



## Evaluation of a new annular capacitance probe for biomass monitoring in industrial pilot-scale fermentations

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Received 7 May 2004; received in revised form 14 October 2004; accepted 6 December 2004

### Abstract

The four-pin electrode capacitance probe has already shown to be a valuable tool for on-line monitoring viable biomass concentration in industrial-type fermentations. A new prototype annular probe was developed and its performance in real-time monitoring the concentration of viable cells during industrial pilot-scale fermentation for the production of an Active Pharmaceutical Ingredient (API) was investigated and compared to the four-pin probe. A set of 14 fermentations was monitored on-line: four of them with the four-pin probe, the remaining with the annular probe. The performance of both the annular and the four-pin electrode probe were compared against each other and against off-line measurements (viscosity and packed mycelial volume).

The prototype annular probe showed to have higher signal intensity and sensitivity than the standard four-pin probe, with higher signal-to-noise ratio. Furthermore, its new design and construction proved to be easier to handle in an industrial environment.

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**Keywords:** Biomass monitoring; Capacitance; Complex media; Streptomyces; Fermentation

### 1. Introduction

One of the most important parameters in bioprocesses monitoring is biomass concentration (Neves et al., 2000). It allows the determination of culture variables like the specific rates of growth and product

formation/substrate consumption and so all mathematical models used to describe microbial growth or product formation contain biomass as an important state variable (Sarra et al., 1996). This explains why the availability of an on-line (viable) biomass-monitoring device is of great relevance to bioprocess research, development, and production (Neves et al., 2000).

Several new techniques for biomass determination based on diverse principles (acoustics, laser light

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scattering, fluorescence, nuclear magnetic resonance spectroscopy, calorimetry, and dielectric spectroscopy or capacitance) have recently been applied to on-line monitoring (Vaidyanathan et al., 1999; Reardon and Scheper, 1991). From these, in situ and on-line steam sterilizable capacitance probes based on dielectric spectroscopy seem best suited for the type of conditions found in large-scale industrial antibiotic fermentations with complex raw materials (Neves et al., 2000; Noll and Biselli, 1998). The operating principle of dielectric spectroscopy has been adequately described elsewhere (Harris et al., 1987; Kell et al., 1990; Spierings, 1998; Yardley et al., 2000).

Normally, capacitance measurements are performed using a four-terminal probe with collinear pins that jut into the fermentation broth or are flush with the end of the probe surface. However, more recently a new annular configuration has been proposed and constructed. The purpose of the present work is to evaluate the performance of the new annular capacitance probe relative

to the standard four-pin electrode probe. In situ and on-line biomass monitoring in industrial-like complex media cultivations was performed with both probes and the capacitance measurements were compared with standard biomass determinations (packed mycelial volume and viscosity).

## 2. Materials and methods

### 2.1. On-line analysis

#### 2.1.1. Capacitance readings

The instrument used throughout this study was the Biomass Monitor™, model 214 M (Aber Instruments, Aberystwyth, UK), dual frequency version (0.2–1.0 MHz, and approximately 9.5 MHz). Both the four-pin electrode and the new annular probe were used for biomass monitoring. The configuration of the two probes is depicted in Fig. 1.

