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# The dielectric permittivity at radio frequencies and the Bruggeman probe: novel techniques for the on-line determination of biomass concentrations in plant cell cultures

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**Summary**

A novel technique is described for the measurement of the volume fraction of biomass in a suspension by the simultaneous measurement of the conductivity of a suspension containing cells and of the medium in which the cells are suspended. The presence of non-conducting particulate matter in a suspension will cause the conductivity of a suspension to be decreased relative to that of the medium in which the particles are suspended. A simple equation (the Bruggeman equation) describes the relationship between the volume fraction of non-conducting particulate matter and the decrease in conductivity. The accuracy of this method for the determination of the biomass concentration of plant cells (*Festuca arundinacea*) in culture was shown. The method was successfully applied to the on-line determination of biomass concentrations during the growth of *F. arundinacea* cultures, and gave good agreement with biomass levels as determined from measurements of the radio-frequency dielectric permittivity of such cultures.

Biomass; Sensor; Conductivity; Bioreactor; Plant cell culture; Dielectric; Permittivity; Capacitance

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## Introduction and Theory

There is a continuing need for a reliable method for the on-line determination of biomass concentration (Harris and Kell, 1985; Clarke et al., 1986; Montague et al., 1989; Kell et al., 1990). It is generally considered (op. cit.) that the most useful approaches would be based on a physical method of measurement. The electrical properties of cell suspensions have already proven to be a rich source of useful biomass detectors (Harris and Kell, 1985; Kell and Davey, 1990). Faradaic reactions at the electrodes, in other words reactions in which charge is transferred across the electrodes can be used to measure the activity of the organisms in a suspension and give an indirect measure of the biomass present (e.g. Wilkins et al., 1978; Holland et al., 1980; Matsunaga et al., 1980; Aston and Turner, 1984). However, changes in the non-faradaic electrical properties can give more direct measures of biomass (Harris et al., 1987; Kell and Todd, 1989; Kell, 1990).

The frequency-dependent, non-faradaic electrical properties of condensed matter, including cell suspensions, are described by the impedance or admittance, the latter being, in effect, the vector sum of the permittivity and the conductivity (see Kell, 1987; Kell and Davey, 1990). Equipment designed for impedance and admittance measurements on biological cell suspensions is commercially available. The Bactometer from Bactomatic (Cady et al., 1978) measures changes in fluid and electrode impedances (Hause et al., 1981) that are related to bacterial growth (Firstenberg-Eden and Eden, 1984). Permittivity changes at radio frequencies are used by the Bugmeter device for biomass measurements, and this method has already proven to be very useful for the estimation of biomass, and its accretion in a number of types of bioprocess (Harris et al., 1987; Davey et al., 1988; Boulton et al., 1989; Stoicheva et al., 1989; Markx and Kell, 1990; Markx et al., 1990; Salter et al., 1990), including studies with plant cell cultures (Markx et al., 1991). The Bugmeter relies on the measurement of the biomass-dependent change in the radio-frequency permittivity, an effect referred to as the beta-dispersion (see Schwan, 1957; Pethig and Kell, 1987) and caused by charge separation across the cell membranes via a Maxwell-Wagner type of mechanism. The overall decrease in the permittivity that occurs when the frequency of measurement is changed from values that are very low and very high with respect to the charging time of the membrane capacitance (which is in the microsecond range) is known as the dielectric increment $\Delta\epsilon$, and (for spherical cells) has a magnitude:

$$\Delta\epsilon = 9PrC_m/4\epsilon_0 \qquad (1)$$

where: $\Delta\epsilon$ = drop in permittivity from "low" to "high" frequency, $P$ = volume fraction of biomass, i.e. the fraction of the total volume of a suspension which is enclosed by the plasma membranes of the cells, $r$ = cell radius (m), $C_m$ = membrane capacitance per unit area (F $m^{-2}$), $\epsilon_0$ = permittivity of free space ($8.854 \times 10^{-12}$ F $m^{-1}$).

The conductivity of a suspension is another useful source of biochemical information which remains relatively unexplored. Many cell types change the conductivity of the medium by the uptake or secretion of ions. This can be used to

monitor cell activity and hence cell biomass (Firstenberg-Eden and Eden, 1984; Ryu et al., 1990); equipment for this is also available commercially, for example that produced by Malthus Instruments (see Richards et al., 1978; Jason, 1983; Easter and Gibson, 1989; Kell and Davey, 1990).

