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Introduction to the dielectric estimation of cellular biomass in real time, with special emphasis on measurements at high volume fractions

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Abstract

The equations that describe the magnitude of the β-dielectric dispersion of biological cell suspensions are introduced. It is then demonstrated how this magnitude can be used to monitor cellular biomass concentrations in real time. These equations are then shown accurately to describe experimental data obtained over a wide range of cell sizes and volume fractions.

Keywords: Cell suspensions; Dielectric estimations

The on-line and real-time measurement of the biomass content of industrial fermentations has long been an area of interest for the development of novel sensing devices. The problems encountered are usually considerable, however. Not only is the biomass often filamentous rather than being discrete spheres or rods but it can also occur as pellets rather than as a uniform suspension. The medium is frequently poorly defined, with a large amount of non-biomass solids which can give a high optical density even prior to inoculation. The fermentors are often aerated and this creates problems of bubbles adding significant noise to the measurements. In addition it is normally the measurement of biomass (as opposed to necromass) that is of interest, and so the sensing device must be able to distinguish the two. Here we show how the dielectric method of measuring biomass can deal with most of these problems. In addition we illustrate the effects of high cellular volume fractions on the linearity of the method.

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Electric fields, capacitance and cell suspensions

For the purposes of this paper a very simplified and intuitive description of the processes involved will be presented. Reviews of the full theory can be found, e.g., in Refs. 1 and 2.

The application of an electric field to a suspension of cells in an aqueous ionic solution is illustrated in Fig. 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 runs from the positively to the negatively charged electrode and pushes the positive ions in the direction of the field and the negative ions in

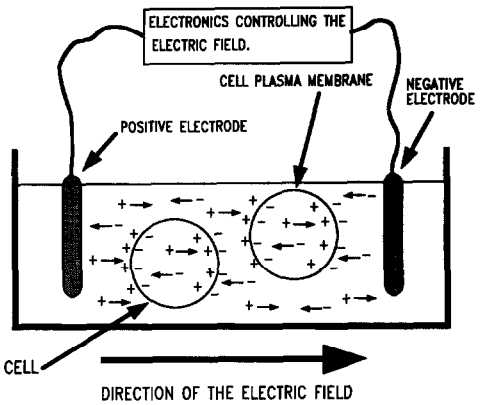

Fig. 1. The effect an applied electric field has on a suspension of biological cells. The ions are shown as point charges.

the opposite direction. As can be seen from the figure, many of the ions both inside and outside the cells can move only 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 there is a large amount of charge polarisation in the suspension in Fig. 1 then one may expect its capacitance to be high. Further, as the volume fraction of cells increases the amount of membranes polarised increases and so the capacitance of the suspension increases further. It is by the measurement of the capacitance of the suspension that one monitors the biomass content [3]. (It is also worth mentioning that the electrodes themselves can display a substantial and frequency dependent capacitance; artefacts of this type are minimised by using a 4-terminal measurement [4].)

A 4-terminal machine that monitors biomass via the radiofrequency electrical capacitance has been developed and commercialised by Aber Instruments (Science Park, Aberystwyth) and is called the Biomass Monitor (BM, formerly called the Bugmeter). It is the theory of how this machine measures biomass that forms the basis of this paper.

Frequency of an electric field and its effect on the capacitance of a cell suspension

So far the field has only been shown going in one direction. Of course one can reverse the field direction. If one does this then the only effect of the field reversal is to change the polarity of the polarisations of the cells but not their magnitude. As capacitance gives a measure of the magnitude of the field induced polarisations then changing the field direction does not change the capacitance of the suspension per se.

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 (Hz). Frequency has a marked effect on the capacitance of a cell suspension, because the ions moving up to and polarising the plasma membranes take a finite time to reach them. Figure 2 shows the typical polarisation induced across a cell as the frequency of the electric field is increased. Also shown is how the capacitance of the suspension changes. At frequency (a) on Fig. 2 the field frequency 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 oppo-