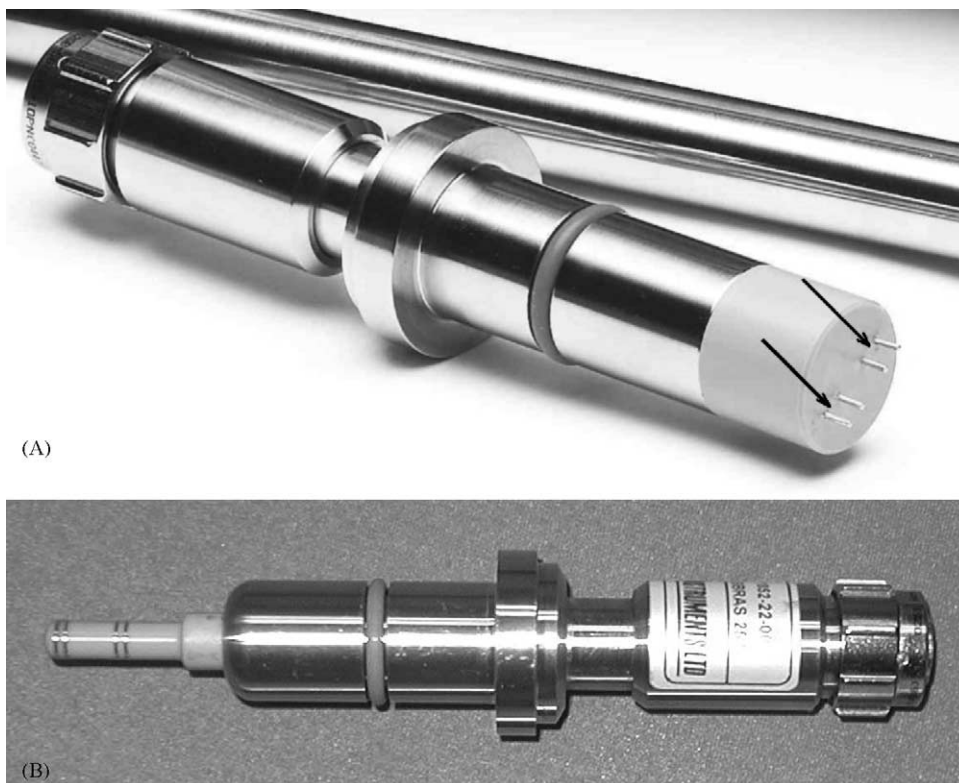


Fig. 1. Image of the two probes tested; (A) four-pin electrode probe (the arrows indicate the location of the electrodes), (B) annular probe.

The on-line measurements were carried out with the capacitance probe inserted directly in the bioreactor through a standard Ingold (25 mm) port located approximately at the stirrer level. Direct sterilization through steam injection was performed in situ at 121 °C. All measurements were performed with the instrument set on the 'low-range', and with the 'low-pass filter' option set on 1 s to smooth variations on signal output and to allow biomass measuring at low conductance (2–16 mS) range. Capacitance and conductance were measured on-line with a frequency of 0.2 min<sup>-1</sup> by an automatic data acquisition system using an excitation frequency of 0.4 MHz.

Digital filtering was applied on the recorded data to reduce the noise in the measurements. The Savitzky–Golay filter (Otto, 1999) smoothes the response curve using a polynomial fit, with the filter window set to provide the best smoothing without significant distortion. The raw capacitance readings were smoothed by the application of a quadratic Savitsky–Golay filter considering 25 data points.

## 2.2. Off-line analysis

### 2.2.1. Packed mycelial volume

Samples of 10 mL from the whole culture media were centrifuged in conic scaled tubes at 3000 rpm for 10 min. The height of the packed centrifuged pellet was used as direct measure of the volume fraction of packed mycelia (pmv, % v/v).

### 2.2.2. Viscosity measurements

The apparent viscosity of the whole culture media was measured using a Brookfield concentric cylinder viscometer (Model LVT, Wilmington, USA). Readings were taken using 500 mL samples at 25 °C, 60 rpm and spindle number 3.

## 2.3. Microorganism and culture conditions

Isolates of *Streptomyces* sp. were supplied by CIPAN, S.A. (Vala do Carregado, Portugal). The cultivation was carried out using a complex medium containing soybean meal, glycerol, dextrin, phosphate and some microelements. The operating conditions used (air flow, temperature, pressure) were typical of those employed routinely in industry for aerobic microbial growth.

A set of 14 fermentations was monitored on-line with a capacitance probe: four of them with the four-pin probe, the remaining with the annular probe. Runs #5, 7, and 13 were non-standard runs, in the sense that different raw materials were used for these fermentations. All the other runs of the experimental set are standard runs once the initial composition of the broth and the operating conditions used were the same for all of them.

### 2.3.1. Inoculum production

Approximately 1.5 mL of a spore suspension (equivalent to a spore concentration of ca. 10<sup>8</sup>/mL) was added to 400 mL of seed medium in 2000 mL high-lap baffled flasks. These were incubated at 25 °C, 220 rpm, for 48 h. The vegetative flask culture was used to inoculate the vegetative medium of the preculture tank. A volumetric inoculation rate of 0.4% (v/v) was used for an operating preculture volume of 100 L. The media used in preculture cultivation was equivalent in composition to the corresponding main culture media.

### 2.3.2. Bioreactor

A fully instrumented bioreactor with a geometric volume of 540 L was used in this study. This bioreactor was fitted with one Rushton turbine of six vertical blades. Cultivations were performed with an initial operating volume of 200 L, using a volumetric inoculation rate of 7% (v/v).

