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The real time measurement of biomass accretion using dielectric spectroscopy.

by

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Summary.

The principles by which biomass may be estimated using dielectric spectroscopy are explained. Examples are given of existing and potential uses of this method for on-line and real-time fermentation monitoring and control.

Introduction

The importance of monitoring and controlling fermentations is constantly increasing. Coupled to the desire to increase the knowledge of what is going on during a fermentation process is the wish to influence that process so that higher and more efficient productivity can be achieved. Together they have brought forth new and more advanced methods for monitoring and controlling fermentations.

Figure 1 gives an outline of a fermentation unit.

Note that the User is the one that finally determines what the total system will do and look like. (One should not see something special in the fact that User is placed under the system.)

Control in the fermentation industry is not primarily different from that in the other chemical industries. However, due to the (non-linear) nature of what is measured, i.e. biological systems, one inherently encounters problems coupled, for example, to time-dependent changes in parameters and bad reproducibility, which brings the need for more specialised and more advanced instrumentation and control strategies. One could say that Life is always more complicated than one thought it to be at first.

Sensors

Sensors are of primary importance for any control system. Only very limited control is possible when no sensors are available. Feedback control is impossible if no information is available about the system.

Sensors may be differentiated into off-line and on-line sensors (Schügerl et al 1985). With off-line sensors part of the system to be measured is separated from the rest and measured. In practice this usually means taking a sample and then doing the measurement.

Disadvantages include the mechanical and sterility problems inherent in sampling systems, and the fact that such approaches have an inherent time-delay between the sampling and the measurement. An improved variant of off-line systems is represented by devices which permit measurements in the medium that has been separated from the fermentation vessel by way of a filter.

With on-line sensors one measures directly in the system. The main advantage of this is that one has (almost) immediate information on the system, in a state exactly representative of that in the fermentor. However the sensor is present in a complex and often agressive environment, so there are quite a lot of prerequisites a sensor has to fulfil before it can be used in fermentation systems.

Table 1 gives a list of the prerequisites.

Not many sensors have been developed that can be used in fermentation systems in situ. Table 2 gives a list of the major parameters that one may wish to measure. The development of new sensors is very slow (Clarke et al 1985), and it is only very recently that sensors for biomass measurements have become available (Kell et al 1987, Clarke 1988).

Now one might say that the above statement is not 100% true. Methods for on-line estimation of biomass, based on principles such as turbidity, have been around but for quite some time, but their main problem was that they were very limited, in particular because they have only a small dynamic range (the Beer-Lambert law fails at very modest biomass densities) and they are very susceptible to interferences e.g. by gas bubbles, particulate matter in the medium and by the formation of biofilms on the optical surfaces. Other more recently developed methods such as the measurement of the acoustic (Clarke 1988) and fluorescent (Beyeler et al 1981) properties of cultures also suffer heavily from interferences.

Dielectric spectroscopy

Measurement of the dielectric properties of a suspension gives a means for the estimation of biomass that is fast and has little problem with interferences (Harris et al 1987, Boulton et al 1989).

The principle of the measurements of the dielectric properties of a material is as follows (Pethig & Kell, 1987). The (passive) electrical properties of all materials can be characterised by their conductance, their capacitance and their inductance. For biological materials in general only conductance and capacitance are of importance. The conductance gives a measure of the ability of a material to conduct electricity, or in other words to dissipate energy, whilst the capacitance reflects the ability of the system to store charge, or in other words to store energy.

Conductance and capacitance are macroscopic properties, which depend upon the size and geometry of the measuring electrodes and are related to the intrinsic properties conductivity and permittivity ("dielectric constant") of the system by the "cell constant". The units of conductivity are $S.m^{-1}$, whilst permittivity is dimensionless.

When measuring the electrical properties of a biological material at different frequencies one generally finds a spectrum such as that in figure 2. Frequency-dependent decreases in permittivity and increases in conductivity are referred to as dielectric dispersions, and take the form of inverted sigmoids. Normally several dispersions occur, which can be ascribed to different mechanisms. In cell suspensions, the dispersion of lowest frequency is called the alpha dispersion, and is due predominantly to tangential movements of ions over the cell surface; it is especially large in Gram-positive organisms. For us the most important one is the beta dispersion, which can be ascribed mainly to the build-up of charge across the cell membrane due to the presence of an electric field. At very high frequencies one can find more dispersions. They are related to dipolar motions of water and other (parts of) molecules.

