

A Sensitive, Compact, In Situ Biomass Measurement System

Controlling and Monitoring Microbial Fermentations Using Radio-Frequency Impedance

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Of all available on-line biomass assays, the radio-frequency impedance (RFI) method has a clear advantage for process development and manufacturing: It is an unambiguous reflection of viable cell biovolume rather than the total number of cells. Viable cell concentration is of prime importance in metabolic studies and others relating to the efficiency of target protein production. RF impedance is also suitable for measuring live cell densities in bioreactors when cells are attached to microcarriers or inert discs.

Measuring optical density (OD) using light absorption (usually at the 600 nm wavelength) is widely used to monitor biomass off-line during bacterial fermentations. In-line optical sensors have been developed for that purpose, and they rely on transmittance, absorbance, and/or backscattering measurements. In general, their performance is acceptable for high-viability cell culture applications, but light-based probes do not distinguish between the viable and nonviable cells. They will also detect debris, aggregates, and other noncellular particles that can collect during fermentation (1, 2). Moreover, the

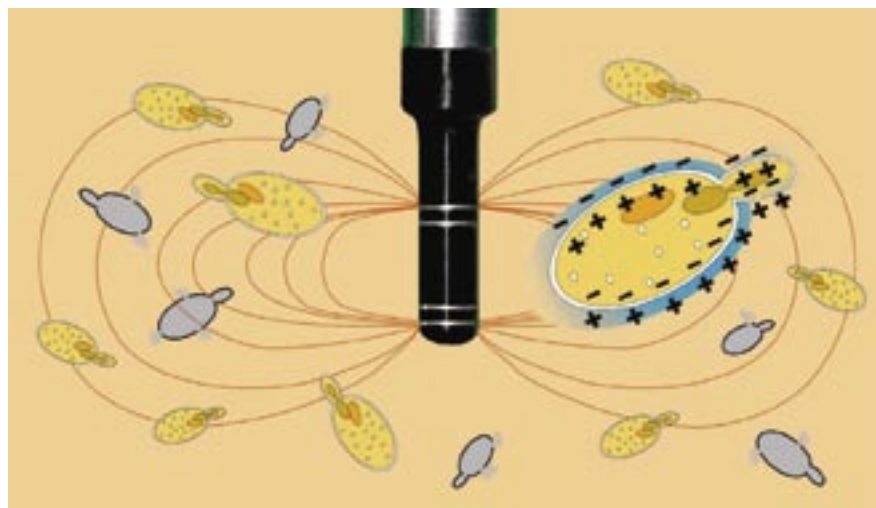


Figure 1: Diagram showing ideal RF impedance electrical field with Aber's annular probe

optical sensors are prone to fouling from the attachment of proteins over extensive production runs. In highly aerated fermentors, light-based sensors are prone to interference from gas bubbles during aeration, and calibration must be conducted by polynomial approximation of the relationship between in-line and off-line optical density.

Here, we describe the development of a more sensitive and compact biomass monitoring system based on radio-frequency impedance. This can be used in bacterial fermentations and other applications with both low and high concentrations of biomass in a fermentor. We show how probe configurations, sensitive electronics, and an advanced electronic filtering system have been used to date. Examples are given for monitoring highly aerated bacterial fermentations at laboratory and production scales using in-line radio-frequency impedance.

PROBE DEVELOPMENTS FOR BACTERIAL FERMENTATIONS

RFI probes for estimating viable biomass by capacitance have traditionally used a four-pin platinum electrode system. For applications such as brewing or mammalian cell culture (in which cell densities are high), such probes have performed well (3–5).

Cells with intact plasma membranes act like tiny capacitors under the influence of an electric field. The nonconducting nature of plasma membranes allows charge to build up. The resulting capacitance can be measured and is usually expressed in picofarads per centimeter (pF/cm). It depends on the cell type and is directly proportional to the membrane bound volume of viable cells.

The Biomass Monitor RFI probe from Aber Instruments is steam-sterilizable in situ and uses four platinum electrodes to apply a radio-frequency field to live cells.