## 3. Results and discussion

### 3.1. Growth curves

The growth curves obtained are very similar in shape, regardless of the probe that was used. For each experiment, three major phases can be identified: a lag phase of about 8 h followed by the exponential growth phase, until ca. 40 h, and then the culture enters a stationary growth phase until ca. 100 h; in the end of the stationary phase some extent of cell lysis is observable in most of the cases. Fig. 2 shows the growth curves obtained for two standard runs from the experimental data set and compares those curves with the measured viscosity and packed mycelial volume (pmv). Run #4 (Fig. 2A) was monitored with the four-pin electrode

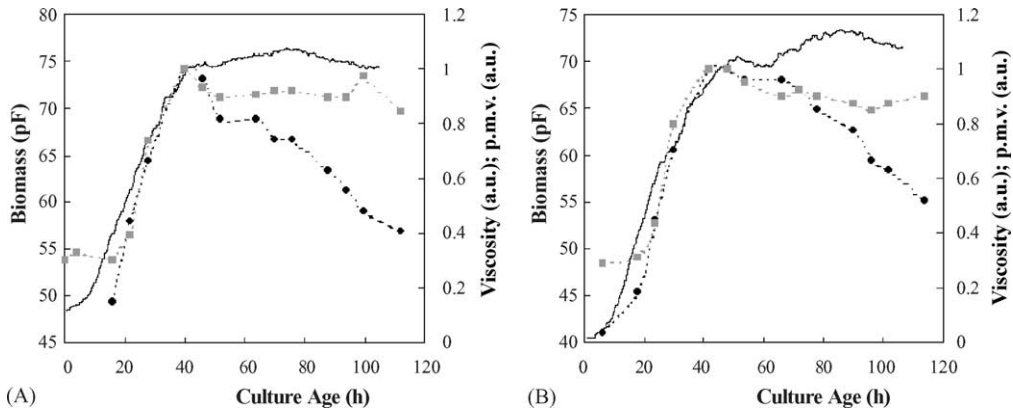


Fig. 2. Time courses of on-line culture capacitance and off-line biomass measurements: (—) capacitance readings, (●) viscosity, (□) packed mycelial volume; (A) run #4, monitored with the four-pin electrode probe, (B) run #14, monitored with the annular probe.

probe while run #14 (Fig. 2B) was monitored with the annular probe.

Viscosity is known to be the most acceptable method for biomass estimation in mycelial systems when more accurate techniques are not available (Sarra et al., 1996). It can be said by observing Fig. 2 that the biomass profile in the first phases of the fermentation is reasonably well described by viscosity measurements. However, at the final stage of the fermentation there is a fast decline in viscosity, which is not visible in the capacitance profile. That decline is most probably associated with changes in morphology, caused by cell lysis or simply by mycelial frag-

mentation, which alter the rheological properties of the broth.

Packed mycelial volume is one of the most common methods employed for biomass measurements due to its simplicity and speed. Nevertheless, it is known not to be reliable once it is influenced by changes in the morphology of the microorganisms and by the presence of insoluble matter. In fact, by observing Fig. 2 we can notice that pmv does not provide an accurate measurement of the viable biomass concentration.

A comparison of the capacitance readings obtained with the two probes during two different standard fermentations (runs #4 and 14) is shown in Fig. 3A.

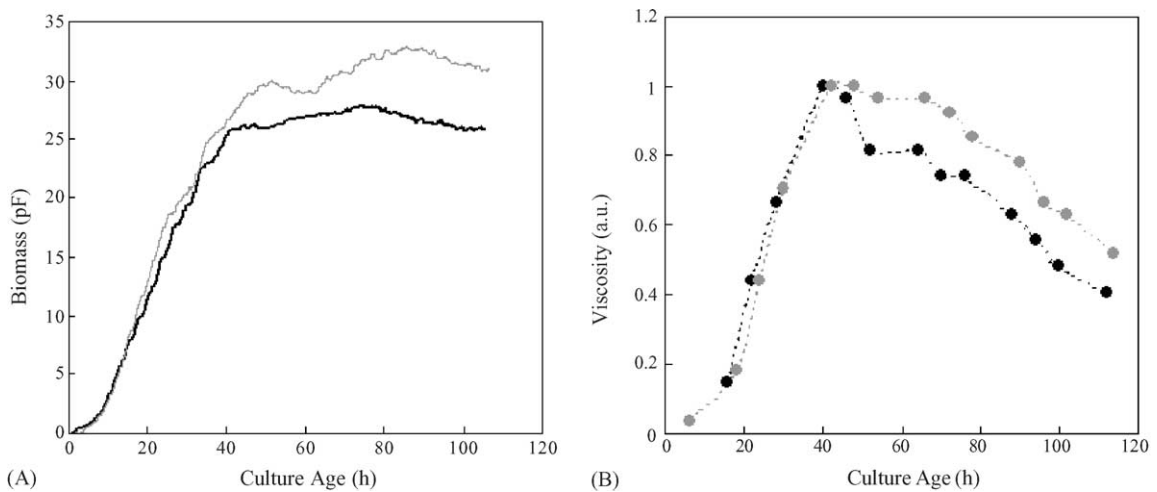


Fig. 3. Comparison of the growth curves obtained with the four-pin electrode probe (run #4, black) and the annular probe (run #14, grey): (A) time courses of on-line culture capacitance, (B) time courses of viscosity measurements.