Another effect of the presence of non-conducting particulate material (e.g. cells) is that the conductivity of a suspension is decreased relative to that of the suspending medium. This effect has also been exploited for the measurement of suspended solids concentrations in sewage sludge (Grune, 1965), in mitochondrial suspensions (Irimajiri et al., 1975) and of the biomass content of immobilised cell systems (Lovitt et al., 1986). The decrease in the (low-frequency) conductivity has been described by the formula (Fricke, 1924; Fricke and Morse, 1925; De La Rue and Tobias 1959):

$$P = X(\sigma_0 - \sigma)/(\sigma + X\sigma_0) \tag{2}$$

where: $P$ = volume fraction of non-conducting particulate material, $\sigma_0$ = conductivity of the suspending medium, $\sigma$ = conductivity of the suspension and $X$ = shape factor ($X = 2$ for spheres, $X$ approaches 1.5 for ellipsoids). Dividing top and bottom of the right-hand-side of Eq. (2) by $\sigma_0$, we have:

$$P = X(1 - R)/(R + X) \tag{3}$$

where $R = \sigma/\sigma_0$.

It may be seen from Eqs. (2) and (3) that the change in conductivity is slightly dependent on the shape of the particles. However, for the volume fractions usually encountered in biological cell suspensions the specific decrease in the conductivity can be regarded as being independent of cell shape. This can be seen in Fig. 1, where $P$ is plotted versus $R$ (the ratio $\sigma/\sigma_0$) for different values of $X$.

For moderate volume fractions ($< 0.3$) the simpler Bruggeman equation (Bruggeman, 1935) can be used (Harris and Kell, 1983):

$$R = \sigma/\sigma_0 = (1 - P)^{(1.5)} \tag{4}$$

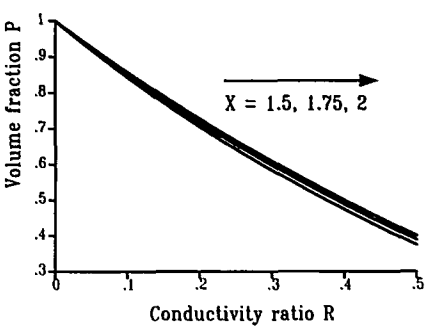


Fig. 1. Effect of different shape factors $X$ on the relation between the volume fraction $P$ and the conductivity ratio $R$. The curves are calculated using Eq. 3. It is evident that for practical purposes the relation between $R$ and $P$ is independent of the shape factor $X$ for $P < 0.3$.

It has been claimed (Irimajiri et al., 1975) that the Bruggeman equation is more accurate for non-spherical particles.

In the above equations it is assumed that the particles are completely non-conducting, which is close to the truth for living cells. Schwan and Foster (1980) and Foster and Schwan (1986) describe the situation when the particles are significantly conductive.

The purpose of the present article is to describe the use of this method for the determination of biomass concentrations and to show the feasibility of its use on-line for following the growth of plant cell cultures.

## Materials and Methods

### *Cell material*

*Festuca arundinacea* (tall fescue) was grown at 25°C, 150 rpm on MS medium (Murashige and Skoog, 1962) with 3% sucrose and 3 mg $l^{-1}$ 2,4-dichlorophenoxyacetic acid. Shake flasks of 250 ml were inoculated by the addition of 20 ml of a 1-week-old suspension to 80 ml medium. Plant cells for the calibration curves of the Bruggeman probe were harvested 18 d after inoculation. By this time cells would be in stationary phase. Cell suspensions for measurement of the growth curve in shake flasks in time were obtained similarly, but the cells were harvested at different times. The cells used had been in culture for over 3 years. For on-line measurements, cells were grown in the same medium in a gas-lift bioreactor described previously (Harris et al., 1987) with a working volume of 1000 ml. The 25 mm Bugmeter probe was inserted through the bottom of the bioreactor vessel, whilst the Bruggeman probe was inserted through the vessel's top plate.

### *Fresh and dry weights*

Fresh weights for the first experiments in which the linearity of the results of the different electrical methods for biomass determination with the biomass present in the suspension were obtained by filtering 5 ml plant cell suspension through preweighed 25 mm 0.22 $\mu$m Whatman filters.

Other fresh weights were obtained by filtering $3 \times 5$ ml plant cell suspension over prewetted, preweighed 25 mm paper Whatman filters.

Dry weights were obtained by drying the above preweighed filters overnight in a drying oven (60°C).