Photo 1: Flush electrode probe developed initially for cell culture and brewing

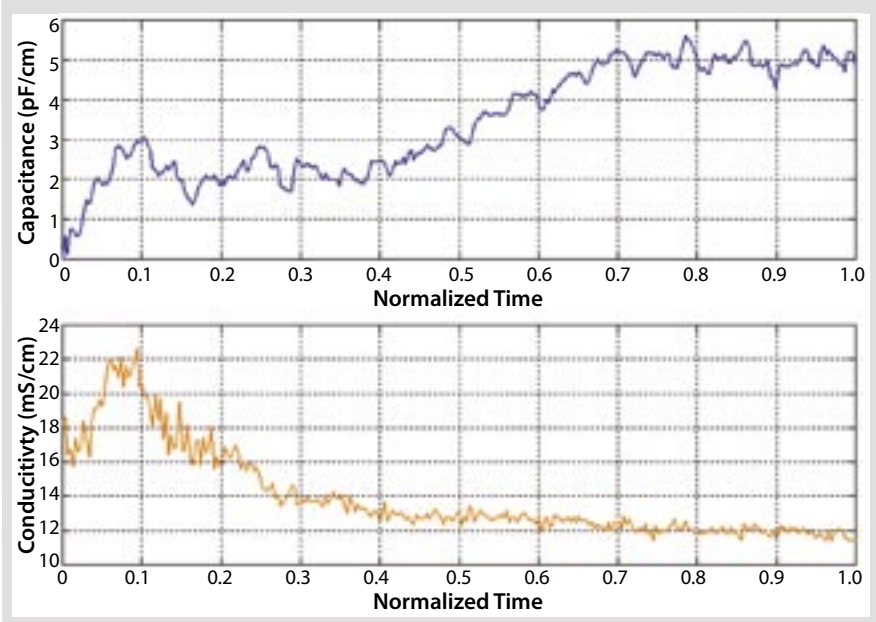
Nonviable and leaking cells are effectively invisible to this detection system. Electronic processing of the resulting electrical signal produces a highly accurate measurement of viable cell concentration. The probes are insensitive to gas bubbles and, moreover, they can monitor very high biomass concentrations in a wide range of complex media.

In brewing applications, concentrations of live yeasts in excess of 10^9 cells/mL found in recovered slurries can provide capacitances well in excess of 50 pF/cm. In animal cell cultures, the large average size of the cells typically leads to a capacitance in excess of 20 pF/cm. For more demanding applications with lower levels of biomass, it became clear that more sensitive electronics were required, with a new probe design, which could accurately measure capacitance values of less than 1 pF in highly aerated and agitated fermentors.

The probe design chosen incorporates four annular-ring electrodes. This provides the optimum radio-frequency field for measuring live cell biomass in microbial fermentations (Figure 1). The probe's sensitivity to cells is distributed over a larger volume than the four-pin electrode design, and it reduces the size of disturbances caused by large gas bubbles in the fermentor. The new annular-ring design is advantageous because it has no sharp or protruding edges, making it ideal for adherent or filamentous cell types. It can be used in 12-mm or 25-mm bioreactor ports. The new design is more sensitive in applications with highly aerated mycelial bacterial processes (6).

In animal cell culture, a 25-mm probe with "flush" platinum electrodes (Photo 1) is the preferred choice for CGMP production applications. The new RFI probe was originally developed for nonaerated brewing fermentors, in which it prevents problems associated with yeasts settling onto pin-type electrodes. But this design is also ideal for "sticky" animal cell lines that can adhere to or wrap around those pin-type electrodes in certain situations. The "flush" probe design also can be used in bacterial fermentors with 25-mm ports. It was used successfully for monitoring viable cell concentrations of *Escherichia coli*

Figure 2: Aber probe (flush) trace in 200-m³ *E. coli* fermentation (SA BIOPRODUCTS, WWW.BIOPRODUCTS.CO.ZA)



growing in a 200-m³ fermentor at SA Bioproducts in South Africa, in an amino acid manufacturing process (Figure 2).

For probes used in a CGMP environment, all wetted materials are required to conform to FDA regulations. Probes used with the latest Aber RFI instruments have a main body constructed of polished 316L stainless steel (with an option for electropolishing and passivation). The electrode end is made from an inert and dielectrically stable polymer. Options include probes using high-performance polymers (polyetheretherketone, PEEK, and polyphenylene sulphide, PPS) and a modified epoxy resin. PEEK and PPS are commonly used in the biopharmaceutical industry and have USP class VI and FDA 21 CFR 177.2415 accreditation for repeated food contact. Tests have shown that such probes can withstand more than 100 steam-in-place (SIP) or autoclave cycles.

