Sedimentation monitoring of the active biomass in bioreactors by electrical impedance spectroscopy

Ernesto Serrano-Finetti

Department of Electronic Engineering
Universitat Politècnica de Catalunya-
BarcelonaTech
Castelldefels, Spain
ernesto.serrano@upc.edu

Núria Mata

Faculty of Bioscience Engineering
KU Leuven
Leuven, Belgium
nuria.m96@hotmail.com

Miriam Cerrillo

GIRO, IRTA – Institute of Agrifood
Research and Technology
Caldes de Montbui, Spain
miriam.cerrillo@irta.cat

Abstract—Sequencing batch reactors are used in wastewater treatment to produce biogas (methane). Currently, when biogas production ceases the active sludge sedimentation process is not monitored but operated on a fixed time basis. One step towards gas production optimization would be to detect sedimentation and no expend time unnecessarily. In this work the use of electrical impedance spectroscopy is explored as a tool for sedimentation monitoring. Two electrodes were placed vertically on the reactor wall and the impedance measurements were used to compute the impedance estimator E_2 . Changes in E_2 have proven to be reliable enough to detect whenever the active sludge fell below the lower electrode position hence enabling the development of impedance probes to monitor sedimentation for this kind of reactors.

Keywords—biomass, electrical impedance spectroscopy, wastewater, bioreactors, active biomass.

I. INTRODUCTION

Before being reused, wastewater goes through a series of processes to remove the pollutants it contains. Bioreactors are employed for this purpose and consist of aerobic or anaerobic tanks where organic and nutrient pollutants are removed through the metabolism of a variety of free suspended microorganisms in a process known as activated sludge.

Besides the reuse of water, it is also possible to recover energy from high organic content wastewater [1]. For example, wastewater coming from swine farms contains soluble organic materials like ammoniacal nitrogen and phosphorus. If methanogens are introduced in such wastewater, and the right environmental conditions are granted, biogas can be produced [2]. Sequencing batch reactors (SBR) are good candidates for biogas production because the microbes can adapt easily and begin producing gas in a relatively short time, and also because they're easy to construct. However, they usually operate in a sequence of timed processes without regard of the viable fraction of microbes present in the reactor hence the biogas outcome might not be optimized. The on-line monitoring of the viable fraction of microbes would become one step forward to the optimization of biogas production in SBRs. Currently, the active biomass (AB) and the substrate are constantly stirred while biogas is produced. When the decline in production is detected, stirring ceases and the AB is left to freely settle during a predefined time. This strategy is not optimal because there's no certitude whether AB has completely settled or not, moreover when the AB and substrate have no visible interface that helps to check the decantation status, as in swine manure wastewater. Therefore, optimization could be achieved by a reliable detection of the completed AB sedimentation.

Electrical impedance spectroscopy (EIS) is a technique that allows to characterize any material (biological and non-biological) by means of electrical parameters that fit into an equivalent circuit model [3]. Variation in any of these parameters can be used to detect changes in the material under test. When dealing with cells or microbes, EIS reflects not only changes in their structure but also changes in their environment. For example, temperature variations or non-homogeneities in the substrate will affect some of the EIS parameters. EIS has been used to monitor biomass in fermentation reactors as in [4] and cell growth reactors as in [5]. In those applications there is a linear relationship between the concentration of biomass and EIS parameters hence enabling an accurate biomass monitoring. Moreover, there exists a mathematical model that explains this relationship whenever the biomass and the substrate are homogeneous. However, wastewater hosts different microbe species, protozoa and even nematodes, and the substrate is rather inhomogeneous hence it is not clear to what extent AB monitoring by EIS will be reliable.

In this work, we evaluate the use of EIS to monitor the sedimentation process of AB in a wastewater SBR. Experiments will be performed in a plastic beaker that is used as a mock reactor. Activated sludge (AS) will be used as a first approach to a complex composition biomass.