### *Permittivity*

Dielectric permittivities were measured using a 4-terminal dielectric spectrometer ("Bugmeter", Aber Instruments Ltd, Aberystwyth, Dyfed, U.K.) designed for the measurement of biomass in bioreactors. Different Bugmeters were used in the

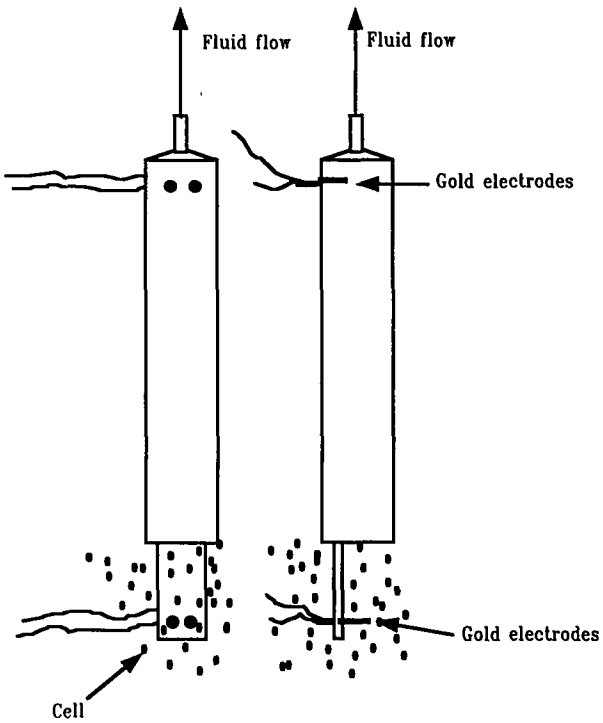


Fig. 2. Diagram of the Bruggeman probe. The probe (of which two elevations are shown) was constructed from a 10 ml syringe and has two pairs of gold electrodes for the conductivity measurements. Separation of medium from the suspensions occurs in the tube by gravimetric settling of the cells.

experiments, with electrodes with cell constants of 0.785 $cm^{-1}$ and 0.830 $cm^{-1}$ (shake flask experiments) and 0.755 $cm^{-1}$ (bioreactor).

*Conductivity*

Conductivities were measured using model ECC 251 conductivity meters from EDT Research (London, U.K.). The conductivities were measured using an electrode of conventional design with parallel platinum black electrodes. The cell constant of the electrode system was determined to be 0.689 by measurement of a KCl solution of known conductivity. The operating frequency of this instrument was 1423 Hz, 86 mV p-p.

*Bruggeman probe*

A "Bruggeman probe" was constructed from a 10 ml syringe, as illustrated in Fig. 2. Electrodes were made from gold wire of 1 mm diameter and had a length of 7 mm. Conductivities were measured by connecting the two pairs of electrodes to 2

ECC 251 conductivity meters as described above. The cell constants were measured to be 0.0439 and 0.0614 for the upper and lower electrodes respectively by the measurement of a KCl solution of known specific conductivity.

A peristaltic pump was used to pump suspension up continuously (flow rate 2.5 ml $min^{-1}$, silicone rubber tubing with 2.5 mm inner bore). The lower electrodes were 2 cm below the bottom of the syringe. Insulation of the connections was achieved using silicone rubber sealer. In the tube the plant cells settle out due to gravity, separating the cells continuously from their suspending medium. Thus the conductivity of the medium can be measured in the top of the tube at the same time as the conductivity of the suspension is measured outside the tube. The medium is in temperature equilibrium with the suspension. For use on-line, the probe was autoclaved in situ.

## Results and Discussion

Fig. 3 shows the volume fraction of plant cells obtained by measurement of the conductivities of suspension and suspending medium vs the dry weight, fresh weight or permittivity at 0.4 MHz of the cells in suspension. The conductivity of the medium was in the range 1–2 mS $cm^{-1}$. Essentially linear plots are obtained between the volume fraction determined from the ratio of conductivities of suspension and the surrounding medium, and the other methods used to determine biomass concentrations. The permittivity measurements show the best correlation between the volume fraction calculated from the conductivities and the other biomass determinations. This is due to the large sampling errors in the determination of the dry and fresh weights of plant cell suspensions, while this is not the case in the permittivity measurements (see also Kell et al., 1990). In terms of the sensitivity of these methods, we estimate that the precision of the Bruggeman probe is of the order of 3%, which from Eq. (4) and the data in Fig. 3A corresponds to an uncertainty in the dry weight of $\pm 1.5$ g $l^{-1}$. Similar reasoning based on a precision of $\pm 1$ pF and the data in Fig. 3C indicates that for the permittivity measurements made with the instrument used, the uncertainty in the equivalent dry weight is approximately $\pm 0.5$ g $l^{-1}$.