RFI INSTRUMENT DEVELOPMENTS FOR BACTERIAL FERMENTATIONS

The Biomass Monitor Model 220 from Aber Instruments of Aberystwyth, UK (www.aber-instruments.co.uk), has been highly specified for flexibility in process development and has a number of features that are not always required for simple bacterial fermentations. Such features include a built-in PC, frequency scanning, multiplexing, and the ability to store individual calibration graphs for each cell type or strain. In 2006 a version of the instrument (Model 200) was introduced



Photo 2: Aber Biomass Monitor Model 200

for simplified use, and it is relatively compact, low in cost, and flexible with communications. Illustrated in Photo 2, this instrument was designed to GAMP4 requirements, with password protection.

Because RFI is a relatively new technology for many companies, there is often a requirement to test an instrument, particularly if it is intended for CGMP production. A communication system and software package was specifically developed to simplify the evaluation process. In its present format, the Model 200 can be connected to an available PC by a USB port. All Lab Workbench software (ideally using Windows XP) allows both capacitance and conductivity to be logged, data which can then be exported into an Excel worksheet.

The instrument is configured to run in four different configuration modes. They allow end users to quickly use preset frequencies and filter settings known to be appropriate for the type of cells being monitored (bacterial, microbial, and cell culture modes). A fourth "custom mode" allows an end user to change those

Figure 3: Correlation between dry cell weight and capacitance (using the Biomass Monitor 200) for *E. coli* culture

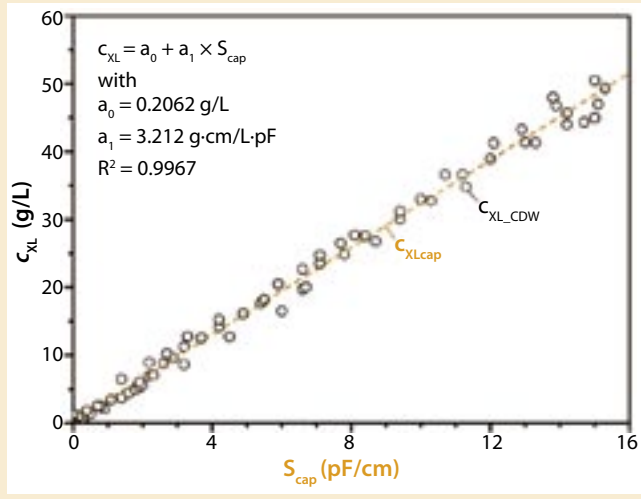
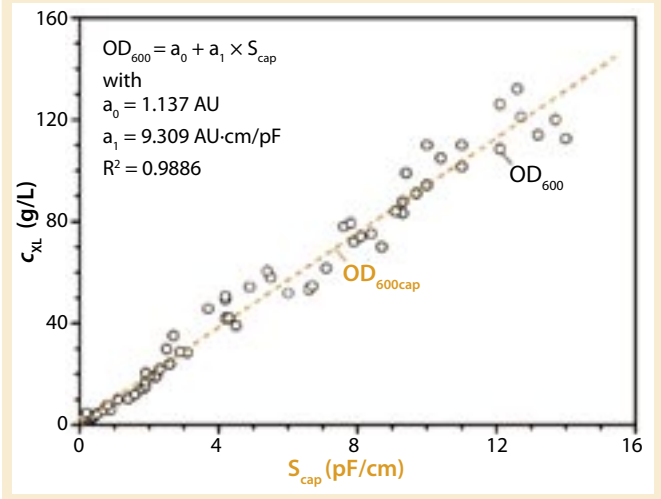


Figure 4: Correlation between OD at 600 nm (off-line) and capacitance (determined by the Biomass Monitor 200) for *E. coli* culture



frequencies and filtering. That mode is also used for checking instrument electronics with a calibrated probe simulator (which traces back to an instrument calibrated to national standards by a third-party calibration house).

MONITORING *E. COLI* FERMENTATIONS

Three different cultivation runs at Hamburg University of Applied Sciences provided the first results from an annular-ring electrode with the Biomass Monitor 200 for monitoring capacitance and conductivity in a genetically modified *E. coli* fermentation grown on minimal M9 media. The fermentations

were performed in a stainless-steel 15-L BIOSTAT C vessel from Sartorius BBI Systems of Germany (www.sartorius.com). Excellent, reproducible correlations were found between capacitance and cell dry weight up to a value of 50 g/L and for the OD at 600 nm up to a value of 120 AU (Figures 3 and 4). Because cell density is a key variable for bioprocess automation and for on-line calculations of cell-specific and volumetric reaction rates, the Biomass Monitor 200 offers a reliable on-line signal for determination of the living cell mass during a cultivation run.

