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REAL-TIME MEASUREMENT OF CELLULAR BIOMASS USING DIELECTRIC SPECTROSCOPY

Whilst much public concern has been aroused in recent years due to a number of outbreaks of food-borne microbial diseases, workers in he fermentation industry normally wish to stimulate the rate or extent of microbial biomass production. It is not difficult [Kell *et al* 1990] to find statements to the effect that 'estimation of microbial biomass is by far the single most important parameter of any fermentation process' [Fung 1988]. To this end, we and others have argued [Clarke *et al* 1985, Harris & Kell 1985] that of all possible approaches only physical methods give one the possibility of estimating microbial biomass in real time. In this paper we describe our implementation of a physical method which provides a solution of the problem of devising a real-time biomass probe. It is understood that biovolume, i.e. the proportion of the total volume that is enclosed by the cytoplasmic membranes of the cells in a suspension, constitutes the most suitable 'operational' definition of biomass for use during fermentations [Harris & Kell 1985].

Physical properties may be divided into optical, mechanical and electrical [Blake-Coleman *et al* 1984, Clarke *et al* 1986, Kell *et al* 1990], only the latter will be considered. Whilst both faradaic and non-faradaic electr(ochem)ical approaches may be used to detect microbial biomass [Ishimori *et al* 1981, Aston & Turner 1984, Blake-Coleman *et al* 1984, Harris & Kell 1985, Clarke *et al* 1986, Kell *et al* 1990], the former is more a chemical than a physical approach; we therefore concentrate on the latter.

THE DIELECTRIC APPROACH TO THE ESTIMATION OF MICROBIAL BIOMASS

The non-faradaic or 'passive' electrical properties of a system, such as a cell suspension, may be completely characterised by its macroscopic capacitance (in Farads) and conductance (in Siemens). These depend in part upon the size and geometry of the electrodes which reflect respectively the system's intrinsic properties permittivity (ability to store electrical energy) and conductivity (ability to dissipate it) [see Kell 1987, Pethig & Kell 1987, Kell & Davey 1990]. Conductivity has the units Siemens/m, whilst permittivity is dimensionless. For plane-parallel electrodes of area A separated by a distance d, the relationship between the conductivity σ' and conductance G is $\sigma' = G.(d/A)$, where (d/A) is known as the cell constant and has units of length^{-1}. The capacitance C is similarly related to the permittivity ε' by $\varepsilon' = C.(d/A\varepsilon 0)$, where $\varepsilon 0$ is an experimental constant equal to $8.854.10^{-12}$ F/m, such that a cubic electrochemical cell of unit dimensions containing water (which has a permittivity of 78.4 at 298K) has a capacitance of some 6.94 pF. A variety of systems have been proposed which can exploit these facts for the estimation of microbial biomass.

The ability accurately to measure the dielectric properties of fermentor broths *in situ* has already proven to be a powerful approach for the on-line, real-time estimation of cellular biomass.

The commonest type of system [Firstenberg-Eden & Eden 1984] relies upon the biomass-dependent changes in bulk conductivity caused by the uptake and excretion of charged compounds, and/or biomass-dependent changes in the polarisation (apparent permittivity) of the electrode-electrolyte interfaces, to sense biomass and do not attempt to distinguish the electrical properties of cells per se [Hause *et al* 1981, Harris & Kell 1985]. Certainly the electrolytic conductivity does provide a generally useful (and greatly under-used) sensor for particular kinds of metabolic activity, although these methods cannot be used accurately to estimate fermentor biomass *in situ* (since of course non-growing cells can carry out these types of reactions). By contrast, direct measurement of the electrical properties of cellular suspensions, under appropriate conditions, can provide a direct and 'instantaneous' reading of biovolume [Harris *et al* 1987, Kell & Todd 1989, Kell 1990].

The passive, non-faradaic electrical or 'dielectric' properties of cellular suspensions themselves (as opposed to those of the suspending medium or the electrodes themselves) are generally characterised by three major areas of frequency-dependence, known

FIGURE 1: BIOMASS MONITOR

VIABLE CELL CONCENTRATION MEASUREMENT

THE BIOMASS MONITOR IS SUITABLE FOR REAL-TIME MEASUREMENT OF VIABLE BACTERIA, YEAST, ANIMAL, PLANT AND OTHER CELLULAR BIOMASS. NON-CELLULAR PARTICLES HAVE A NEGLIGIBLE EFFECT ON READING.

