UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Escola Superior d'Agricultura de Barcelona

ONLINE BIOIMPEDANCE MEASUREMENT: CHARACTERISATION AND APPLICATION IN BIOSYSTEMS ENGINEERING

Bachelor Thesis

Degree in Biological Systems Engineering

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September 2016

Abstract

Biological materials can be characterized by electric impedance and its variation within a range of frequencies. Specifically, the electrical behaviour of biological materials is described by the β-dispersion. This methodology has been deeply developed in different sectors including biomedicine, biotechnology and food industry.

In this work it is studied its application in environmental engineering for wastewater treatment purposes. It is an *'On line', 'In situ' and 'Label free' system* that could overcome the main drawbacks that conventional analytics present. To achieve that, activated sludge samples have been measured through an impedance analyser (Agilent 4294A) with the main objective to detect presence or absence of microorganisms. Since the electrical behaviour of such a heterogenic biomaterial was unknown, the experimental design has been defined along the work, describing all the followed steps from equipment configuration till the own measurement methodology.

Through the obtained results, a simpler interpretation of bioimpedance readings is being suggested, rather than β-dispersion. It has been observed that there is a variation of the impedance modulus along a determined range of frequencies for biomass measurements, while it remains almost constant in absence of biomass. This simpler explanation of the method could take interest in all those Biosystems which knowing the biomass concentration in a determined point and moment would increase the efficiency of the biological process.

Results show that bioimpedance correlates very well with VSS (volatile suspended solids), the usual unit expressing biomass concentration in biological wastewater treatment plants.

Resum

Els materials biològics poden ser caracteritzats per la impedància elèctrica i la seva variació al llarg d'un marge de freqüències. Concretament, el comportament elèctric dels materials biològics ve descrit per la dispersió-β. Aquesta metodologia s'ha desenvolupat en diferents sectors com són la biomedicina, la biotecnologia i la indústria alimentària.

En aquest treball s'estudia la seva possible aplicació en enginyeria ambiental, en el tractament d'aigües i residus, ja que és una tècnica '*On line*', '*In situ*' i 'l*abel free*', que podria resultar més avantatjosa que els sistemes de mesura de biomassa habituals. Per això, s'han mesurat fangs de depuradora amb un analitzador d'impedàncies (Agilent 4294A) amb el principal objectiu de detectar presència o absència de microorganismes. Com es desconeixia el comportament elèctric d'un material biològic tan heterogeni, el disseny experimental s'ha anat definint al llarg del treball, descrivint els passos seguits des de la configuració de l'aparell al propi mètode de mesura.

A través dels resultats obtinguts, es suggereix una interpretació de les lectures de bioimpedància més simple que no pas la β-dispersió. S'ha observat que hi ha una variació del mòdul de la impedància en mesurar biomassa, mentre que roman quasi constant per a absència de biomassa.

Aquesta simple explicació del mètode pot tenir interès en tots aquells biosistemes en els quals conèixer la concentració de biomassa en un determinat punt i moment incrementaria la eficiència del procés biològic.

Els resultats mostren que la bioimpedància es correlaciona molt bé am bels SSV (Sòlids Suspesos Volàtils), que és la unitat de mesura de concentració de biomassa habitual en plantes de tractament d'aigües residuals.

Resumen

Los materiales biológicos pueden caracterizarse por impedancia eléctrica y la variación de ésta a lo largo de un margen de frecuencias. El comportamiento eléctrico de los materiales biológicos concretamente, viene descrito por la dispersión –β. Esta metodología de análisis biológico está siendo utilizada y desarrollada en diferentes sectores como la biomedicina, biotecnología e industria alimentaria.

En el presente trabajo se pretende estudiar la posible aplicación de dicha metodología en la ingeniería ambiental, en éste caso, para el análisis en el tratamiento de aguas residuales. Puesto que es una técnica 'o*n line*', 'i*n situ*' i '*label free*', podría resultar ser una tipo de medida ventajosa respecto a las técnicas actuales de medida de biomasa. Con el fin de estudiar esta posibilidad se han analizado lodos de depuradora con un analizador de impedancias (Agilent 4294A) con el objetivo de detectar presencia o ausencia de microorganismos. Debido al desconocimiento ante el comportamiento eléctrico de un material tan heterogéneo, el diseño experimental se ha ido definiendo a medida que se han ido obteniendo resultados, des de los pasos seguidos para el diseño del aparato de medida al propio método y al análisis de estas medidas.

Los resultados obtenidos sugieren una interpretación más simple de las lecturas de bioimpedancia que la habitual β-dispersión. Se ha determinado que existe una variación del módulo de la impedancia al haber presencia de biomasa, mientras que éste no varía cuando no hay presencia de biomasa. Esto puede ser de gran interés para todos aquellos biosistemas en los que conocer la concentración de biomasa en un determinado momento incrementaría la eficiencia del proceso biológico.

Los resultados muestran que la bioimpedancia se correlaciona muy bien con los SSV (Sólidos Suspendidos Volátiles), que es la unidad de medida de concentración de biomasa habitual en las plantas de tratamiento de aguas residuales.

Agraïments

Voldria agrair a totes aquelles entitats i persones que han col·laborat en la elaboració d'aquest treball.

En primer lloc, a Carles Aliau, per iniciar aquest projecte i fer-me'n part. Sense ell, aquest treball no hagués estat possible. A més, agrair la seva total disposició a ensenyar-me (i repetir-me, les vegades que fes falta) sobre teoria de la bioimpedància. Tampoc mai m'ha faltat l'ajuda de Marcos Quilez com a cotutor, a més d'acompanyar-me en totes les mesures.

Al meu tutor Xavier Flotats, per la seva incansable dedicació. Gràcies a ell he arribat més lluny del que en un principi esperava.

A la E.D.A.R de Rubí per l'accés a totes les mostres, especialment a Belén Lafuente, qui m'ha facilitat molt la feina i a Teresa Balanyà per a establir el contacte.

Al grup ISI per donar-me la possibilitat de fer mesures amb l'analitzador d'impedàncies.

