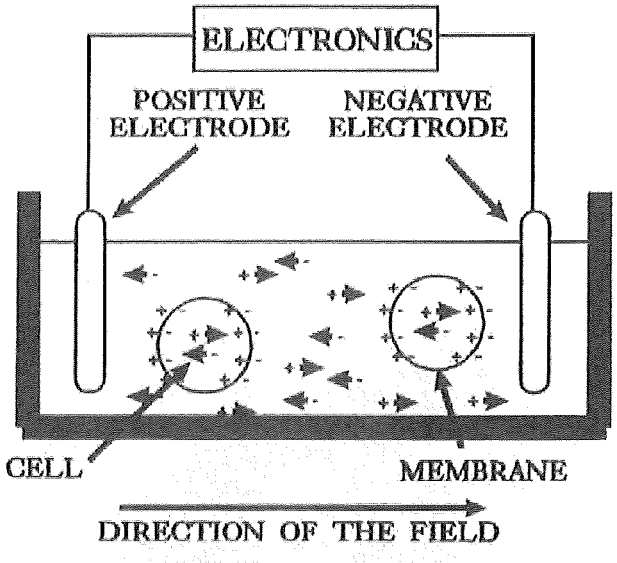

Figure 1. The effect of an electric field on a suspension of cells. Ions are shown as point charges.

So far the field has only been shown going in one direction. If one reverses the field direction then the only effect is to change the polarity (but not the magnitude) of the polarisations of the cells and so the capacitance of the suspension is unchanged.

One can of course change the rate at which the field changes direction. The number of times the field changes direction per second is measured by its frequency (units Hertz, Hz). The faster the field changes direction the higher will be its frequency.

Frequency has a profound effect on the capacitance of a cell suspension, since the ions moving up to and polarising the plasma membranes take a finite time to reach them. Figure 2 shows the typical polarisations induced across cells as the electric field frequency is increased. Also shown is how the capacitance of the suspension changes. Frequency (A) on Figure 2 is low and so a lot of ions have time to reach the plasma membranes and polarise them before the field changes direction and moves the ions the

opposite way. Thus the capacitance of the cell suspension is high. At higher frequencies (B on the figure) less ions have time to reach the membranes and so the capacitance of the suspension is also lower. At very high frequencies (C on the figure) very few ions have time to polarise the membranes and so the induced membrane polarisation is very small. At these frequencies one mainly measures the background capacitance of the medium.

From figure 2 it is seen that the capacitance of the suspension goes from a high low-frequency plateau (maximal cell polarisation, Figure 2A) to a low high-frequency plateau (minimal cell polarisation, Figure 2C). This fall in capacitance of a suspension due to the loss of induced membrane polarisation with increasing frequency is called the β–dispersion. The residual high frequency capacitance due to the medium is called C_∞ and the height of the low-frequency plateau above this is the ΔC (capacitance increment) of the β–dispersion.

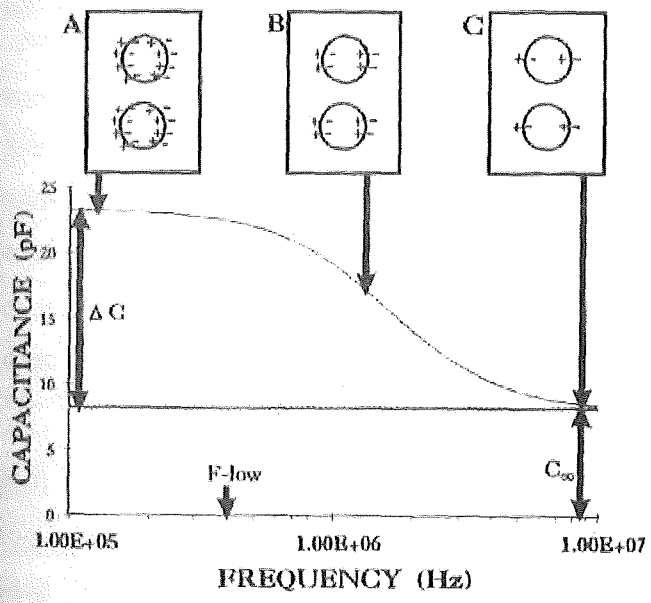

Figure 2. How the capacitance of a cell suspension varies with frequency. Also shown are the polarisations (in one direction only) of the plasma membranes of some typical cells.