II. SEDIMENTATION MONITORING BY EIS

A. Impedance estimator E_2

The electrical impedance $Z(f)$ of a homogeneous generic cell suspension is usually modelled as a simplified RC network whose electrical parameters are correlated with the cell volume fraction p , the intracellular and extracellular conductivities, the cell membrane and the cell radius. For this simplified model, $Z(f)$ is described according to the Cole-Cole impedance model (1)

$$Z(f) = R_\infty + (R_0 - R_\infty) / \left[1 + (jf/f_c)^\alpha \right] \quad (1)$$

where R_0 is the equivalent resistance at dc, R_∞ is the resistance at an infinite frequency and f_c is the characteristic frequency of the biological sample in the so-called β -dispersion zone [3]. Usually, $Z(f)$ is measured over a frequency range –ideally including f_c – and the experimental impedance data is fitted to (1). When dealing with microbes, their diameter is usually in the tenths of μm to few μm that are usually matched with f_c values above 10 MHz. Therefore, this scheme requires a calibrated high performance impedance analyzer. The impedance estimator E_2 has been introduced in [4] and used in [6], [7] to monitor biomass in different situations. This

Research funded under grant RTA2015-00079-C02-01 from the National Institute for Agricultural and Food Research and Technology (INIA), Spain.

estimator is based on the measurement of $Z(f)$ at two frequencies, one well above the characteristic frequency f_c ($Z(HF)$) and the other well below it ($Z(LF)$),

$$E_2 = (1 - Z(HF)/Z(LF)) \cdot 100\% . \quad (2)$$

This estimator has proven to increase linearly with p for a biomass concentration above 0.3 g/L (dry weight) –or higher for smaller cell radius– for cultures of the same microorganism.

In SBRs, biomass comes from the AB. One possible scheme is to place the measurement electrodes at a fixed position, i.e. around half of the total reactor height. There, settling would eventually translate into a decrease of AB concentration that would be detected as an E_2 decrease. To accurately know the AB concentration the four-electrode impedance measurement technique performs best as compared to the two-electrode technique [8]. However, such accuracy might not be necessary to detect AB settling hence only two electrodes will be used to measure $Z(f)$. In turn, this would relax any future development of a custom measurement hardware.

B. Electrodes

Polarization of electrodes manifests as an added impedance $Z_p(f)$ in series with $Z(f)$ when measuring with two electrodes. $Z_p(f)$ is usually modelled as a parallel RC network with C larger for large electrode area. It is reported that $Z_p(f)$ could mask $Z(f)$ even for high frequencies, but electrodes are usually small. For this work, the electrodes used are two high grade metric 3 stainless steel acorn nuts that are cheap, readily available and do not easily corrode. Above 100 kHz their impedance has proven to be negligible [9].

III. EXPERIMENTAL SETUP

A. Hardware

A 1-liter plastic beaker (15 cm diameter, 20 cm height) was modified to hold the measuring electrodes. Each electrode consisted of an M3 acorn nut (A4 grade) tightened to its corresponding bolt that acted as the electrical terminal where a wire was connected. Initially, three of such electrodes were placed on the beaker, as shown in Fig. 1: two on the same vertical (adjacent electrodes configuration), 5 cm apart, and one placed on the diametrically opposed vertical (opposed electrodes configuration). This allows to check whether there is an optimum electrode configuration or not. Cabling was glued to the beaker in order to keep its influence (e.g. stray capacitance) constant between measurements.

$Z(f)$ was measured with an HP4192A (Agilent, USA) impedance analyzer from 100 kHz to 50 MHz in a logarithmic scale (201 points). The excitation signal was set to a peak-to-peak amplitude of 100 mV. A pluggable three-terminal female block was used to switch the measurement between adjacent and opposed electrodes whereas a pluggable two-terminal male block was attached to the HP4192A measurement terminals. This is done in order to easily reproduce the beaker placement between wastewater samples and to avoid disturbances in the measurement setup when changing between electrode configurations.

Fig. 1. Left: Plastic beaker showing the adjacent electrodes and their terminals. Right: the same beaker showing the diametrically opposed electrode.