A Jaume Montiel per considerar aquesta línia d'investigació en el seu TFG i als companys de biosistemes amb qui he compartit les reunions de seguiment i ajudes especials al laboratori. També a Maria Rodríguez per la seva total disponibilitat i ajuda al laboratori d'aigües de l'ESAB.

A la meva família i amics que m'han fet més amè tot el procés i que m'han ajudat, com sempre, a no perdre la calma. I per últim, però per ser el meu pilar indiscutible, al Joan Pol.

Summary

List of figures

List of tables

Abbreviations

AS = Activated Sludge

CW = Clarified Water

WT = Water Treatment

AC = Alternating current

CC = Continuous current

R = Resistance [Ω]

C = Capacitance [F]

V = Voltage [V]

I = Current Intensity [A]

Z = Impedance [Ω]

σ = Electrical conductivity [S/m]

EC = Electrical conductivity [S/m]

ε = Electrical permittivity [F/m]

kcell = cell constant [m]

f = Freq = Frequency [Hz]

Θ = phase shift [rad]

ω = Angular frequency [rad]

ϕ = Angular phase [rad]

j = Imaginary unit

UV =Ultra Violet

IR = Infra-red

PET= Polyethylene Terephthalate

TS = Total Solids [g TS/l]

VS = Volatile Solids [g VS/l]

TSS = Total Suspended Solids [g TSS/l]

VSS = Volatile Suspended Solids [g VSS/l]

COD = Chemical Oxygen Demand [mg O_2/L]

CI = Confidence Interval [%]

el = electrode

1. Introduction and objectives

1.1. Introduction

Bioimpedance is an electric measurement technique widely used in different industries as an indicator of the variation of cellular content of a particular medium. The biomass concentration is determined by the electrical impedance, whose value is modified by variations of conductivity caused by the presence or absence of microorganisms in a medium. In this way, different biological materials, such as tissues, organs or cellular suspensions, can characterized without interfering in the measured object, which means that it is not a destructive method. Another important advantage is that the equipment can be calibrated to obtain an 'online' reading, which means that results can be shown at the same time the measurement is performed. All this makes the bioimpedance analyser a promising tool in different industries and sectors.

For several decades, electrical bioimpedance technology has played an important role in electrophysiology and biophysics. Currently, bioimpedance is a means of non-invasive, quick and relatively affordable assessment of tissue composition as well as of volumes of various body water compartments that are difficult to assess by other techniques (Schneditz, 2006). Bioimpedance applications for skin cancer detection (Aberg et al., 2005) or dry weight determination (Kuhlmann et al., 2005) have increased during the last decade (Buendía, 2011). Nowadays its use in nutrition and sports medicine is becoming more widespread.

Although bioimpedance techniques are mainly being developed in biomedicine, they are also of interest to the food industry as a quality control tool, for example for meat and seafood. There, changes in water content or the presence of particular microorganisms can be quickly detected. Electrical impedance has been used as tool to investigate the dielectric properties of cells and organelles for over 75 years (Feldman et al., 2003). For example, impedance techniques have been used to investigate changes in the membranes of biological cells in suspension, to measure micromotion of cells on a small electrode, to monitor enzyme reactions, to detect immunological reactions, to detect microbial metabolism, and to detect protein adsorption to supported lipid bilayers (Ehret et al., 1998).

Its use in monitoring animal cell culture, plant cell culture and microbial fermentation has been reported since 1991 (Cannizzarro *et al*., 2003). It also has potential to be used to automatize the fermentation process in brewing industries. With regard to environmental issues, soil quality and humidity, as well as some geophysical properties, have been measured with immittance (which can be described as the opposite of impedance) since 1920. Moreover, volcanic activity is monitored by impedance in Iceland (Grimmes and Martinsen, 2008).

As can be seen, bioimpedance is a technique used several sectors with a long history. So, why does it not seem to have become popular in industrial or environmental engineering? The complexity of its readings and all the factors involved in electromagnetic phenomena make it difficult to be used on an industrial scale. Temperature, the position of the electrodes, materials and parasite capacitances are some of the factors involved in impedance measurements. All this means that this technique requires state of the art technology and strict performance conditions, which are usually expensive to implement and difficult to automatize in non-sterile environments.

In biosystems engineering, it is essential to know the amount of biomass in a bioreactor, either to control the growing biomass or to check the generated products. Generally, the most used sensors are those that work with chemical and physical variables related to microorganisms' metabolism, which in some cases may not be reliable. Also it can be said that biologic sensors are far more complex because their response is dependent on many requirements that limit the benefits (Bragós, 1997).

Bioimpedance could be developed as a very useful tool in biosystems engineering but, to encourage its use, a simpler yet clear method needs to be developed so that it can be used in an easy and reliable way. Similarly, it is vital to give another point of view and propose a simpler methodology, one that is demonstrably less accurate but that meets all the requirements.

1.2. Objectives

The main objective of this work is to probe that bioimpedance technique can be used to measure biomass and its application in the automatization of a bioreactor is possible. For that, it is required to determine if bioimpedance is sensitive to biomass concentration.

By describing the observed electrical behaviour of such a heterogenic biomaterial like wastewater products, this work is intended to be a first contact between bioimpedance technique and bioreactors for wastewater treatment in biosystems engineering.

Since bioimpedance is sensitive to environmental conditions, necessarily external and internal factors affecting the readings are studied in order to detect possible error sources, considering this as secondary objective. Moreover, a measurement cell and a measurement protocol is developed, taking into account the most relevant factors which affects the readings.

1.3. Thesis outline

This thesis has been structured in the standard form for scientific documents, – introduction, objectives, materials and methods, results and conclusions – but other points have been included that are not so conventional:

- The first chapter introduces the use of the bioimpedance technique, some relevant moments in its history and its main applications, as well as some concerning factors about its development in Biosystems engineering.

- The second chapter “Theoretical basis of bioimpedance” is focused on the fundamentals of bioimpedance. Since it is a complex term that can be explained from a wide perspective, some aspects that help explain the conclusions of this work are given here. It has been developed through bibliographic research. There, electrical impedance is defined as is its particular use in the study of biological materials. To end with, in “state of the art” the importance of biomass

detection, as well as other techniques used, is detailed. Also the main uses of bioimpedance for biomass detection and its possible application into a SBR reactor are examined.