If one changes the biomass concentration (volume fraction) of cells in the suspension then the only effect on the β-dispersion curve in Figure 2 is to increase the size of ΔC, which increases as the biomass content does. *Thus the problem of measuring the biomass content of a cell suspension reduces to one of measuring the magnitude of the* ΔC *of the β–dispersion* [1]. The way ΔC was estimated for the animal cell studies to be described later was by using the single frequency marked f-low on Figure 2. At zero biomass concentration the capacitance at f-low equals C_∞; thus one can measure the capacitance of the medium at f-low prior to inoculation and then back this to zero (i.e. set C_∞ to zero). This means that any change in capacitance at f-low during a culture must reflect changes in ΔC and hence biomass concentration. For a real culture one normally produces a calibration curve of ΔC versus biomass concentration (e.g. as viable cell numbers/ml or dry weight).

3. Experiments with Chinese hamster ovary (CHO) cells

Following earlier dielectric measurements of LS-L929 mouse fibroblasts [14], our current studies relate capacitance to the number concentration of viable cells in batch cultures of CHO 320 cells genetically-engineered to produce interferon-γ [15]. These are used to demonstrate the potential of the capacitance probe as a general on-line assessor of biomass in animal cell cultures.

CHO cells were cultivated in a 3-L Bioreactor System (Applikon Dependable Instruments, Schiedam, Netherlands) with controlled parameters: temperature (37°C), pH (7.25) and pO_2 (50% of the air saturated medium). A marine-type impeller was used for stirring the culture at 60 rpm. On-line computer data acquisition was by Biowatch v.2.26. Zero point of the biomass probe was calibrated with a capacitance simulator provided with the instrument. Capacitance was continuously recorded after the inoculation of the culture. Total cell number was measured by a Coulter counter, Model D (Luton, Beds). Viable cells were stained by fluorescein diacetate (FDA) and their percentage of the total was immediately determined using a Skatron Argus flow cytometer. Both total and viable cell numbers were checked by haemocytometer using FDA and ethidium bromide [16].

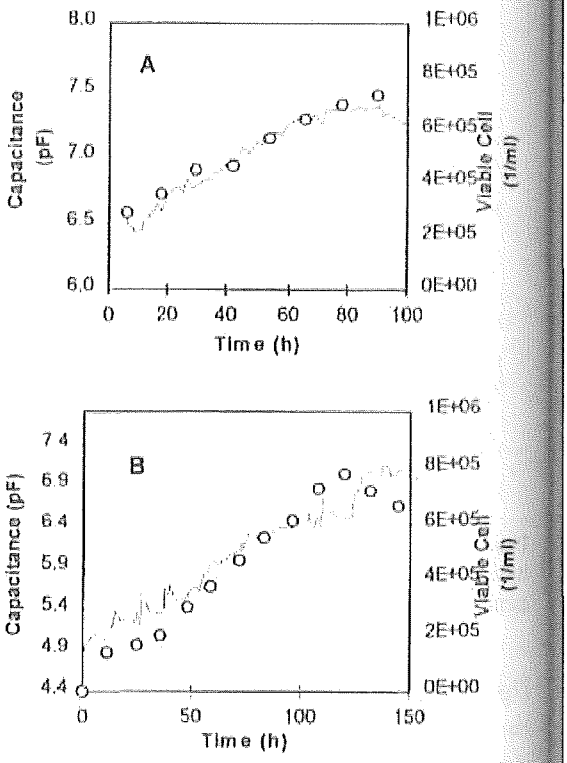

Figure 3 The relation between the number concentration of viable CHO cells in different RPMI-based defined media [15] and the on-line capacitance signal: (A) the medium is buffered by 20 mM HEPES and 4 mM biocarbonate, (B) the medium is buffered only by 20 mM biocarbonate. (o) viable cell, (—) capacitance.

Results in Figure 3 show an acceptable correlation between the viable cell number and the recorded capacitance. Other tests (data not shown) also revealed that a moving average of the Biomass Monitor signal over 1 h is an appropriate way to reduce noise. Although the theoretical relationship between the cell number concentration and the capacitance is more complex, empirically one may still explore a quantitative correlation between them, a simple form of which is:

$$\Delta C = \Delta C_0 + K N_v$$

in which ΔC and C_0 are the capacitance increments of the β-dispersion (pF) for the cell-containing and cell-free media respectively, N_v is the viable cell number (cells/ml), and K is a constant mainly determined by the type of cell and apparently also by their physiological conditions. For the results shown, the value for K is in the range of (4 - 7) X 10^{-6} pF.ml.cell^{-1}.