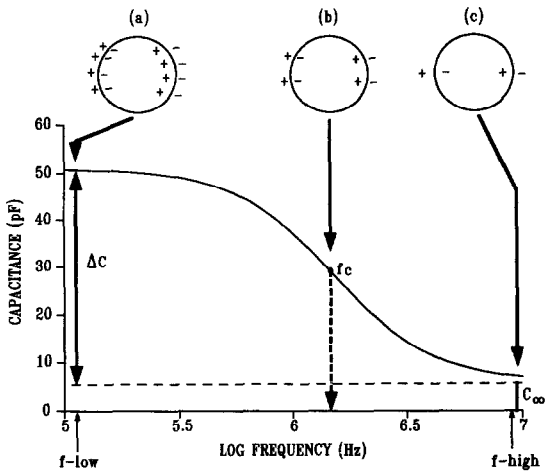

Fig. 2. How the capacitance of a suspension varies with frequency. Also shown are three cells from a suspension like Fig. 1 at a (a) low, (b) medium and (c) high frequency with respect to the β-dispersion. For convenience the induced polarisations of the plasma membranes are shown only with the field going in one direction (left to right). See the text for a full discussion of the annotation on this figure.

site way. Thus the capacitance of the cell suspension is high. At higher frequencies (b on the figure) fewer ions have time to reach the membranes and so the extent of the induced transmembrane polarisation is less and the capacitance of the suspension is also lower. At very high frequencies (c on the figure) very few ions have time to move to and polarise the membranes before the field changes direction, and so the induced membrane polarisation is very small. At these frequencies the cell's contribution to the capacitance of the suspension is very small and one just measures the background capacitance of the medium (mainly due to water dipoles).

From Fig. 2 it is seen that the capacitance of the suspension goes from a high low-frequency plateau (maximal cell polarisation, Fig. 2a) to a low high-frequency plateau (minimal cell polarisation, Fig. 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. The frequency when the fall in capacitance is half completed (i.e. the frequency when capacitance equals $C_\infty + (\Delta C/2)$ is called the critical frequency (f_c).

As one is aiming to measure the biomass content of a cell suspension then one needs to see what effect this has on the β-dispersion curve shown in Fig. 2. Figure 3 shows the β-dispersion of cell suspensions with different biomass contents. The figure shows that the f_c is not changed by the biomass content, nor is C_∞. What does change as a function of biomass content is the magnitude 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 [3].

Effect of necromass and non-biomass material on the biomass measurements

So far it has been stated that one can measure the biomass content of a suspension using dielectric means, and by the measurement of the ΔC of

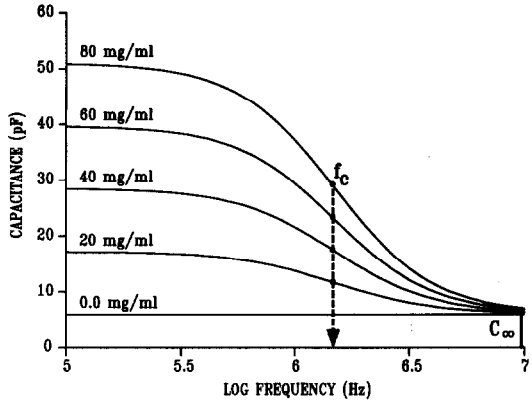

Fig. 3. The effect of increasing biomass concentrations (in mg wet weight ml^{-1}) on the β-dispersion of a cell suspension. See the text for a full discussion.

the β-dispersion in particular, but no justification for this statement has been given. If the cells in a suspension have badly ruptured plasma membranes then the ions in the solution just move through the holes in the membranes and fail to cause polarisation of the membranes even at low frequencies. Thus dead cells do not have a significant β-dispersion (i.e., a ΔC). Thus if one uses the ΔC of the β-dispersion to measure the biomass content of a cell suspension one can expect to measure only cells with intact plasma membranes (i.e., living cells) as opposed to ruptured (dead) cells [5].

Since the basis for biomass measurement via the ΔC of the β-dispersion depends on the polarisation of the plasma membranes by the application of an electric field one can expect, and indeed finds, that the mechanism works for intact cells of any size and shape.

If non-biomass solids, oil droplets or gas bubbles are present in the medium then their effect on the ΔC of the β-dispersion must be considered. The ions in a growth medium either travel straight through the non-biomass material if it is permeable to ions or just move round them if it is not. In neither case are significant charge polarisations produced (since they have no plasma membranes) and so these materials will not produce a significant ΔC term. Thus non-biomass materials are not expected to contribute to the

ΔC of the β-dispersion in a real fermentation medium.