Fig. 4 shows the volume fraction of suspended plant cell material obtained from the conductivities by the use of the Bruggeman equation plotted against the conductivity of the medium. The volume fraction obtained is relatively independent of the conductivity of the medium over most of the range of conductivities investigated. A rather large dependence on the conductivity is seen at very low conductivities ($< 1$ mS $cm^{-1}$), while a slight decline is seen with increasing conductivity at higher conductivities ($> 4$ mS $cm^{-1}$). Both declines arguably reflect the effect of the conductivity of the suspension on the turgor of the cells (Glaser and Donath, 1988).

Fig. 5 shows a plot of the volume fraction, obtained by measurement of a plant cell suspension using the Bruggeman probe as described in Materials and Meth-

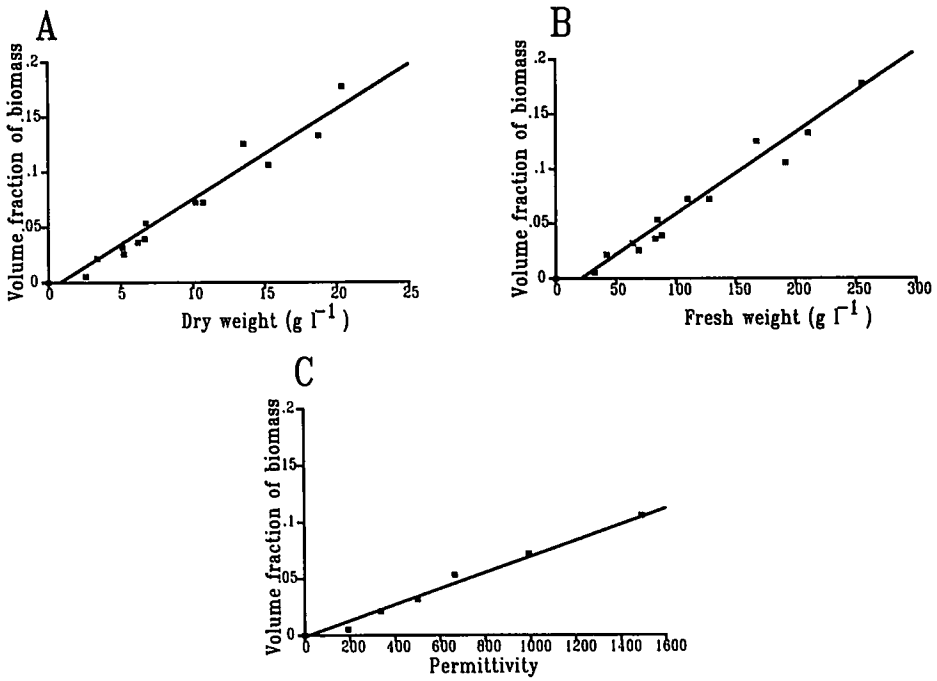


Fig. 3. The volume fraction of plant cell biomass calculated from the conductivities of medium and cell suspensions using the Bruggeman relation (Eq. 3), as a function of (A) the dry weight (volume fraction = $8.61\times10^{-3}\times$dry weight $-1.18\times10^{-2}$, correlation coefficient = 0.96), (B) the fresh weight (volume fraction = $7.19\times10^{-4}\times$fresh weight $-1.32\times10^{-2}$, correlation coefficient = 0.96) and (C) the dielectric permittivity at 0.4 MHz (volume fraction = $7.55\times10^{-5}\times$permittivity $-3.7\times10^{-3}$, correlation coefficient = 0.99). The best correlation exists with permittivity, since sampling errors are the lowest with this method.

ods, vs the dry weight of plant cells. Again a good relation can be seen between the two methods of biomass determination.

In the next experiment displayed, we studied the changes in biomass level during the batch culture of *Festuca* in shake flasks in terms of dry weight, fresh weight, permittivity and the Bruggeman method. The results are plotted in Fig. 6. The results show that for most of the growth period the fresh weight, permittivity and Bruggeman probe give biomass levels which are very similar, but that the dry weight does not show as large an increase as the other methods. It is a well known phenomenon in plant cell cultures that cells increase their size at certain stages of growth, particularly in the stationary phase of carbon-limited cultures (Yeoman and Street, 1977). This increase in cell size will be detected by the measurement of the fresh weight, the permittivity and the Bruggeman probe measurements, as they are methods that give signals that are related to the volume fraction of biomass in the suspension. Dry weight however, is not directly related to the volume fraction of cellular material.