THE LIMITATIONS IMPOSED BY CONVENTIONAL OPTICAL MEASUREMENT TECHNIQUES HAVE BEEN OVERCOME AND HIGH BIOMASS CONCENTRATIONS CAN BE MONITORED, EVEN IN OPAQUE AND SOLID MEDIA.

THE RUGGED ELECTRODE PROBE MAY BE INSERTED INTO PROCESS PIPEWORK OR DIRECTLY INTO A FERMENTOR VESSEL. THE PROBE ITSELF IS FULLY STERILISABLE AND THE NEED TO REMOVE PROBES FOR CLEANING IS FURTHER MINIMISED BY THE UNIQUE ANTIFOULING CLEAN PULSE SYSTEM.

AUTOMATIC ON-LINE CONTROL IS EASILY ACHIEVED BY THE USE OF THE COMPREHENSIVE OUTPUT FACILITIES PROVIDED BY THE BIOMASS MONITOR.

(in order of increasing frequency) as the α-, β- and γ-dispersions [Pethig & Kell 1987]. The β-dispersion, centred in the radio-frequency region of the electromagnetic spectrum, is caused in large measure [Ferris *et al* 1990] by the charging of the rather large membrane capacitance C_m displayed by all intact cells. This is typically of the order 1 $\mu F.cm^{-2}$, and is due to the possession by cells (and by nothing else likely to be found in a fermentor) of non-micellar phospholipid membranes of molecular thickness. For spherical cells of radius r, present at a volume fraction P, the permittivity at low radio-frequencies exceeds that of the background by a value given to a close approximation by $9PrC_m/4\varepsilon 0$. For non-spherical cells the factor ¾ is different. Thus, by measuring the dielectric permittivity of cell suspensions at low radio-frequencies, it is possible to design a biomass probe that is specific for viable cells (since necromass, particles, emulsions and gas bubbles do not have intact bilayer-type cell membranes).

Based on these principles, we have developed a biomass probe (the Aber Instruments Biomass Monitor, *Figure 1*) suitable for the real-time estimation of biomass in fermentors *in situ*. The (steam-sterilisable) probe consists of four gold electrodes in an insulating matrix suitable for insertion in a standard 25 mm port. The outer two electrodes apply alternating current of a suitable frequency in the range 0.1 to 10 MHz whilst the inner two pick up the alternating potential difference, an arrangement that more-or-less completely avoids artefacts due to electrode polarisation phenomena. Biofouling is obviated by the manual or automatic application of

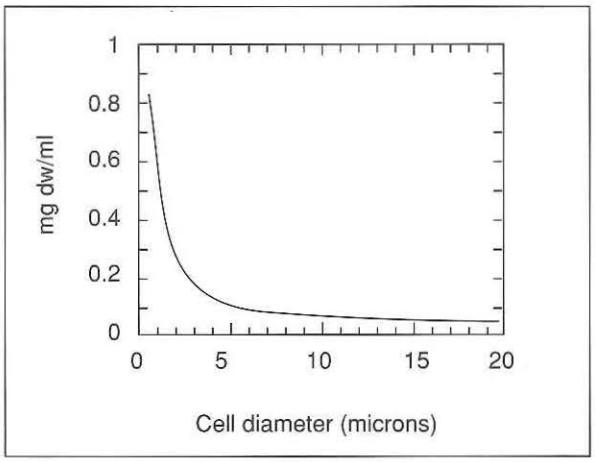

FIGURE 2: BIOMASS MONITOR – LIMITS OF DETECTION

THE LIMIT OF DETECTION OF THE BIOMASS MONITOR DEPENDS UPON A NUMBER OF FACTORS; PREDOMINANT AMONG THESE ARE: THE CONDUCTIVITY OF THE SUSPENSION, THE CELL RADIUS AND THE MODE ('HI', 'LO', OR, FOR SPECIAL PURPOSES, INCREASED GAIN). THE FOLLOWING GRAPH IS FOR CONDITIONS IN WHICH THE BIOMASS MONITOR IS SET IN 'LO' MODE, AND ASSUMES A CONDUCTANCE AS DISPLAYED OF 10 MS, AND THAT THE DRY WEIGHT OF A CELL IS ONE QUARTER OF ITS WET WEIGHT. THEREFORE TO CALCULATE THE EQUIVALENT LIMIT OF DETECTION IN TERMS OF WET WEIGHT, MULTIPLY THE VALUES SHOWN BY FOUR.