Figure 5 shows the time course of a typical fed-batch production process

using the common host system *E. coli*.

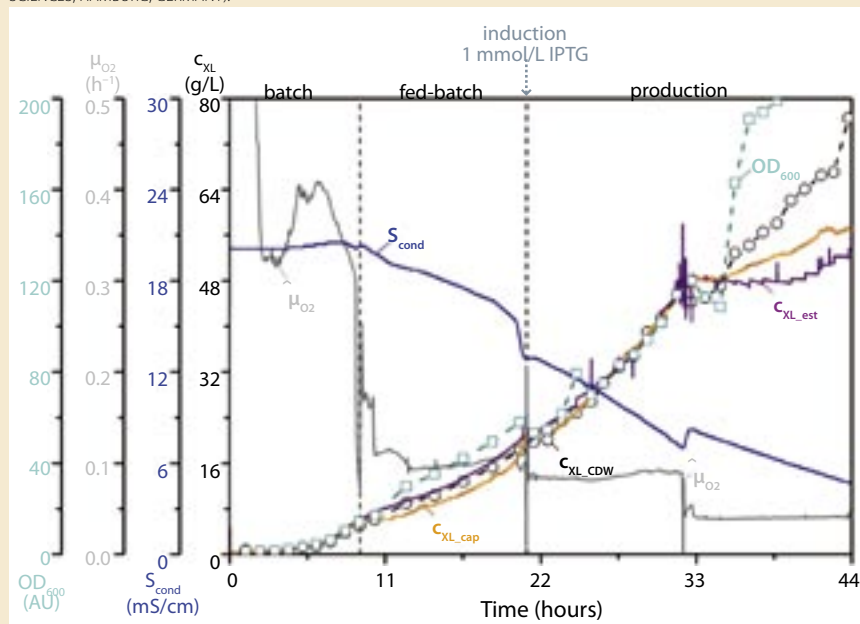
Data include

- profiles for OD at 600 nm
- off-line cell density c_{XL_CDW}
- calculated cell density c_{XL_cap} using capacitance (converted to g/L using the calibration graph in Figure 3)
- estimated cell density c_{XL_est} using off-gas balances
- a reconstructed cell-specific growth rate μ_{O_2} using off-gas balances (7).

Conductivity determined by the Biomass Monitor 200 is also included because it can provide valuable additional information on the consumption and/or production of dissolved nutrients. Cell density calculated from capacitance and the estimated cell density show excellent correlation with the off-line-determined cell dry weight. About 31 hours after inoculation, the cell-specific growth rate (controlled by an exponential open loop control) had to be reduced to prevent oxygen limitation (data not shown). Also, a sterile ammonium stock solution was added to ensure optimal nitrogen supply.

Those changes to the culture significantly influenced the various methods for cell mass determination. Presumably that influence was caused by cell death during recombinant protein production as well as a very low carbon supply. Change in the cell membrane composition and the shape or size of cells would also influence in-line capacitance measurements. Off-line determination of cell density by OD does not differentiate between viable and dead cells, so that method provides the highest values. Release of components from cells during lysis will cause a sharp increase in OD and a subsequent overestimation of the biomass present.

Figure 5: Genetically modified *E. coli* DH5alpha (pTrcHisB_T-Sapphire) grown on M9 media in a 10-L Biostat C fermentor from Sartorius BBI Systems — comparing capacitance and cell dry weight. (C. KAISER, RESEARCH CENTER OF BIOPROCESS ENGINEERING AND ANALYTICAL TECHNIQUES, HAMBURG UNIVERSITY OF APPLIED SCIENCES, HAMBURG, GERMANY).



NEW AND IMPROVED

The sensitivity of RFI measurements has been greatly improved by using probes designed with smooth annular-ring (or flush) electrodes. The new probe design is suitable for highly aerated bacterial fermentations. A new compact instrument for monitoring RF impedance has been found ideal for monitoring bacterial fermentations. Measured capacitance was found to correlate well with cell dry weight and off-line absorbance.

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