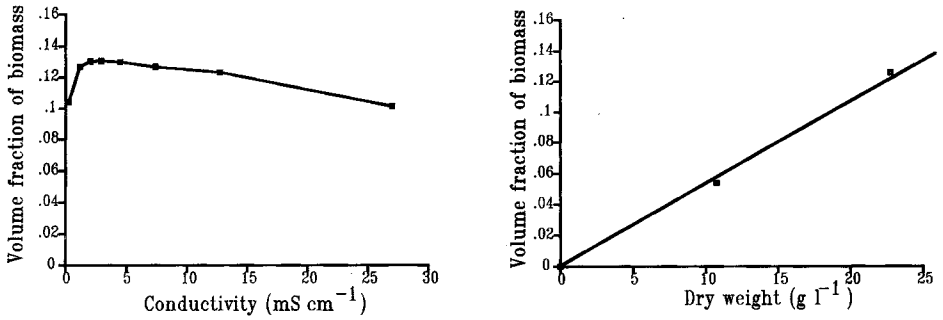


Fig. 4. Change in the volume fraction calculated from the conductivities of plant cell suspensions and suspending medium, plotted vs the conductivity of the medium. Changes in the volume fraction measured remain limited over the range of conductivities measured.

Fig. 5. Results of the on-line measurement of cell biomass using the Bruggeman probe. Experiments were carried out as described in Materials and Methods. The volume fraction of plant cells is plotted against dry weight. The results show that on-line measurement of plant cells using the Bruggeman probe is feasible.

Having shown in shake flasks that the method can in principle be used for the on-line determinations of volume fractions of biomass in bioreactors, a culture of *Festuca arundinacea* was started in which biomass was estimated using both dielectric permittivity and Bruggeman probe measurements. Earlier experiments had shown that the cell constant of the Bruggeman probe system was not stable in long-term experiments when the conductivity meters were left on all the time. This was presumably due to the fact that the currents produced by the conductivity meters, which were not really designed for continuous measurements, changed the cell constants of the electrode. This meant that the Bruggeman probe could only be used discontinuously during this experiment. By selection of more suitable electrodes or meters it should be possible to overcome this problem.

Figs. 7A, B show the changes in the permittivity and the conductivity in the frequency range 0.1–10 MHz at different times during the growth of *Festuca* in the

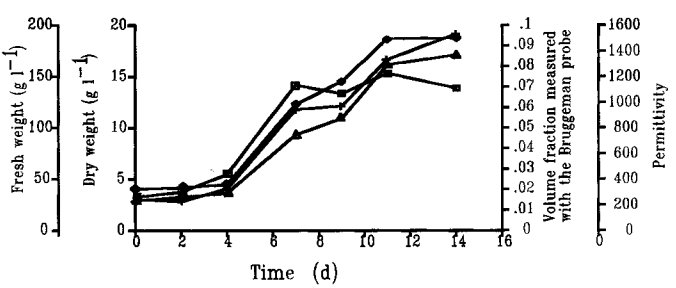


Fig. 6. Measurement of the biomass levels during the growth of *Festuca* in shake flasks, using different methods. [■] Dry weight, [+] Fresh weight, [▲] Permittivity, [◆] Volume fraction measured with the Bruggeman probe.