- The third chapter concerns materials and methods. Since different methodologies have been used, they are described in different sections (bioimpedance measurements, analytical methods and statistic methods). Before this, the nature of the samples is briefly described, as are the steps followed to develop the measurement cell and to configure the impedance analyser.

- In "Results and discussion" different assays have been performed during the work to test the kind of information that can be obtained through bioimpedance measurements, starting with the first biomass measurement and the chosen measurement protocol. They are all detailed here in the same chronological order as they were performed, so new experiments were required at the moment that a common pattern in the results was observed.

- Finally, the fifth chapter contains the conclusions of this work. But also, as a conclusion itself, clear suggestions for further research needed to be made, as this thesis is the first-contact for the bioimpedance technique in the wastewater treatment sector.

2. Theoretical basis of bioimpedance

2.1. Definition of impedance

In a case study, measurements of impedance can be understood as measuring a "black box", where a reading is obtained but without any term to be referred to. So, for its electrical measurement, the content must be characterized, where data is used to describe the electrical behaviour and perhaps even to explain the real nature of the inside of the box. The description must necessarily be based on some form of model, for example in the form of an equivalent electric circuit, mimicking the measured electric behaviour (Grimnes and Martinsen, 2008). But, to understand it, some previous definitions must be given, considering a simple electric measurement as shown in Figure 1.

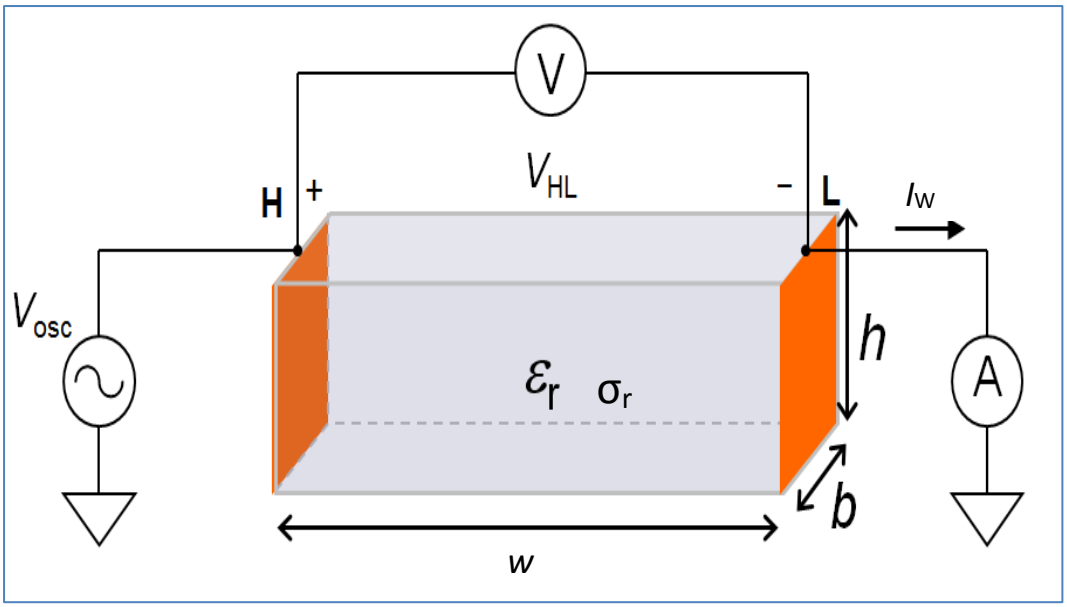


Figure 1 Scheme of an electrical measurement: An altering voltage V_{OSC} is injected through a medium with specific conductivity (σ_r), permittivity (ε_r) and volume (b·h·w ,m^3). Voltage V_{HL} is measured with a voltmeter (V) from the higher potential point (H^+) to the lower potential point (L^-) and current (I_w) is measured with an ampere metre (A) (Aliau, 2013).

When an electrical current (I_W) flows between two electrodes (H and L points) through a material with a determined resistance, a voltage gradient V_{HL} is generated between them. A directly proportional relationship is established in which the voltage increases with resistance. This concerns the well-known Ohm's Law:

$$V_{HL}(t) = I_W(t) \cdot R \ . \qquad (1)$$

Since resistance can be defined as a material's opposition to current, it must be dependent on the intrinsic properties of the measured material, such as its ionic concentration, type of ions and temperature, as well as the geometry of the measured area. From that, it can also be said (Aliau, 2013):

$$R = \frac{1}{\sigma_r} \cdot \frac{w}{b \cdot h} = \frac{1}{\sigma_r} \cdot k_{cell} \ , \qquad (2)$$

where k_{cell} is a geometric factor (w/b·h) and σ_r is the electrical conductivity of the medium, which can be defined as its aptitude to let current flow or as the relationship between current density and the power of the electric field.

Another material property that is shown in Figure 1 is the electrical permittivity (ε_r), which is the ability to permit storage of electric energy by charge polarization from an electric field. In other words, the permittivity of a material defines its susceptibility to polarization by an electric field. The relationship between these quantities are based on the equations of Maxwell (1873) and they establish a direct link between charge density and induced current with the electric field applied (Grimnes and Martinsen, 2008).

The term "Bio" is used since the electrical behaviour of a biological material is studied. To connect both concepts, the biological material is described in the form of an equivalent electric circuit. In this case, the biological material consists of microorganisms suspended in a liquid medium and it can be analysed as a circuital model of cellular suspensions (Bragós, 1997).

To design an electric circuit there are used three main components which are described in figure 2: resistors, capacitors and inductors. A capacitor consists of two parallel conductive plates separated by a dielectric, whose response to an electric current causes the plates to become electrically charged. That means it has the ability to store electrical energy. Because of their nature, inductances present the opposite effect, but they are not used in this case study because resistor and capacitor behaviour are dominant.