Fig. 2. Plastic beaker showing the reference levels where the impedance measurement was performed. Left: Initial state (level 0); middle: Interface between AB and clear water passing between the adjacent electrodes (level 1); right: Interface well below the lower electrode (level 2).

B. Activated sludge samples

Samples of AS were obtained from a nutrient elimination reactor of two different municipal sewage treatment plants, one located at Sant Feliu de Llobregat, Barcelona, Spain (SF1 and SF2) and the other at Rubí (RU1), also in Barcelona, Spain. All samples were obtained on different days, separated by a month or more. The dry weight of the biomass was estimated from its volatile suspended solids (VSS) content that was measured in the following days (never more than seven days) after the impedance test was performed. Then, a total of 12 samples with different VSS concentrations were prepared: three for SF1 and for SF2, and six for RU1.

Sample electrical conductivity and temperature was measured with a Multi-340i (WTW-Xylem Analytics GmbH, Germany) handheld electrical conductivity meter. Temperature was not controlled and showed values between 18.5 °C and 23.3 °C. During each individual sedimentation test, temperature deviated by no more than 0.2 °C. Electrical conductivity ranged between 1.35 mS/cm and 2.25 mS/cm, values in the usual range for a wastewater treatment plant.

C. Impedance sedimentation tests

Each sample was stirred until it became a homogeneous mixture and then it was left to freely settle. Three reference levels labelled 0, 1 and 2 were marked on the beaker as shown in Fig. 2. At the time the interface between clear water and AS arrived to a marked level, an impedance measurement was performed. In this way, it is possible to check whether the proposed electrode placement was able to discern between the homogeneous state (interface above the upper electrode) and the decanted state (interface below the lower electrode). The HP4192A was controlled by means of a LabVIEW® virtual instrument using a GPIB-USB-HS interface. The impedance test was performed 3 consecutive times to check its

repeatability. For three first RU1 samples the impedance sedimentation test was repeated after 4 and 14 days from the first measurement without any further supply of nutrients or oxygen. The viable biomass is expected to decrease, mimicking the possible decrease of viable microbes in the AS when all the nutrients have been metabolized.

IV. IMPEDANCE DATA ANALYSIS

All data was analyzed offline with MatLab® (ver. 2017b). Two different data analysis were performed: data fitting to an equivalent circuit and computation of E_2 .

A. Data fitting

The equivalent circuit of Fig. 3 is one possible electrical representation of (1) for $\alpha = 1$ and is widely used to model the electrical impedance of biological tissues. A constant phase element was discarded because the analysis of preliminary tests consistently showed α values very close to 1 hence a capacitor is enough and only three electrical parameters need to be fitted.

R_e and R_i represent the extracellular medium and the intracellular medium respectively. C_m represents the cell membrane hence any decrease in the biomass concentration – the active sludge in our case – should be detected as a decrease in C_m . This analysis was carried out for each sedimentation test.

B. Computation of the impedance estimator E_2

E_2 should be computed according to (2) hence only two measurements needed to be selected from the whole impedance magnitude spectrum. $|Z(LF)|$ is the impedance measured at 100 kHz and $|Z(HF)|$ is the impedance measured at 10 MHz. Sensitivity loss would arise because of this frequency selection as explained in [4] but this was necessary due to an observed resonance occurring above 10 MHz that distorted the impedance spectrum. Such resonance is related to the stray capacitance from the material under test to ground, which in turn depends on the volume of the material and its distance to ground [10]. As in the data fitting analysis, E_2 was computed for all the sedimentation tests.

V. RESULTS AND DISCUSSION

A. SF1 and SF2 tests

The total dry weight of each sample as estimated by its VSS is shown in Table I. These values are low and near to the threshold of detectability using this setup [4].

Fig. 3. Equivalent circuit modelling the impedance of any AS sample. C_m depends on the concentration of biomass and R_e and R_m depend on the conductivity of the liquid fraction.