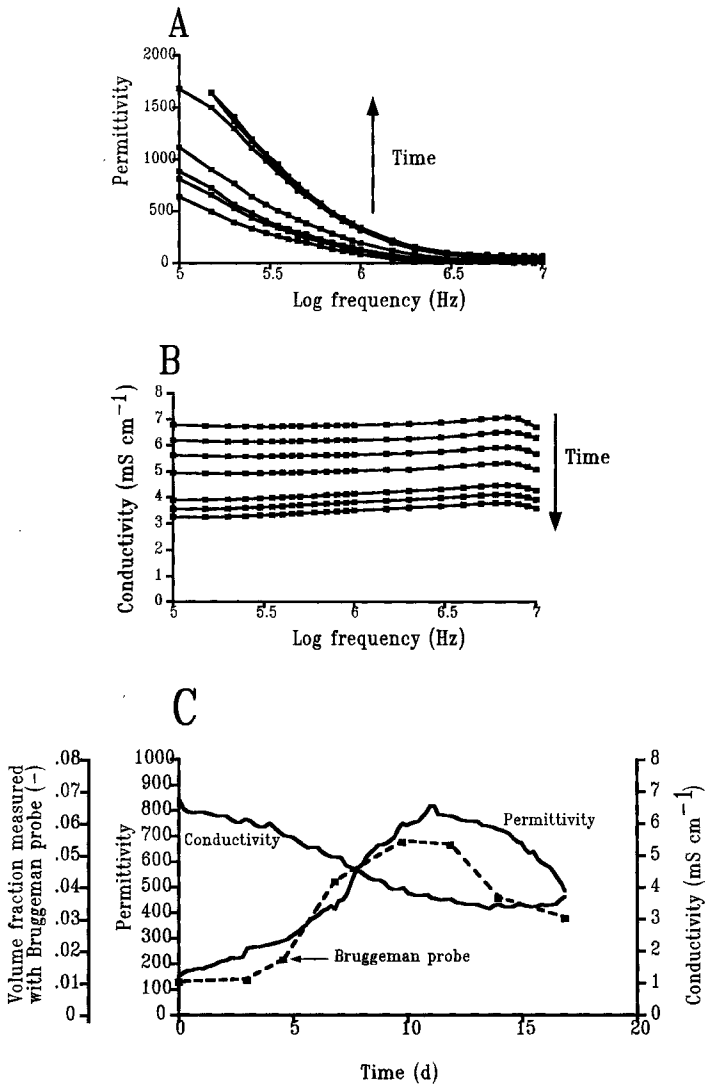


Fig. 7. On-line measurement of biomass during a batch culture of *Festuca*. The biomass levels were measured by permittivity measurements, low-frequency conductivity measurements and with the use of the Bruggeman probe. (A) Frequency-dependent permittivity and (B) conductivity measurements at 0.1–10 MHz in the bioreactor after periods of 0, 2, 4, 7, 9, 11 and 14 d after inoculation; later samples have greater low-frequency permittivities and smaller conductivities. (C) Time-dependent changes in the permittivity and conductivity at 0.4 MHz and in the volume fraction estimated by means of the Bruggeman probe.

bioreactor. The results clearly show the increase in the permittivity at lower frequencies due to the increase in cell volume fraction and the decreases in the (low-frequency) conductivity when the cells take up ions from the medium (and of course because of the increase in volume fraction of the cells per se).

Fig. 7C shows the time-dependent changes in the permittivity and conductivity of the bioreactor medium at 0.4 MHz, and the volume fraction of biomass as measured with the Bruggeman probe. The changes in the permittivity and the volume fraction measured with the Bruggeman probe all show an increase in the biomass level during the first 12 d. After day 12 growth stops and the culture becomes stationary. A phase of cell lysis may then be seen in the Bruggeman and permittivity measurements, though it is not reflected in the low-frequency conductivity measurements.

## Conclusion

The initial experiments with plant cells confirmed that the Bruggeman equation can be used to estimate the volume fraction of biomass in suspension up to very high volume fractions. The method can be used over a large range of conductivities. On-line application of the method for the measurement of the volume fraction of plant cells by use of the Bruggeman probe, based on the simultaneous measurement of plant cell suspension and suspending medium by gravimetric separation, was demonstrated.

More general on-line applications of the Bruggeman method will always rely on the possibility of measuring the conductivity of the suspension and the suspending medium separately. Thus one always has to have some way of separating the medium from the suspension. With smaller organisms, such as bacteria and to some extent yeasts, continuous separation of the medium is an arduous task (unless the cells have been immobilised (Lovitt et al., 1986)), which would make the application of the method to on-line determination of biomass concentration difficult. However, application of this method to the on-line determination of the biomass concentration of large cell types such as fungi, plant, and (perhaps) animal cells seems to be more straightforward. The "Bruggeman probe" built for the on-line measurement of the volume fraction of plant cells in suspension in the present work relied on the separation of plant cells from the medium by gravity. Other on-line methods for the separation of medium from a suspension, such as dialysis or filtration, can easily be envisaged.

Whilst the Bruggeman method gave generally excellent agreement with the measurements based on dielectric permittivity, the latter were much more convenient to use for continuous, on-line measurements, in that no mechanical pumping system was necessary. In addition, the sensitivity of the dielectric measurements is significantly greater. However, the Bruggeman approach is somewhat cheaper to implement. Notwithstanding, we have shown in the present work that both types of approach can provide novel and extremely convenient means for the on-line determination of biomass in plant cell cultures.

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