In figure 2 it can be seen that electrical responses for capacitances and inductances produce a defacement between voltage and current. Hence, going back to equation 1, it must be said that Ohm's Law cannot be applied in these terms. To be able to work with these complex elements, there are used sinusoidal signals, which are mathematical easier forms to describe them. Hence, intensity and voltage are now described by:

$$I(t) = I_{MAX}\, sin(\omega_0 t + \varphi) \quad , \tag{3}$$

$$V(t) = V_{MAX} \sin(\omega_0 t + \varphi'') \ , \tag{4}$$

where I_{MAX} and V_{MAX} are current and voltage maximum values of I and V (see figure 2), ω_0 is the angular frequency of the signal (equal to $2\pi f$) and φ and φ'' their defacements. This terms can be seen in figure2.

Now that all these concepts have been introduced, impedance can be defined as a complex number formed by a modulus and a defacement or phase shift that connects both sinusoidal voltage and current.

$$Z = |Z| + e^{j\,\theta} \qquad , \tag{5}$$

where,

$$|Z| = \frac{V_{MAX}}{I_{MAX}} \qquad \text{and} \tag{6}$$

$$\theta = [\varphi'' - \varphi'] \ . \tag{7}$$

It can also be described in the Cartesian coordinates as a complex number of two components, the real and the imaginary part:

$$Z = R + j\,X \quad , \tag{8}$$

where R is the real part of impedance or the resistance value, and X is the imaginary part or reactance. Then, impedance modulus will also be described by:

$$|Z| = \sqrt{R^2 + X^2} \quad , \tag{9}$$

and its phase by:

$$\theta = \tan^{-1}\frac{X}{R} \quad . \tag{10}$$

In figure 2 there are represented all the described terms. Note that red and green circles corresponds to maximum values for voltage (V_{MAX}) and intensity (I_{MAX}), respectively. Hence, A) describes the voltage (V) and intensity (I) response for a resistor and shows that there is no defacement (ϕ) between them, so there is no real part and impedance (Z_R) is the resistance value (R) in itself; B) represents the V/I response for a capacitor, which presents a phase defacement of ϕ=-90° between them; there, imaginary part (X_C) is inversely affected by the angular frequency (ω) and the capacitance value (C); lastly, C) defines the electronic response for an inductor, which produces a defacement of ϕ=+90° between voltage and intensity, but here imaginary part (X_L) is directly proportional to ω and the inductance value (L). For this reason, the impedance modulus of a capacity tends to decrease while its inductance increases, as can be seen in Figure 3.

To plot impedance, different models have been developed to represent this complex number along a range of frequencies and time. In this work, a type of plot that consists of an axial representation with log frequency on the X-axis and both the absolute values of the impedance (|Z|) and the phase-shift on the Y-axis is used. Here only the impedance modulus is represented.

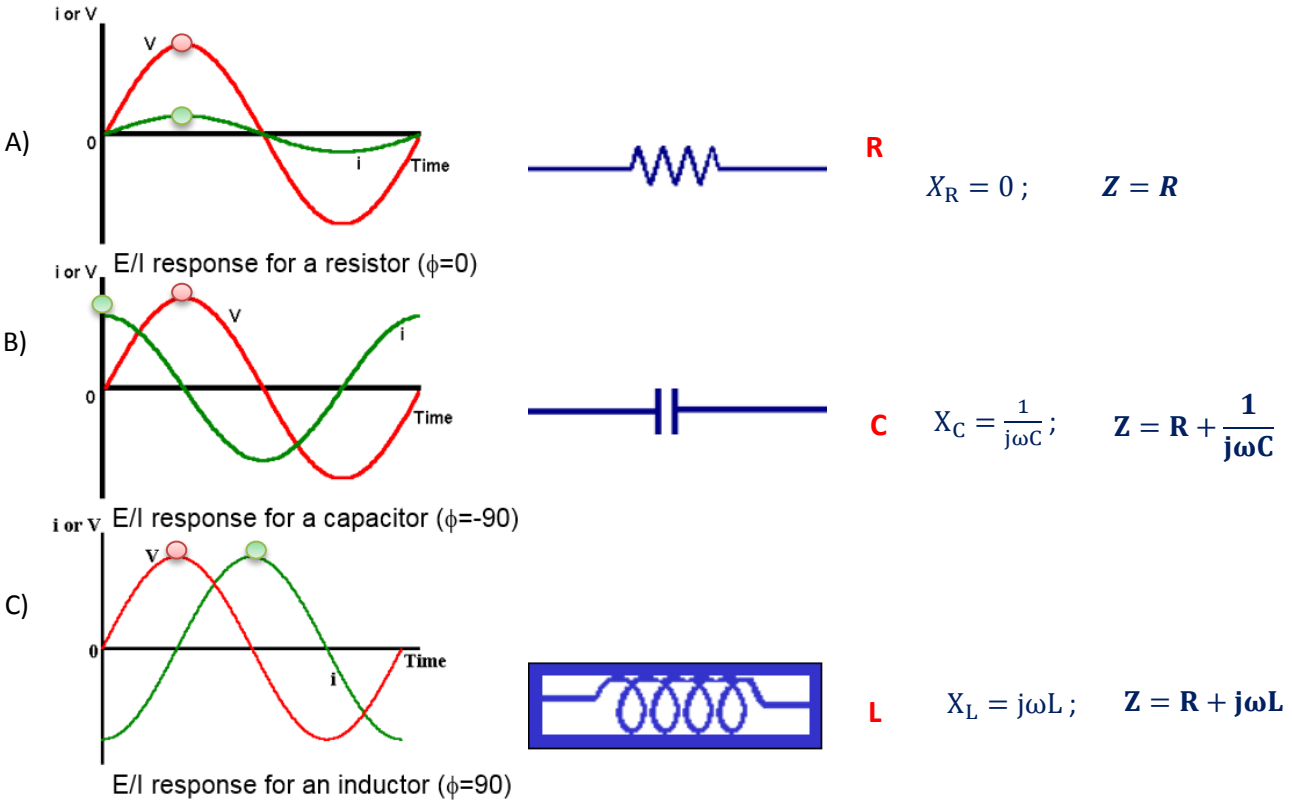


Figure 2 Explanation of the different electrical responses for resistor, capacitor and inductor through Ohm's Law (Gamry instruments, Inc., 2010):