The beta dispersion is so important because it can be described by the following formula:

$$\varepsilon_l = \varepsilon_\infty + 9PrC_m/4\,\varepsilon_0$$

where

ε_l = permittivity at low frequency
ε_∞ = permittivity at high frequency
P = volume fraction of biomass present
r = equivalent radius of a cell (m)
C_m = membrane capacitance ($F.m^{-2}$)
ε_0 = permittivity of free space ($8.854, 10^{-12}$ F/m)

Biological membrane exhibit a rather constant value for C_m of some 10^{-6} $F.cm^{-2}$. **This means that the drop in the permittivity from lower to higher frequencies is directly related to the volume fraction of intact membranes present.** Thus dielectric spectroscopy can be used to estimate the biomass present. In collaboration with Aber Instruments and ICI Biological products, we have developed a biomass-sensing instrument (The Bugmeter™), based on the above principles. The Bugmeter incorporates anti- fouling, electrolytic cleaning pulses, and makes measurements of the dielectric properties of fermentation broths, using a standard 25 mm probe, at frequencies between 100 kHz and 10 MHz. It provides analogue and (if required) RS232 outputs, and is commercially available from Aber Instruments (Science Park, Aberystwyth SY23 AH).

For a given biomass concentration, the Bugmeter signal is mainly dependent on:

-the frequency chosen
-the size of the organism
-the intactness of its membranes (its "viability")
-(to some extent) the conductance of the medium

The effect of increasing the conductance is mainly that it shifts the characteristic frequency (midpoint) of the dispersion to higher frequencies. Thus the whole curve shifts to higher frequencies. The effects of the conductance can be minimised by choosing the correct frequency, in or near the 'plateau' region between the alpha and beta dispersions.

The Bugmeter does not significantly measure components in the medium that do not consist of two aqueous phases separated by a molecularly thin, non-conducting membrane (and are thus biological cells). Thus antifoam, particles from the medium and gas bubbles are not (or are barely) measured. In fact the Bugemeter has successfully been used to measure biomass entrapped in ceramic beads (Salter et al 1990) and in solid-substrate fermentations (CLD, W. Peñaloza, J.N. Hedger & DBK, in preparation).

Organisms which are not spheres but rods, filamentous organisms and so on may also be measured. In some cases information concerning cell size distributions may be obtained. Most cell types have already been measured, including bacteria (L. Ferris, CLD & DBK, submitted), yeast (Harris et al 1987), fungi (CLD, W. Peñaloza, J.N. Hedger & DBK, in preparation, animal (Davey et al 1988) and plant cells.

Applications of the Bugmeter.

The operation of the Bugmeter was automated using a Blackstar 2308 interface and an OPUS II IBM compatible PC. A control program was written in GWBASIC that allows one to log data at two different frequencies and to make frequency scans.

Examples of the use of the Bugmeter in this set-up and others are:

- monitoring of biomass production in batch fermentations
- monitoring of the occurrence of cell differentiation, such as occurs during the secondary growth phase.
- detection of infections
- control of yeast pitching in the brewing industry
- control of the biomass level in the fermentor at a steady state (the "permittistat", a novel form of turbidostat)
- measurement and control of biofilm development
- study of the toxicity to cells of organic solvents (Stoicheva et al 1988)

Data to illustrate most of these examples will be presented during the talk.

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Table 1.

Prerequisites for sensors for on-line use in fermentation systems

- selective (no interferences or interferences can be compensated for)
- adequate sensitivity
- adequate response time
- low drift
- robustness
- sterilisibility
- simple to use

Table 2.

Process variables to be measured during fermentation

Physical	(physico)chemical	Biological
-gas flow	-CO_2, O_2, other gases in liquid and gas phase	-(active) biomass
-bioreactor volume	-pH	-excreted biochemicals and metabolites
-conductivity	-inorganic ions	-intracellular components
-permittivity	-other nutrients (e.g. sugars)	DNA
-foaming	-redox potential	RNA
-liquid flow	-osmolarity	NAD(P)H
-OD		ATP
-power input (agitator speed)		proteins
-pressure		amino acids
-temperature		others
-viscosity		-morphology
		-microbial population