Table 1  
Linear relationships between capacitance readings (Cap) and viscosity measurements (visc)

		Equation	$R^2$
Four-pin probe	Run #4	$\text{Cap} = 0.22 \times \text{visc} + 53.4$	0.99
	Average	$\text{Cap} = (0.20 \pm 0.05) \times \text{visc} + (56.8 \pm 9.4)$	0.99
Annular probe	Run #14	$\text{Cap} = 0.30 \times \text{visc} + 46.5$	1.00
	Average	$\text{Cap} = (0.29 \pm 0.03) \times \text{visc} + (48.2 \pm 1.0)$	0.99

The initial capacitance ( $t=0$ , no biomass present in the fermentor) was subtracted from the capacitance profiles to enable signal comparison. Fig. 3B depicts the corresponding viscosity measurements. Observation of Fig. 3A might indicate that the signal intensity of the annular probe is higher than the four-pin probe but, from Fig. 3B, viscosity in the stationary phase is also higher during run #14. The linear relations between viscosity and capacitance during the exponential phase (15–30 h) of the fermentation were calculated for each run. Results for runs #4 and 14 and the average results for fermentations monitored with each probe are displayed in Table 1. The slope is consistently higher for the annular probe indicating that for the same broth viscosity, capacitance readings will be above those obtained with the four-pin electrode probe. The signal intensity of the prototype probe is slightly superior to the currently employed probe so it will be more sensitive to lower biomass concentrations.

The growth curves of four different standard experiments monitored with the prototype annular probe are depicted in Fig. 4. It can be seen that all the profiles are very similar though they were conducted several weeks or months apart from each other. This means that given the same initial conditions and using the same operating conditions during the course of the fermentation, the measured capacitance provides a consistent indication of the viable biomass concentration.

### 3.2. Specific growth rate

The Biomass Monitor<sup>TM</sup> measures biomass by the capacitance of the cell suspension,  $C$ . According to the

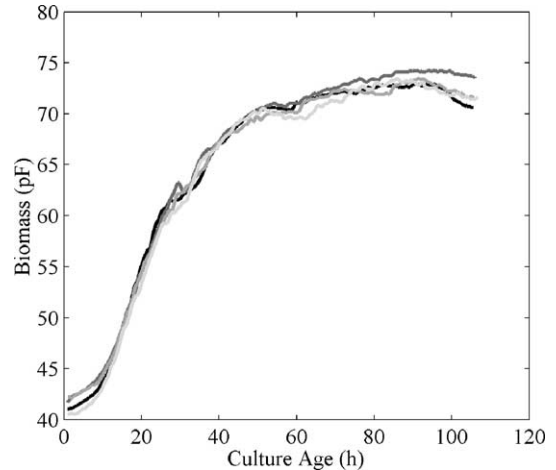


Fig. 4. Growth curve of four different standard runs monitored with the annular probe—from the darkest to the lightest: runs #6, 8, 9, and 14.

theory of dielectrical spectroscopy, capacitance readings have a linear correlation with the concentration of biomass inside the bioreactor,  $X$ .

$$X = \alpha C \quad (1)$$

The calibration coefficient  $\alpha$  is the slope of the linear relationship between capacitance and biomass and can be determined by an off-line calibration procedure (Spierings, 1998), which is outside the scope of this paper. The relation expressed in Eq. (1) enables the calculation of the specific growth rate,  $\mu$ , by the following equation:

$$\mu = \frac{1}{X} \frac{dX}{dt} = \frac{1}{C} \frac{dC}{dt} \quad (2)$$

The evolution of the specific growth rate during the entire course of runs #4 and 14 is displayed in Fig. 5A. This rate was calculated from the smoothed capacitance data and the result was also smoothed by application of a quadratic Savitsky–Golay filter with a 25 points width window to obtain the data shown in the figure. The specific growth rate observed in the first 20 h of the fermentation is displayed in Fig. 5B. It is clear that the specific growth rate presents oscillations during that period. Until 16–18 h of fermentation, the process is carried as a batch with no alterations of the operation conditions. From that moment on, the process is run as fed-batch. Both probes capture these oscillations and