For SF1 and SF2, a total of 27 impedance spectra were acquired and their C_m and E_2 values were computed. Fig. 4 shows the Bode plots of a typical impedance spectrum (magnitude and phase) while Fig. 5 shows its corresponding Nyquist plot. Phase is especially susceptible to this distortion hence it affects the imaginary part at frequencies between 10 MHz and 20 MHz. For this reason, the impedance data that were finally used to fit the equivalent circuit was restricted to 15 MHz. E_2 computation used a maximum frequency of 10 MHz, as explained before.

TABLE I. VOLATILE SUSPENDED SOLIDS, ELECTRICAL CONDUCTIVITY (EC) REFERENCED TO 25 °C AND TEMPERATURE FOR EACH ONE OF THE TESTED

SAMPLES				
AS sample	VSS (g/l)	T (°C)	EC25 (mS/cm)	
SF1	1	2.5	18.5	2.06
	2	3.2	18.6	2.05
	3	4.9	19.8	1.36
SF2	1	4.6	21.2	2.14
	2	4.9	21.6	2.14
	3	3.1	21.6	2.14
RU1	1	2.1	22.6	1.78
	2	2.3	23.2	1.76
	3	2.0	23.3	1.77
	4	3.5	16.4	1.76
	5	3.4	16.5	1.76
	6	3.6	17.5	1.76

Fig. 4. Bode plots of the impedance magnitude (up) and impedance phase (down). It can be clearly seen the effect of the resonance at about 20 MHz.

Table II shows the mean value of the three consecutive measurements for both C_m and E_2 at each marked level. In all cases, the changes produced by the sedimentation process on both estimators are small. However, the changes in E_2 are larger than those of C_m and allows to reliably detect sedimentation. One issue with data fitting is that the available data did not cover the whole arc that is expected in the β -dispersion zone of biological tissues hence larger uncertainty in the fitted parameters could reasonably be expected. Anyway, the changes in C_m allow to associate the observed changes in the estimator to changes in the biological content of the samples. On the other hand, repeatability of E_2 is quite good as can be observed in Fig. 6. All the values corresponding to a certain level are tightly packed, with a standard deviation of around 1% of the measured value. This

makes it a good candidate for sedimentation monitoring even though it is not being measured with an optimum sensitivity.

Fig. 5. Typical Nyquist plot of the measured impedance. The distortion caused by the resonance is more obvious here than in the Bode plots.

TABLE II. C_m AND E_2 VALUES FOR THE IMPEDANCE SEDIMENTATION TESTS OF SF1 AND SF2 AS SAMPLES (ADJACENT ELECTRODES)

Sample	Level 0		Level 1		Level 2	
	C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)
SF1-1	14.6	10.0	14.1	9.2	13.8	8.7
SF1-2	14.2	10.0	13.8	9.2	13.5	8.8
SF1-3	16.5	17.2	16.3	16.2	16.2	15.9
SF2-1	14.3	8.4	14.4	8.3	14.0	7.5
SF2-2	13.8	7.8	13.8	7.7	14.0	7.4
SF2-3	13.8	7.6	13.8	7.5	13.4	6.8

TABLE III. C_m AND E_2 VALUES FOR THE IMPEDANCE SEDIMENTATION TESTS OF SF1 AND SF2 AS SAMPLES (OPPOSED ELECTRODES)

Sample	Level 0		Level 1		Level 2	
	C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)
SF1-1	14.4	10.5	13.4	9.3	13.2	8.5
SF1-2	13.0	9.9	13.5	9.8	13.8	9.6
SF1-3	16.0	19.0	15.8	17.4	15.6	16.8
SF2-1	12.4	8.0	12.2	7.5	12.1	7.0
SF2-2	14.0	8.3	13.9	8.0	14.2	8.0
SF2-3	14.0	8.0	13.9	7.7	13.8	7.4