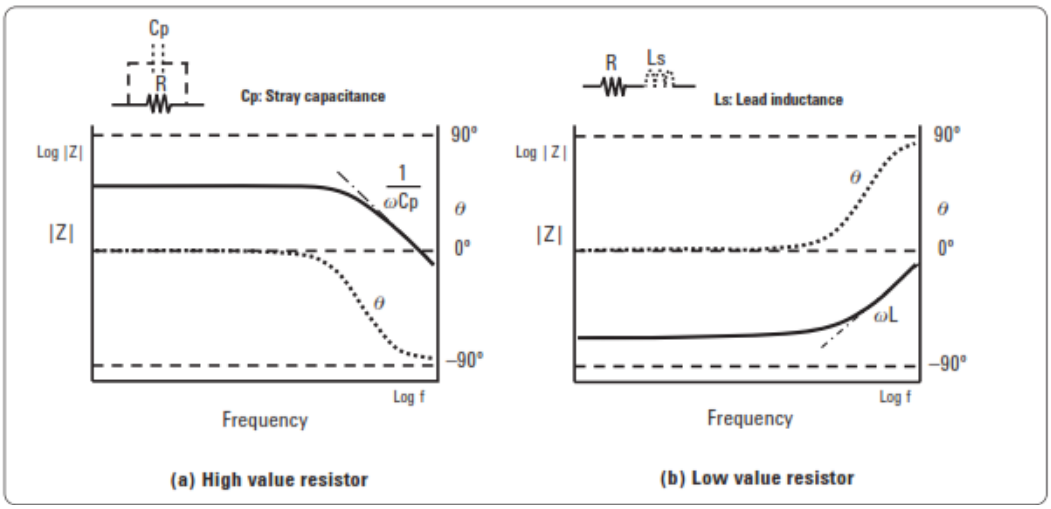


Figure 3 Two plots of Impedance modulus vs. frequency obtained from the Agilent 429A Manual, both represent the resistor frequency response. It can be observed the impedance tendency of the effect capacitor (a) and the inductor (b) (Agilent, 2013)

2.2. Impedance of biological materials or bioimpedance

As has already been mentioned, characterization of impedance measurements must be based on the form of an electrical model. In this case study, the biological material is described as cellular suspensions. They are composed of cells surrounded by a cellular membrane which isolates their inner ionic medium from the extracellular fluid. Despite the high permittivity of water, electrolytes behave like ohmic resistors and membranes form capacitive elements due to their high resistance, where the typical time constant for charging cell membranes is of the order of a microsecond (El Khaled et al., 2015). Evidently, this explanation describes a very simple structure for these biological materials and its frequential behaviour depends on other many factors (Bragós, 1997).

As can be observed in Figure 4, if a low frequency current is injected through the material, it will circulate via extracellular because there is a high capacitive effect from the membranes produced by cell polarization (Cannizzarro et al., 2003). Moving to higher frequencies, the capacity will decrease because of incomplete cell polarization (Cannizzarro et al., 2003), showing a short circuit effect from the capacities which enables the current to flow through the intra cellular space.

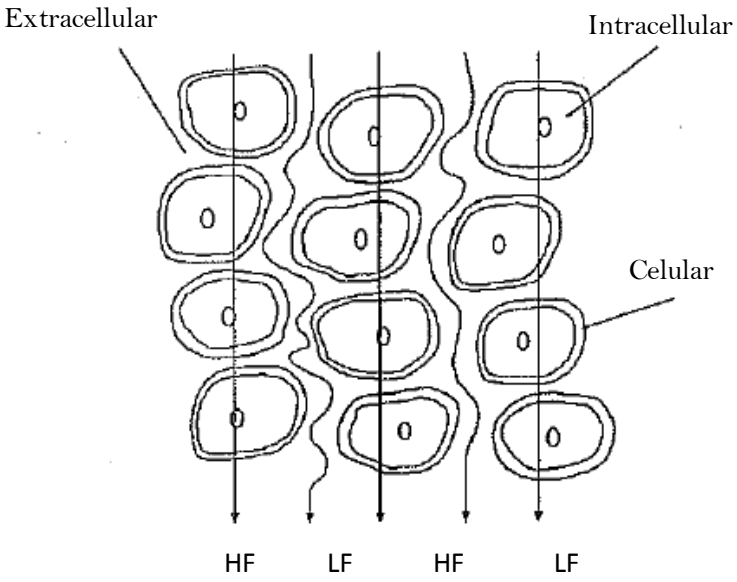


Figure 4 Simplistic representation of the high frequency (HF) and low frequency (LF) currents through a biological material composed of cells, with cellular membrane and intracellular fluid, immersed in an extracellular fluid (Bragós, 1997).

As a simple analysis of the system: electrical current is unable to cross the cell membrane at low frequencies, and is confined to an extracellular pathway, whereas the current will flow intracellularly at high frequencies due to the shortcut from the capacitive effect of the cell membrane. This produces an impedance variation along a range of frequencies, which in the study of biological materials is known to produce specific curves or relaxations. Of great interest for cellular suspension monitoring is the β-relaxation (Spiller et al., 2006), which is produced by the shortcut of the cellular membrane. There are two other main relaxations involved with other phenomenon, shown in figure 5. The lowest frequency range contains the α-relaxation, which is produced mainly by the ionic environment that involves each cell as well as the ionic surface of the membrane (E. Spiller et al., 2006). On the other side, up to 10 GHz, there is γ-relaxation, produced by the effect of the free water molecules in the medium (Bragós, 1997).

