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REAL-TIME ESTIMATION OF MICROBIAL BIOMASS DURING FERMENTATIONS, USING A DIELECTRIC PROBE

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Summary

The problem of estimating microbial biomass during industrial fermentations is considered. Biovolume, or the volume fraction enclosed by the cytoplasmic membrane of the organisms, constitutes the most appropriate definition of biomass for this purpose. The dielectric permittivity of cellular suspensions is a linear function of their biomass content, due to their possession of various dielectric dispersions (usually termed α-,β- and γ-), i.e. frequency-dependent changes in permittivity. The magnitude of the β-dispersion, which results predominantly from the existence of a relatively ion-permeable cytoplasmic membrane surrounding the cells, is proportional to the cell radius and volume fraction. Thus measurement of the permittivity at (radio-)frequencies substantially between the α- and β-dispersions allows the convenient estimation of biovolume and hence biomass. Since the (four) electrodes used are metallic, they may be autoclaved, and their fouling may be prevented by electrochemical cleaning.

Introduction

The estimation of microbial (or other cellular) biomass during laboratory and industrial fermentations, especially in situ and in real time, remains an outstanding and generally unsolved problem (CARLEYSMITH & FOX /1/, HARRIS & KELL, /5/, PIRT /12/). The first difficulty relates to deciding what even in principle constitutes an appropriate definition of biomass, since the usual

definitions (e.g. POSTGATE /13/), such as the number of cells capable of growth, metabolism and/or division, cannot be realised in real time. However, since it is known that cellular viability depends upon the possession by the cells of a relatively ion-impermeable cytoplasmic membrane, this and other considerations lead to the view (HARRIS & KELL /5/) that biovolume, i.e. the volume fraction of the culture enclosed within the cytoplasmic membrane of the cells, represents the most useful reflection of biomass, i.e. one that is relatively independent of the physiological state of the culture and which may yet be used in real time. Similarly, we and others (CLARKE et al./2/, HARRIS & KELL /5/) have concluded that physical methods alone, in contrast to say chemical methods, can provide an adequate approach to the real-time microbial biomass.

A biophysical approach to the estimation of cellular biomass

As part of an investigation of the dielectric behaviour of microorganisms, we have developed an automated, dielectric spectrometer, based upon commercially available equipment and capable of operating in the frequency range 5 Hz - 13 MHz (HARRIS & KELL /4/). Whilst the use of this equipment has allowed us to uncover certain novel mechanisms of dielectric dispersion in membranous systems of known dry weight or protein content (HARRIS & KELL /6/, KELL /8/, KELL & HARRIS /10/, /11/), the major importance of these studies for the present purpose has been to show (a) that the radio-frequency dielectric properties of these systems are dominated by the charging of the cell membrane capacitance (i.e. by the so-called β-dispersion (SCHWAN /14/)), and (b) that the magnitude of the β-dispersion is linear with the membrane-enclosed volume fraction P up to very high values of P. Thus, and since the measurement of the dielectric behaviour of cell suspensions requires only (autoclavable) metallic electrodes as an interface between the measuring system and the culture, it occurred to us that the estimation of the dielectric properties of cultures, preferably at one or more frequencies between the α- and β-dispersions, would provide a novel, useful and non-invasive means for the real-time estimation of biomass during laboratory and industrial fermentations (HARRIS et al./7/).

Our earlier work was confined to low conductivities (say $\lesssim$5 mS/cm), since our instrumentation was capable of being used only as a 2-terminal system, so that electrode polarisation (e.g. GRANT, SHEPPARD & SOUTH /3/, KELL /9/, SCHWAN /15/), which is exacerbated at higher conductivities, would also contribute to the measured dielectric properties at the high conductivities possibly

characteristic of fermentations. We have therefore developed a dedicated four-terminal instrument (the Dulas Bug Meter, patents pending) suitable for the real-time estimation of microbial biomass, and in what follows we give its specification.

The instrument measures the capacitance (and conductance) of a sample using a four-terminal cell and at selectable frequencies in the range of 0.2 MHz - 12 MHz. Frequency, conductance and capacitance are displayed and presented as output voltages. Calibration to obtain the cell constant is with solutions of known permittivity and/or conductivity, whilst back-offs are provided to make the capacitance output proportional to, rather than linear with, biomass content. Bipolar 10V electrode cleaning pulses may be applied manually (to prevent the build-up of biofilm on the electrodes). The permittivity output is linear with biomass up to at least 150 mg dry weight/ml, and (despite an erroneous expectation to the contrary (CARLEYSMITH & FOX /1/)) is essentially unaffected by the presence of large amounts of particulate matter (which does not possess a cell membrane and hence lacks a sizeable β-dispersion). Our measurements to date (HARRIS et al./7/) suggest that this instrument indeed possesses all the properties of an ideal biomass probe as defined (HARRIS & KELL /5/) in an earlier review of this problem.

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References

1. CARLEYSMITH, S.W. & FOX, R.L. Adv. Biotechnol. Proc. 4 (1984), 1
2. CLARKE, D.J., CALDER, M.R., CARR, R.J.G., BLAKE-COLEMAN, B.C., MOODY, S.C. & COLLINGE, T.A. Biosensors J. 1 (1985), 213
3. GRANT, E.H., SHEPPARD, R.J. & SOUTH, G.P. Dielectric Behaviour of Biological Molecules in Solution. Oxford University Press, Oxford (1978)
4. HARRIS, C.M. & KELL, D.B. Bioelectrochem. Bioenerg. 11 (1983), 15
5. HARRIS, C.M. & KELL, D.B. Biosensors J. 1 (1985), 17
6. HARRIS, C.M. & KELL, D.B. Eur. Biophys. J. 13 (1985), 11
7. HARRIS, C.M., TODD, R.W., BUNGARD, S.J., LOVITT, R.W., MORRIS, J.G. & KELL, D.B. Enz. Micr. Technol. (1986), submitted
8. KELL, D.B. Bioelectrochem. Bioenerg. 11 (1983), 405

9. KELL, D.B. (1986) in Biosensors: Fundamentals and Applications (A.P.F. TURNER, I. KARUBE & G.S. WILSON, eds.) Cambridge University Press, Cambridge, pp. 429-470

10. KELL, D.B. & HARRIS, C.M. Eur. Biophys. J. 12 (1985), 181

11. KELL, D.B. & HARRIS, C.M. J. Bioelectricity 4 (1985), 317

12. PIRT, S.J. Principles of Microbe and Cell Cultivation. Blackwell, Oxford (1975)

13. POSTGATE, J.R. Symp. Soc. Gen. Microbiol. 26 (1976), 1

14. SCHWAN, H.P. Adv. Biol. Med. Phys. 5 (1957), 147

15. SCHWAN, H.P. in Physical techniques in Biological Research, Vol. VIb (W.L. NASTUK, ed.), Academic Press, New York, pp. 323-407

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