Biomass concentration of viable yeast during the fermentation process of alcoholic beverages is commercially available. There, the extracellular medium is held relatively controlled in order to favor the grow of biomass. Because of this, the usual concentrations are much larger and the expected changes in E_2 (or C_m) would also be larger and depend linearly on concentration. From the EC25 data in Table I, the extracellular medium seems not to be comparable hence the E_2 values would neither be comparable. This means that similar values would not necessarily imply that the biomass concentration would be the same. For example, VSS values for SF1-2 and SF2-3 are 3.1 g/l and 3.2 g/l respectively and their E_2 values are quite different (10.0 % and 7.6 % respectively), and their EC25 was 2.05 mS/cm and 2.14 mS/cm. For this set of samples, the correlation factor was $R^2 = 0.1$. Moreover, two random samples were put under a microscope to check their contents. As expected, microbial flocs were present but also few nematodes, which can become a source of uncertainty.

The resistance fitted values of the equivalent circuit also showed changes during the settling process, but they are influenced by factors different than cell concentration. For example, settling of the substrate would lead to changes in R_e but not in C_m . As a matter of fact, the electrical conductivity

of water showed slight changes during the sedimentation process.

Fig. 6. Repeated tests show that the detection of the AS sedimentation is reliable. The difference between level 0 and level 2 are sufficiently apart as compared to the deviation standard.

Although temperature is an interfering variable in impedance measurements, its influence did not prevent the detection of sedimentation. Moreover, real-world SBRs are either covered with a thermal jacket or are placed on a thermostatic bath that allows temperature control. Therefore, temperature is not expected to be of concern.

Finally, Table III shows the results for the opposed electrodes. This electrode configuration yielded equivalent results to the adjacent configuration, showing no further improvements hence it was discarded for the rest of the experiments.

B. RU1 impedance tests

Table IV summarizes the results for E_2 and C_m . Samples RU1-4 to RU1-6 yielded similar changes on E_2 to SF1 and SF2 samples and sedimentation could also be detected reliably. For the 3-day tests, the VSS values obtained on the 4th day and on the 14th day were slightly higher. This result was paradoxical as it was expected that the aerobic AS would not thrive on the anaerobic conditions with lack of nutrients. On the other hand, the EC25 mean values increased with time from a 1.76 mS/cm to 1.83 mS/cm on the 4th day and to 1.93 mS/cm on the 14th day (not shown on Table III).

Taking a closer look at the E_2 and C_m values obtained over the 3-day test, sedimentation produced increasingly smaller changes. For example, in RU2 the change detected for sedimentation was 1.0 % on the first day, 0.3 % on day 4 and 0.2 % on day 14. The other samples showed similar trends. C_m also showed smaller changes, arriving at practically no changes on the last day. Apart from corroborating that sedimentation is still detectable, no other conclusions could be drawn at this point as the specific microbe species are not known and so is their behavior in front of nutrient deprivation.

VI. CONCLUSIONS

The AS of a bioreactor for wastewater treatment contains viable biomass that might be monitored by means of the electrical impedance estimator E_2 . However, it is difficult to discern the exact concentration of biomass only from the E_2 values. This is due basically to the fact that the bacterial flocs contained in wastewater consists of different species that exhibit different diameters. On the other hand, the medium where microbes grow can differ greatly in terms of electrical conductivity, particle contents and can also include larger organisms as nematodes. Therefore, the usual models used to

relate biomass impedance and concentration might no longer hold for wastewater.

TABLE IV. C_m AND E_2 VALUES FOR THE IMPEDANCE SEDIMENTATION TESTS OF RU1 AS SAMPLES

Sample	Level	Day 1		Day 4		Day 14	
		C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)	C_m (pF)	E_2 (%)
RU1-1	0	9.4	16.2	12.0	15.8	11.7	15.7
	1	9.2	15.6	11.9	15.8	11.7	15.8
	2	9.1	15.3	10.3	14.7	11.6	15.3
RU1-2	0	11.8	17.9	11.5	14.9	10.8	14.8
	1	9.9	17.3	11.5	14.9	10.8	14.7
	2	9.7	16.9	11.5	14.6	10.7	14.6
RU1-3	0	9.5	15.5	12.3	15.4	11.7	15.6
	1	9.3	15.3	12.3	15.4	11.7	15.4
	2	9.2	15.0	12.2	15.0	11.6	15.3
RU1-4	0	10.5	20.8	-	-	-	-
	1	10.0	19.1	-	-	-	-
	2	10.1	18.4	-	-	-	-
RU1-5	0	11.4	22.8	-	-	-	-
	1	10.9	19.9	-	-	-	-
	2	9.9	18.1	-	-	-	-
RU1-6	0	11.1	19.8	-	-	-	-
	1	10.7	18.5	-	-	-	-
	2	10.0	17.2	-	-	-	-