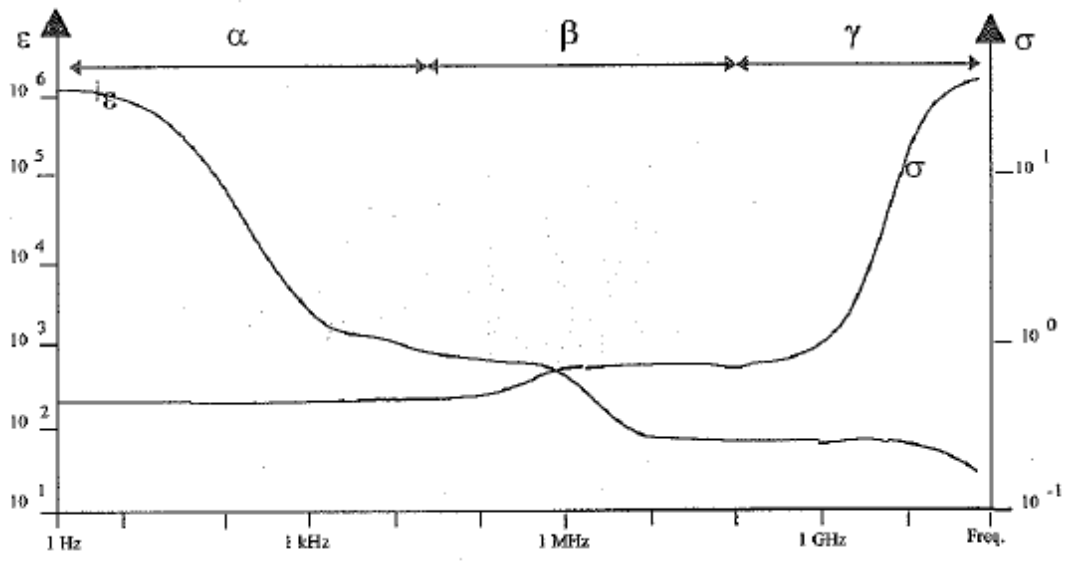


Figure 5 The three relaxations of σ and ε along a range of frequencies defined to understand the dielectric properties of biological cells (Bragós, 1997). There are three regions: α, β and γ-dispersions.

Continuing with the analysis of the cellular suspensions system, an electrical model can be applied, like the one shown in Figure 6. It considers both internal and external medium resistances (R_i and R_e), the membrane capacity (C_m) and its resistance (R_m).

There can be used other circuital models, depending on the studied biomaterial and the studied electrical behaviour. This case contemplates an ideal homogeneous cellular suspension, and although it does not correspond to activated sludge case, it can be accepted. For this reason, there might be observed other electrical behaviours that are not explained through this model.

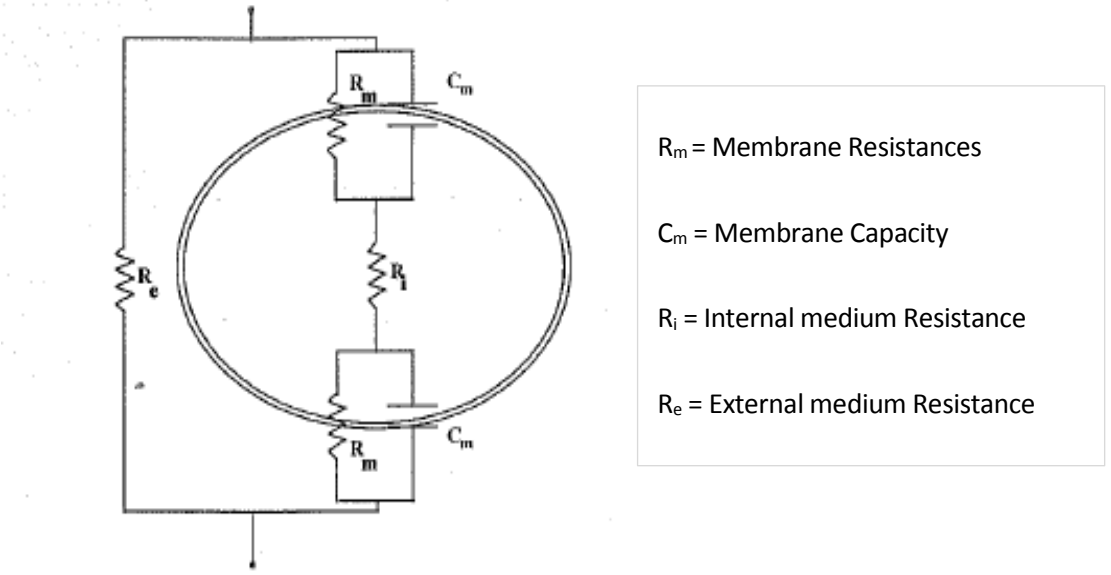


Figure 6 Circuital model for cellular suspensions (Bragós, 1997)

2.3. About impedance measurements

There are three main components in impedance measurements which can introduce uncertainty: the circuital model chosen, the parasitic or stray impedances of the connections and the instrumental error (Pallàs, 2008).

All real-world devices have parasitic or unwanted inductance in resistors, unwanted resistance in capacitors, unwanted capacitance in inductors, etc. The principal attributes of L, C, and R components are generally represented by the nominal values of capacitance, inductance or resistance at specified or standardized conditions. However, all circuit components are neither purely resistive nor purely reactive. They involve both of these impedance elements (Kølvoy et al., 2009).

There is a parasitic capacitance between the material under test and earth ground, and its effects are different depend on the measuring system. This parasitic capacitance is produced at high frequencies whereas electrodes have influence at the lower frequencies (Aliau, 2015). In the middle range, there is a flat frequency band where impedance of the medium can be measured.

There are different electrode configurations that allow the user to reduce uncertainty in the measurement, but these usually require more complex systems. Measurements with the impedance analyser can be performed with 2, 3 or 4 electrodes. The 2-electrode configuration is the simplest method, but contains many sources of error (Agilent, 2013). By moving to 3 and 4-electrode configurations, measurement error can be reduced, but more complex systems and specific electrical components (front end) are required.

The impedance analyzer can also be configured to measure the impedance modulus (|Z|) or the real part of impedance (R). Although impedance variations are better percieved in the real part, it is easier to measure |Z| and it enables to design a simpler impedance analyser specific for this purpose.

As has already been said, there may be polarization potentials at the interfaces between electrodes and the material that could affect the true potential across the material. Moreover, current is distributed along the medium following equipotential lines, flowing from one electrode to the other. Equipotential lines stay close near the zone of the electrodes and become more

extended moving far from the electrodes. In Figures 8 and 9 there are represented the two possible electrodes distributions for this case study, in whose the distribution of the equipotential lines can be observed.

By this way, different positioning and dimensions of the electrodes will affect the current density distribution (Baker, 1989), as will the non-homogeneity of the measured material.