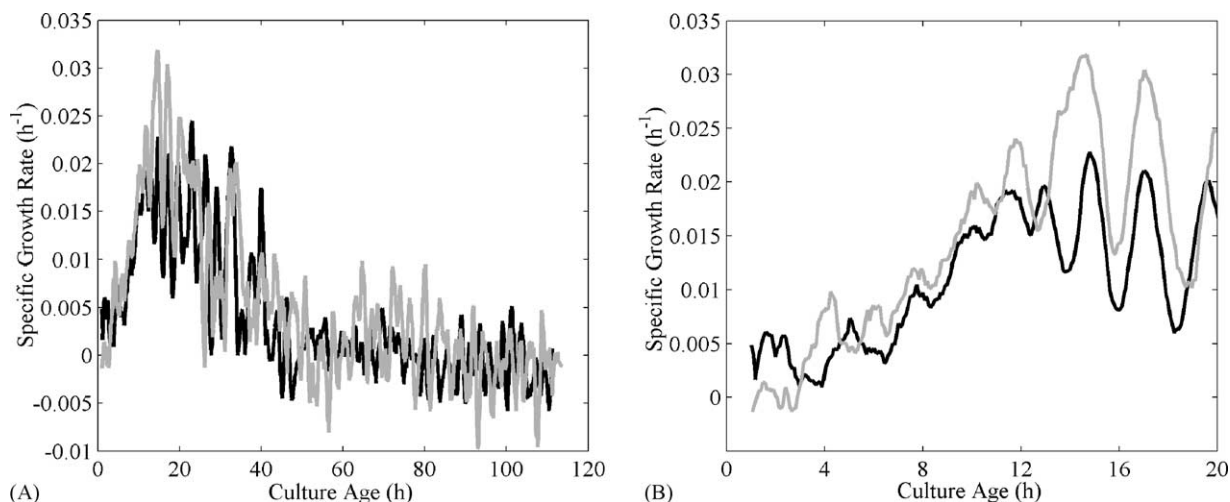


Fig. 5. Calculated specific growth rate: (A) whole fermentation, (B) first 20h of fermentation; black-run #4 (four-pin probe), grey-run #14 (annular probe).

we would stress that they are not due to mathematical artefacts (that could be introduced by the filter used to smooth data) but are observed as a consequence of the continuous readout that is available (Davey et al., 1996). It is possible that these oscillations in the specific growth rate during the beginning of the exponential growth phase reflect metabolic oscillations of the culture in that phase and further work will be required in this area.

### 3.3. Signal-to-noise ratio

The assessment of the signal-to-noise ratio,  $S/N$ , for each probe is important when comparing the probe's performance. For that purpose the maximum capacitance signal during the course of a fermentation was divided by the average standard deviation of the raw capacitance signal in the same time, according to the formula:

$$S/N = \frac{Cap_{max}}{\sigma_{average}} \quad (3)$$

The average signal-to-noise ratio for each probe was calculated by averaging the observable ratios for all the fermentations monitored with that probe. The average  $S/N$  ratio was 346 for the four-pin probe and 407 for the annular probe and so the signal-to-noise ratio for the new annular probe is about 17% higher than the ratio observed for the old pinned probe.

## 4. Conclusions

The use of an in situ capacitance probe was demonstrated for the on-line monitoring of a pilot-plant mycelial streptomycete process run under the same cultivation conditions as the industrial process—viz., similar cell densities, culture media, plant environment. Biomass determinations obtained by standard techniques (packed mycelial volume, viscosity) were presented and compared with capacitance measurements. Capacitance readings can successfully replace the former measurements giving an accurate and on-line description of the biomass profile during the entire process time. Moreover, it enables the on-line computation of the specific growth rate and thus the development of more accurate and reproducible process operational control strategies.

The growth curves measured with each of the two probes are different, both in intensity as well as sensitivity, and the prototype annular probe proved to yield a stronger signal and more sensitive measurements. It was demonstrated that the new annular probe has a better signal-to-noise ratio, and so, the lower noise level enables more accurate readings. The new probe yields essentially the same process knowledge, but its new design avoids the need of taking care not to bend the probe's pins making it more rugged and easier to handle by plant personnel. There are reasons to expect that the prototype probe will be accepted by the bioprocesses

industry as alternative to the four-pin electrode probe for biomass monitoring.

### Acknowledgements

The authors wish to express their gratitude to CIPAN S.A. for supporting this work and for permission to publish the above results. The authors also want to thank Dr. John Williams, Engineering Director and Dr. John Carvell, Sales and Marketing Director, from ABER Instruments (Aberystwyth, UK) for the opportunity of testing ABER's new probe and for the guidance in conducting this research. APF gratefully acknowledges financial support from the Portuguese Foundation for Science and Technology (research grant SFRH/BD8807/2002).

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