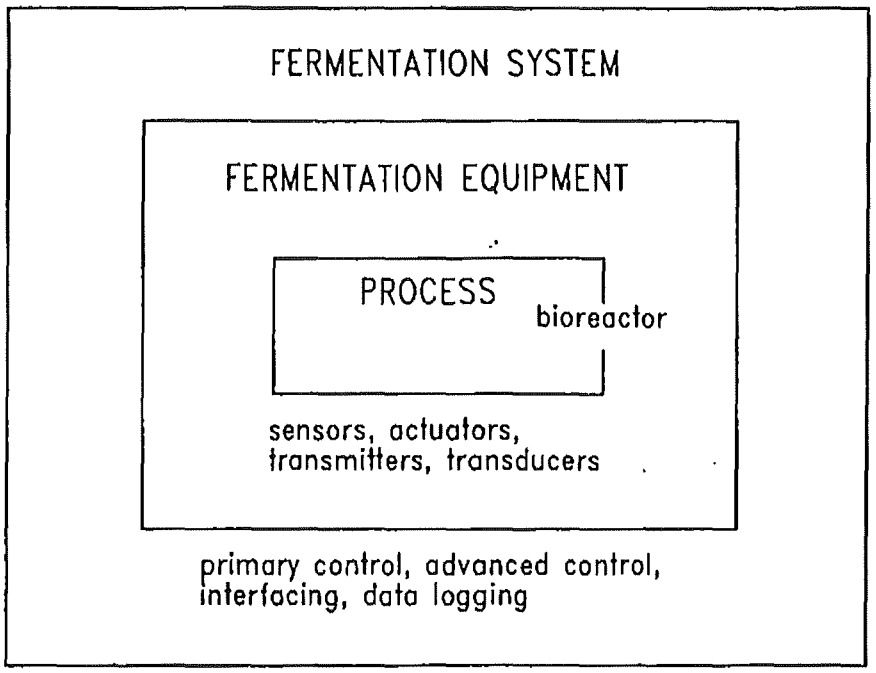

Figure 1. Outline of a fermentation unit

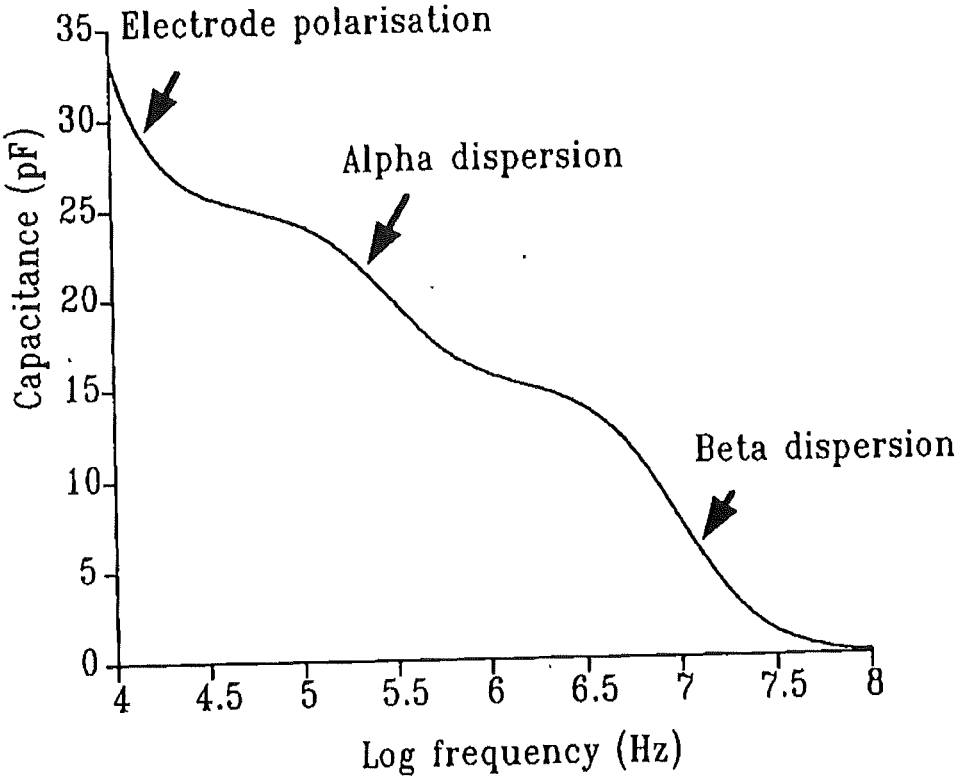

Fig 2. Typical dielectric spectrum of a biological cell suspension