In Figure 7 it can be observed the distribution of the current flow if electrodes were located at one side of the cell. Equipotential lines are concentred at that side of the cell, and they distance themselves at the other side. This allows a general characterization of the medium but presents more measurement error due to parasitic capacities. On the other hand, in Figure 8 electrodes are located at both sides of the cell, producing that equipotential lines remain horizontally uniform along the cell, having a more precise reading around the zone between the two electrodes.

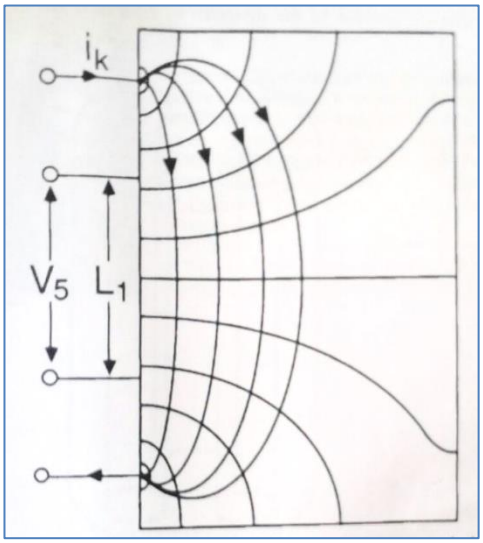


Figure 8 Current and potential distribution if the current is introduced into the measurement cell using small electrodes on one side of the cell (Baker, 1989).

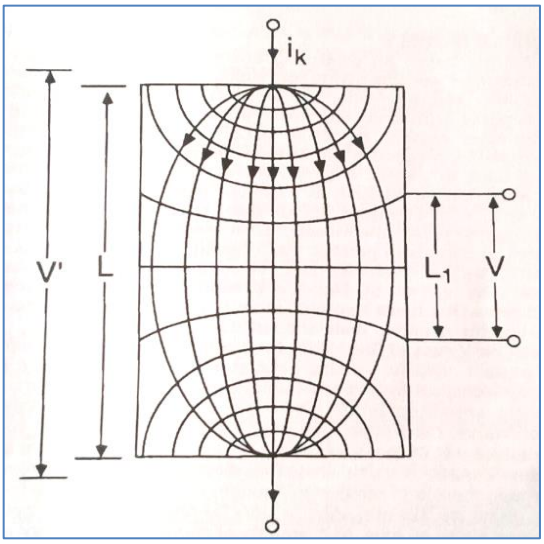


Figure 7 Current and potential distribution if the current is introduced into the cell using small electrodes on both sides of the cell (Baker, 1989).

2.4. State of the art

In the last 30 years, biotechnology and biotechnological processes have exploded from a traditional craft into a dynamic cutting edge technology (Vojinović et al., 2006). The number of bio-products and applications is increasing constantly and the ability to control the desired bioprocess results in increased efficiency and productivity. In this regard, biomass concentration is one of the most important parameters in bioprocesses monitoring. Several new techniques for biomass determination based on diverse principles have recently been applied to online monitoring (Ferreira et al., 2005). The most frequently used methods included optical based methods, such as optical density, UV-Vis spectrophotometry, IR spectroscopy, fluorescence resonance energy transfer (FRET) and Raman spectroscopy; heat exchange methods, and electrical methods, which include bioimpedance, capacitance and electrical conductivity. Their operating principles and main uses are adequately described elsewhere (Vojinović et al., 2006; Ferreira et al., 2005; Kell, et al., 1990;). In industrial applications, viscosity is known to be the most acceptable method for biomass estimation in mycelial systems when more accurate techniques are not available (Ferreira et al., 2005).

Real-time bioprocess monitoring techniques can provide information during the process, but they are relatively scarce on an industrial scale (Vojinović et al., 2006). They need to be implemented for very specific purposes and adapt their working mechanisms to specific conditions. Most successful applications are in biomedicine, biophysics, biochemistry and microbiology, where sampling volumes are in microlitres. However, industrial scale prototypes have been successfully applied to on-line monitoring of viable biomass concentration in industrial-type fermentation for the production of an active pharmaceutical ingredient (Ferreira et al., 2005), using a four-terminal probe which is showed in Figure 9. Moreover, other prototypes have been developed using similar principles to bioimpedance, which include capacitance and electrical conductivity measurements (Ferreira et al., 2005; Vojinović et al., 2005). For example, the Biomass Monitor ™ (Aber Instruments ©) measures biomass by correlating the capacitance of the cell suspension to the concentration of biomass, added to viscosity measurement. It needs to be calibrated with the specific growing rate of the studied microorganisms (Ferreira et al., 2005).

Figure 9 A four-pin electrode probe to detect biomass through capacitance (Ferreira et al., 2015).

All these applications have been developed on a small scale and taking into account very specific variables, such as the production of a specific metabolite or the controlled production of a microorganism. No applications have been found to measure heterogenic microbial cultures as found in industrial wastewater treatment.

There are different types of bioreactors for wastewater treatment. Of these, the Sequential Batch Reactor (SBR) is currently used in wastewater treatment due to its ability to tolerate charge and flow variations, and to control the duration of each process. As can be observed in Figure 10, it performs all the transformation processes through filling and emptying as the microbial cycles are developed. This case is of great interest for bioimpedance application, as by knowing the exact biomass concentration, more accurate cellular retention time could be predicted, leading to a better operation and efficiency. Here, biomass control is a key element for the proper functioning of the bioreactor due to the need to temporize different devices to complete the cycles (Montiel, 2015).