However, to detect the AS sedimentation it is not necessary to accurately know biomass concentration but its deviation from a homogeneous state. E_2 has proven to consistently detect this deviation with a pair of adjacent electrodes placed vertically on the reactor wall. Nevertheless, the electrode placement used in this work would not be practical as it needs machining of the reactor. One alternative would be to design a cylindrical impedance probe that can be inserted through the reactor cap as for other submersible probes used in similar applications. For this application, it seems enough to use only two-electrodes, despite its reduced sensitivity. In a real situation, it is impractical to use a large impedance analyzer as the HP4192A hence a custom-made measurement system should be build. The possibility of using only two electrodes would relax the design requirements of the electronic circuits compared to a four-electrode technique.

ACKNOWLEDGMENT

This work was funded by the National Institute for Agricultural and Food Research and Technology (INIA),

Spain, under grant RTA2015-00079-C02-01. The authors would like to thank Mr. Francis López for his technical assistance.

REFERENCES

- [1] H. Liu, R. Ramnarayanan, and B. E. Logan, "Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell," *Environ. Sci. Technol.*, vol. 38, no. 7, pp. 2281–2285, 2004.
- [2] R. E. Speece, "Anaerobic biotechnology for industrial wastewater treatment," *Environ. Sci. Technol.*, vol. 17, no. 9, pp. 416A–427A, 1983.
- [3] C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues : I . Literature survey," vol. 41, pp. 2231–2249, 1996.
- [4] R. Bragós, X. Gámez, J. Cairó, P. J. Riu, and F. Gòdia, "Biomass monitoring using impedance spectroscopy," *Annals of the New York Academy of Sciences*, vol. 873, pp. 299–305, 1999.
- [5] C. Xiao and J. H. T. Luong, "On-line monitoring of cell growth and cytotoxicity using electric cell-substrate impedance sensing (ECIS)," *Biotechnol. Prog.*, vol. 19, no. 3, pp. 1000–1005, 2003.
- [6] E. Sarró *et al.*, "Electrical impedance spectroscopy measurements using a four-electrode configuration improve on-line monitoring of cell concentration in adherent animal cell cultures," *Biosens. Bioelectron.*, vol. 31, no. 1, pp. 257–263, 2012.
- [7] J. Martínez-Teruel, T. García-Sánchez, A. Fontova, and R. Bragós, "Electrical impedance spectroscopy cell monitoring in a miniaturized bioreactor," *19th IMEKO TC4 Symp. - Meas. Electr. Quant. 2013 17th Int. Work. ADC DAC Model. Test.*, pp. 446–450, 2013.
- [8] R. Bragós *et al.*, "Four versus two-electrode measurement strategies for cell growing and differentiation monitoring using electrical impedance spectroscopy," *Annu. Int. Conf. IEEE Eng. Med. Biol. - Proc.*, pp. 2106–2109, 2006.
- [9] E. Serrano-Finetti, C. Aliau-Bonet, O. López-Lapeña, and R. Pallàs-Areny, "Cost-effective autonomous sensor for the long-term monitoring of water electrical conductivity of crop fields," *Comput. Electron. Agric.*, vol. 165, no. July, p. 104940, 2019.
- [10] C. Aliau-Bonet and R. Pallàs-Areny, "Effects of stray capacitance to ground in bipolar material impedance measurements based on direct-contact electrodes," *IEEE Trans. Instrum. Meas.*, vol. 63, no. 10, pp. 2414–2421, 2014.