4. Conclusions.

These results show that dielectric spectroscopy can fill the need in animal cell culture for an on-line and real-time assay for biomass and will be particularly important in the coming field of culture on macroporous microcarriers where conventional means of assessing biomass are not possible, even off-line.

References.

1. Harris C.M., Todd R.W., Bungard S.J., Lovitt R.W., Morris J.G. and Kell D.B. (1987) The Dielectric Permittivity of Microbial Suspensions at Radio-Frequencies: A Novel Method for the Real-Time Estimation of Microbial Biomass. Enz. Microb. Tech. 9, 181-186.
2. Fehrenbach R., Comerbach M. and Petre J.O. (1992) On-Line Biomass Monitoring by Capacitance Measurement. J. Biotechnol. 23, 303-314.
3. Markx G.H., Davey C.L., Kell D.B. and Morris P. (1991) The Dielectric Permittivity at Radio Frequencies and the Bruggeman Probe: Novel Techniques for the On-Line Determination of Biomass Concentrations in Plant Cell Cultures. J. Biotechnol. 20, 279-290.
4. Davey C.L., Penaloza W., Kell D.B. and Hedger J.N. (1991) Real-Time Monitoring of the Accretion of *Rhizopus oligosporus* Biomass During the Solid-Substrate Tempe Fermentation. World J. Microbiol Biotechnol. 7, 248-259.
5. Salter G.J., Kell D.B., Ash L.A., Adams J.M., Brown A.J. and James R. (1990) Hydrodynamic Deposition: A Novel Method of Cell Immobilisation. Enz. Micr. Technol. 12, 419-430.
6. Boulton C.A., Maryan P.S. and Loveridge D. (1989) The Application of a Novel Biomass Sensor to the Control of Yeast Pitching Rate. Proceedings of 22nd European Brewing Convention. Zurich, pp653-661.
7. Markx G.H., Davey C.L. and Kell D.B. (1991) The Permittistat: A Novel Type of Turbidostat. J. Gen. Microbiol. 137, 735-743.
8. Cerckel I., Garcia A., Degouys V., Dubois D., Fabry L. and Miller A.O.A. (1993) Dielectric Spectroscopy of Mammalian Cells 1. Evaluation of the Biomass of HeLa- and CHO cells in Suspension by Low-Frequency Dielectric Spectroscopy. Cytotechnol. 13, 185-193.
9. Degouys V., Cerckel I., Garcia A., Harfield J., Dubois D., Fabry L. and Miller A.O.A. (1993) Dielectric Spectroscopy of Mammalian Cells 2. Simultaneous In-Situ Evaluation by Aperture Impedance Pulse Spectroscopy and Low-Frequency Dielectric Spectroscopy of the Biomass of HTC Cells on Cytodex 3. Cytotechnol. 13, 195-202.
10. Beving H., Eriksson L.E.G., Davey C.L. and Kell D.B. (1994) Dielectric Properties of Human Blood and Erythrocytes at Radio Frequencies (0.2-10 MHz); Dependence on Cell Volume Fraction and Medium Composition. Eur. Biophys. J. 23, 207-215.
11. Foster K.R. and Schwan H.P. (1986) Dielectric Properties of Tissues. In: Polk C. and Postow E. (eds.) CRC Handbook of Biological Effects of Electromagnetic Fields. CRC Press, Boca Raton FL., pp27-96.
12. Davey C.L. and Kell D.B. (1995) The Low-Frequency Dielectric Properties of Biological Cells. In: Walz D., Berg H. and Milazzo G. (eds.) Bioelectrochemistry of Cells and Tissues. Birkhauser Verlag, Basel, Switzerland, pp159-207.
13. Stoicheva N.G., Davey C.L., Markx G.H. and Kell D.B. (1989) Dielectric Spectroscopy: A Rapid Method for the Determination of Solvent Biocompatability During Biotransformations. Biocatalysis 2, 245-255.
14. Davey C.L., Kell D.B., Kemp R.B. and Meredith R.W.J. (1988) On the audio- and radio-frequency dielectric behavour of anchorage-independent, mouse L929-derived LS fibroblasts. Bioelectrochem. Bioenerg. 20, 83-98.
15. Hayter P.M., Curling E.M.A., Baines A.J., Jenkins N., Salmon I., Strange P.G. and Bull A.T. (1991) Chinese hamster ovary cell growth and interferon production kinetics in stirred batch culture. Appl. Microbiol. Biotechnol. 34, 559-564.
16. Kemp R.B. (1995) Cytotoxicity in an anchorage-independent fibroblast cell line measured by a combination of fluorescent dyes. Methods Mol. Biol. 43, 211-218.