If the volume fraction of the non-biomass material is very high then it may contribute in a negative sense to the β-dispersion curve. This is because one is replacing a finite volume fraction of polarisable material (cells and water) with non-polarisable material (non-biomass solids, oil, bubbles etc). The effect of this is to reduce C_∞, as the volume fraction of water has been reduced. ΔC is also reduced because one has reduced the volume fraction of biomass present.

Measurement of the ΔC of the β-dispersion for the estimation of cellular biomass

Earlier it was stated that the problem of biomass measurement reduces to one of the estimation of the ΔC of the β-dispersion. Thus one needs a convenient means of measuring ΔC during a fermentation. There are two ways of achieving this. Figure 2 showed a β-dispersion and marked on it were two spot frequencies labelled f-low and f-high. The capacitance at f-high is approximately equal to C_∞ whilst that at f-low approximately equals $(C_\infty + \Delta C)$. Thus one can see that if one measures the capacitance at f-high and f-low "simultaneously", and then subtracts the capacitance at f-high (C_∞) from that at f-low $(\Delta C + C_\infty)$ one gets ΔC, which is what is related to the biomass concentration. This is the principle of dual-frequency biomass measurements. The second method of estimating ΔC, and hence biomass concentration, uses the capacitance at f-low alone. At zero biomass concentration the capacitance at f-low equals C_∞ (see Figs. 2 and 3); thus one can measure the capacitance of the medium at f-low prior to inoculation and then back off this capacitance to zero (i.e., set C_∞ to zero). This means that any change in capacitance at f-low during a fermentation must reflect changes in ΔC and hence biomass concentration. This is how single-frequency biomass measurements work.

For a real fermentation one normally produces a calibration curve of ΔC (estimated by single- or dual-frequency measurements) versus biomass concentration (e.g., as dry weight). Figure 4 is such a calibration curve for a yeast. Dual- and

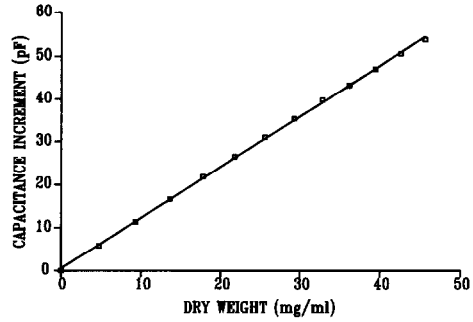

Fig. 4. A calibration curve of ΔC (capacitance increment), as judged by single frequency measurements (f-low = 0.4 MHz, cell constant = 78 m^{-1}), versus mg dry weight ml^{-1}. The linear regression fit had a gradient of 1.179, a Y-intercept of 0.547 and an r^2 of 0.9995.

single- frequency measurements using a Biomass Monitor (BM) have been successfully used to monitor the biomass concentrations in a variety of systems [6]. These include bacterial and yeast cultures [3,7,8], bacterial biofilms [9], cultured cells [10], immobilized cells [11] and filamentous cells in liquid and solid substrate fermentations [12,13]. Mishima et al. [14] have studied 2-terminal capacitance measurements as a method for biomass estimation, apparently in ignorance of the above work.

Capacitance and relative permittivity

To consider the relationship between the ΔC of the β-dispersion and the structure of the cells in the suspension giving rise to it, one cannot work easily with capacitances. The reason for this is that capacitance is a macroscopic measurement that depends on the geometry of the electrodes used. For biomass measurements this is fine because the electrode geometry remains constant. However for physical calculations the need to adjust the capacitances read to allow for the electrode geometry is an inconvenience. Thus one requires a way of quoting capacitances so that they are independent of the geometry of the electrodes. To do this one converts capacitance to relative permittivity (ϵ'). Just like capacitance, ϵ' gives a measure of the extent of the polarisations induced in a material by the application of an electric field. The capacitance (C in Farads) of a

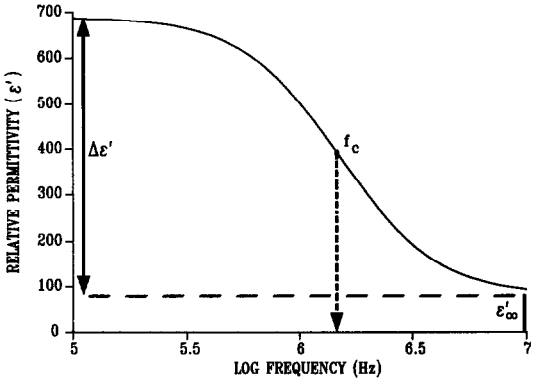

Fig. 5. The data of Fig. 2 (cell constant 120 m^{-1}) converted to ϵ' using Eqn. 1. The annotations on the figure are discussed in the text.