electrolytic cleaning pulses, although a similar system without cleaning pulses may be used to assess the extent of such biofouling [Markx & Kell 1990]. The output of this device may be chosen in terms of absolute capacitance, capacitance minus that upon inoculation, or (via previously determined calibration) mg/ml. An output of the conductance of the broth is also provided. The Biomass Monitor is suitable for use in all kinds of fermentations, and has been applied to a variety of prokaryotic and eukaryotic microbes [Harris *et al* 1987], pitching control in breweries [Bolton *et al* 1988], plant cells [Markx *et al* 1991b,c], animal cells [Davey *et al* 1988], immobilised cells [Salter *et al* 1990], solid-substrate fermentations [Davey *et al* 1991, Peñaloza *et al* 1991], and (since it measures biomass possessed of an intact cell membrane, and not necromass lacking one) in assessing cytotoxicity [Stoicheva *et al* 1989]. We have also shown this to be a particularly convenient means of controlling a turbidostat [Markx *et al* 1991a].

As mentioned above, the dielectric increment $\Delta\varepsilon$ due to the β-dispersion is given, for spherical cells, by $\Delta\varepsilon = 9PrC_m/4\varepsilon 0$, where cells of radius r and membrane capacitance per unit area C_m occupy a volume fraction P (for non-spherical cells the factor $\frac{9}{4}$ is modified). This equation allows one, given a knowledge of the minimum permittivity or capacitance change measurable, to calculate the limit of detection of the method, for cells of different sizes [Kell *et al* 1990]. This depends in part on the ionic conductivity of the medium of interest [Kell 1987]; however, the present Biomass Monitor instrument is usable in media of conductivity up to some 20 $mS.cm^{-1}$. The limit of detection (in terms of cell number or dry weight) also depends on the cell radius, such that 1 mg dry $weight.ml^{-1}$ of bacteria, yeast and plants cells give dielectric increments of 1-2, 3-6 and 25-50 permittivity units respectively. Given the cell constant usually used, this equates for yeast to some 0.5-1 pF, which is easily measurable, since the drift of the instrument is only some 0.05 pF per day [Markx & Kell 1990]. Whilst this does not reflect the absolute precision of the instrument in the face of changes in parameters such as cell size, temperature, pH, conductivity, etc, taking the figure of 0.05 pF equates to a change in biomass for yeast of some 0.1 mg dry weight ml^{-1}. Based on these considerations, the limits of detection of the present instrument are given in *Figure 2*.

'Estimation of microbial biomass is by far the single most important parameter of any fermentation process.'

FUTURE WORK

The ability accurately to measure the dielectric properties of fermentor broths *in situ* has already proven to be a powerful approach for the on-line, real-time estimation of cellular biomass (see above). It is clear that by carrying out measurements at different frequencies one may extend the information available using this approach, and hence, by suitable signal analysis, the number of determinands which may be estimated. We have already shown [Kell & Davey 1992] that neural networks may be exploited in the accurate fitting of such dielectric data to the pertinent ('Cole/Cole' equation), although nonlinear least-squares approaches are likely to prove more suitable in practice. Our present work is centred on the development of this approach, and on the integration of the measurement and data analysis system.

AVAILABILITY

The Biomass Monitor is presently available from Aber Instruments in two main configurations. The Model 316B is a single-frequency instrument (0.3 MHz) designed particularly for the control of yeast pitching in breweries, and incorporates a microprocessor. The model 214 is a research-type instrument covering the frequency range 0.1-10 MHz, and may also be interfaced to a microcomputer. A recent development is the model 214 2f instrument, which rapidly scans between the set frequency and a high frequency (ca 10 MHz), permitting the on-line subtraction of any changes in background. A multiplexer device, permitting the control of eight probes by a single Biomass Monitor, is also available. ■

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