material is related to the equivalent relative permittivity (ϵ') by:

$$\epsilon' = C(k/\epsilon_o) \quad (1)$$

ϵ_o is the permittivity of free space and is a constant with a value of 8.854×10^{-12} F m^{-1}. k is known as the cell constant and has units of m^{-1}, reflecting the geometry of the electrodes. The important point is that electrodes of different geometries (different k values) will record different capacitances for the same cell suspension, but will all produce the same ϵ' value.

Relative permittivity (ϵ') of a cell suspension in the region of the β-dispersion

From Eqn. 1 it is clear that the relative permittivity of a material (e.g., a suspension) is equal to the capacitance multiplied by a constant for a given electrode system of (k/ϵ_o). Taking this into account then a β-dispersion plot like Fig. 2 is reflected in the permittivity plot of Fig. 5. ΔC becomes $\Delta\epsilon'$ (dielectric increment) and C_∞ becomes ϵ'_∞; all that has happened is that the capacitance terms have been multiplied by (k/ϵ_o) to convert them to ϵ' values. The f_c is the same in both plots. The formula that relates the magnitude of $\Delta\epsilon'$ (and hence the ΔC) to the properties of the biological cells in the suspension is:

$$\Delta\epsilon' = (9PrC_m)/4\epsilon_o \quad (2)$$

Here P (which is unitless) is the volume fraction of cells present (i.e., the volume of material bounded by a plasma membrane, per unit volume of suspension), r is the radius of the nominally spherical cells from the cell centre to the plasma membrane (in metres) and Cm is the plasma membrane capacitance per unit of membrane area (F m^{-2}). This gives a measure of the ability of the plasma membrane to store charge. For biological systems C_m typically has a value of 0.01 F m^{-2}.

For a given cell suspension, r and C_m are constant and ϵ_o is a physical constant anyway. Thus a plot of $\Delta\epsilon'$ versus cellular volume fraction is a straight line of gradient $(9rC_m/4\epsilon_o)$. This relationship explains why ΔC is linearly proportional to biomass concentration.

$\Delta\epsilon'$ (and ΔC) of the β-dispersion at high volume fractions

Equation 2 holds only for "low" volume fractions, in which the electric field impinging on a given cell in the suspension has not been distorted by the cells around it. At high volume fractions this does not apply, and a plot of $\Delta\epsilon'$ vs. volume fraction begins noticeably to plateau out (at say $P > 0.15$, i.e., approximately 150 mg wet weight ml^{-1}). From the point of view both of academic studies on cell structure (e.g., estimating C_m using Eqn. 2) and the industrial measurement of biomass concentrations, one needs to be able to model and compensate for this loss of linearity.

Schwan and Morowitz [15] suggested a modification to Eqn. 2 that allowed for this non-linearity at high volume fractions (P):

$$\Delta\epsilon' = (9rC_m)/(4\epsilon_o) \cdot P/[1 + (P/2)]^2 \quad (3)$$

This is Eqn. 2 with the additional term $1/[1 + (P/2)]^2$ that models the non-linearity at high values of P. This additional factor depends only on the volume fraction of cells present and is independent of the cell radius. Although this equation is potentially of great use, it had never been checked thoroughly with real cells. By making careful and independent measurements of P (by two different methods), r and $\Delta\epsilon'$ for a number of bacterial and yeast suspensions, we have recently shown that this equation does indeed hold true over a wide range of cell sizes and volume

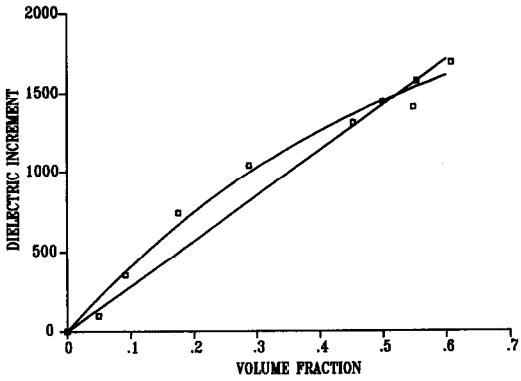

Fig. 6. The dielectric (permittivity) increment ($\Delta\epsilon'$) as a function of volume fraction for Distillers Co. Distillery yeast. The straight and curved lines are for the best fits of Eqns. 2 and 3 respectively to the data using non-linear least squares fitting. See Ref. 16 for a full description.

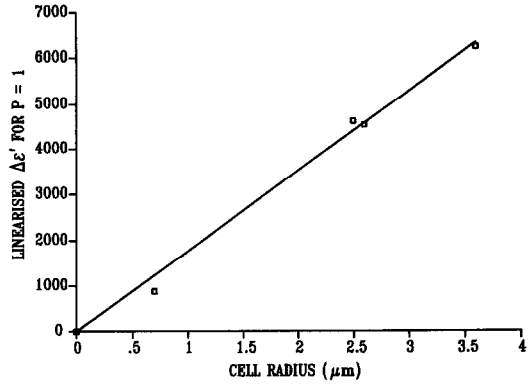

Fig. 8. A plot of $\Delta\epsilon'$ as linearised for a volume fraction of 1 vs. the radius of the cells used. Data are shown for one bacterial strain (Micrococcus luteus) and three strains of the yeast Saccharomyces cerevisiae (DCL Baker's and Distillery yeasts, and a strain called BB 11). See Ref. 16 for details.

fractions [16]. Figure 6 shows how the $\Delta\epsilon'$ varies as a function of volume fraction for a strain of the yeast *Saccharomyces cerevisiae*. Also shown on the plot are the least squares fits of Eqns. 2 and 3 to the data (see Ref. 16 for full experimental details). For both fits $\Delta\epsilon'$ and P were measured for the cell suspensions used and so the fits were achieved by iteration of the $(9rC_m/4\epsilon_o)$ terms in each equation. Equation 3 produced a visually as well as a statistically better fit to the data than did Eqn. 2.

Equation 3 can be modified by replacing all the volume fraction terms P by $V_{sp}C_n$. Where V_{sp} is the specific enclosed volume of the cells (i.e., the volume enclosed by the plasma membranes of the cells, per unit biomass) in ml (mg dry weight)$^{-1}$. C_n is the dry weight of the cells in mg ml^{-1}. That the correction factor for the nonlinear relationship between $\Delta\epsilon'$ and P is indeed independent of the cell radius has the important and useful consequence that a simple calibration curve of dielectric increment vs. dry weight or cell numbers permits one to determine the specific enclosed volume of the strain of interest, i.e., the volume enclosed by the cytoplasmic membranes of the cells per unit biomass (Fig. 7).

From Eqn. 2 one would expect that a plot of $\Delta\epsilon'$ per unit volume fraction (either measured at low P or linearised) vs. the cell radius to give a straight line. This is shown in Fig. 8.

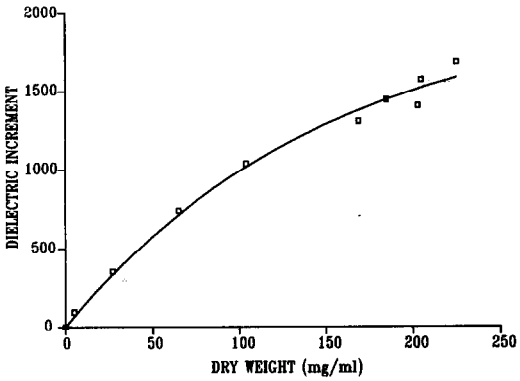

Fig. 7. The $\Delta\epsilon'$ data in Fig. 6 replotted versus cell dry weight ml^{-1}. The curve on the plot is the non-linear least squares best fit of Eqn. 3 with $V_{sp}C_n$ replacing the P terms. See the text for details. The best fit for V_{sp} was 3.5 ml mg^{-1}.

Conclusions

This paper has discussed the theory behind how the Aber Instruments Biomass Monitor measures biomass concentrations. In addition data were presented to demonstrate that the $\Delta\epsilon'$ (ΔC) of the β-dispersion, which is what is measured by the BM to monitor biomass, is linear with volume fraction to very high levels. At concentrations where linearity is lost it is shown that this is predictable using a simple equation that can be implemented as part of a linearising routine.

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