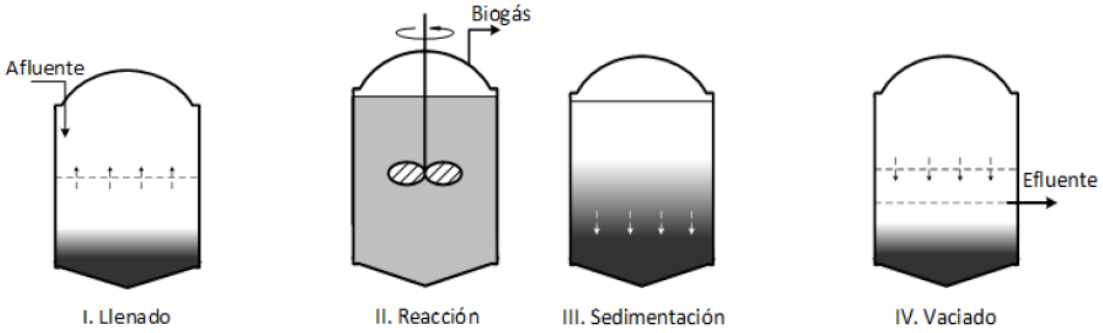


Figure 10 Scheme of the 4 phases that occur in an SBR: I. Filling, II. Reaction, III. Sedimentation and IV. Emptying (Montiel, 2015)

Here it is suggested that bioimpedance sensors could be located at different parts of the bioreactor, enabling different readings and purposes. The scheme of Figure 11 indicates the possible electrodes location. With electrodes located in the reactor walls and radially oriented, bioimpedance measurements would allow the biomass concentration to be known at the same horizontal plane of the electrodes. Another possibility would be to insert one electrode in the upper part of the wall and to locate the other electrode on the bottom, in order to know the global state of the microorganisms (mixed or settled, dead or alive,...).

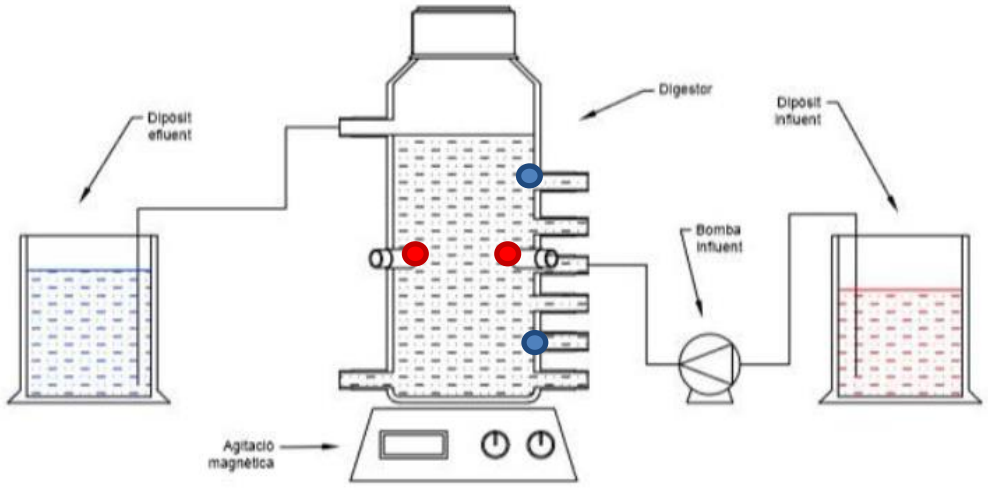


Figure 11 Working scheme of a bioreactor (Montiel, 2015). Two red dots indicate the possible location of radially-oriented impedance electrodes, and two blue dots, the location of vertically-oriented electrodes.

3. Materials and methods

Experiments have been performed following two lines: on the one hand Bioimpedance measurements that have been performed at EETAC (Escola d'Enginyeria de Telecomunicació I Aeroespacial de Castelldefels) and, on the other hand, conventional laboratory analytic determinations, which have been done at ESAB (Escola Superior d'Agricultura de Barcelona). They have been performed separately following different methodologies that will be described below.

In order to ensure the same conditions and state of the samples, both measurements were performed within 24 hours.

All the pictures shown in this work were done by the author, otherwise they will be properly referenced.

3.1. Samples

All the samples used for experiments were kept in fridge for no more than 24 hours in order to ensure that the microorganisms were alive.

Samples of activated sludge and clarified water were obtained from the Water Treatment Plant of Rubí (E.D.A.R.-Estació Depuradora d'Aigües Residuals), which treats wastewater from the 3 surrounding municipalities, Rubí, Valldoreix and Sant Cugat, and was designed to treat a water flow of 27,000m^3/day (ACA, 2015). The location of the water treatment processes can be seen in Figure 12.

Like many municipal wastewater treatment plants, it performs a secondary biological treatment with activated sludge, which involves the production of an activated mass of microorganisms capable of consuming organic matter under aerobic conditions. This mass consists of a heterogeneous microorganism population that frequently changes as a function of the varying composition of the wastewater and environmental conditions. These microorganisms are mainly bacteria, fungi, algae, protozoans and rotifers.

Figure 12 Satellite image of Rubí E.D.A.R. Samples of activated sludge were taken from the secondary pond, while clarified water samples were obtained from the outflow of the water treatment plant (www.arcgis.com)

This wastewater micropopulation heterogeneity is significant when analysing through bioimpedance, as the measurements will surely be different each time and it will be partly responsible for the different results obtained.

Samples of activated sludge were taken from the aeration tank or secondary pond, which is shown in Figure 12. Before measurement, the sludge was settled and clarified water was also used for the experiments. Finally, samples of the outflow water from the treatment plant were taken in order to have samples with similar conductivity as the activated sludge samples but with extremely low biomass concentration.

3.2. Experiment procedure

As the bioimpedance behaviour of activated sludge samples was uncertain, the experimental design was defined during the work, since each result obtained determined the next experiment.

Before starting to measure the bioimpedance of biomass, some prior tests were performed in order to determine the feasibility of the project. There are many publications about bioimpedance measurements, but there is a high level of uncertainty in terms of the interpretation of the results. So, at the beginning, the potential result was completely unknown.

The first previous assay was carried out by measuring a sample of activated sludge and a sample of clarified water in order to observe the differences. Taking Bragós (1997) as the main reference for bioimpedance measurements of cellular suspensions, the aim was to observe the characteristic β-relaxation of biologic materials, supposed to be around a frequency of 1MHz. However, it was not observed, probably due to the capacitive effect produced after 1MHz and the low sensitivity of the instrument used. In his work, Bragós (1997) measured in a range of 10kHz to 20MHz and used a more accurate system with 4 electrodes, with which a sensitivity of 0.1% can be achieved.

Even though the β-relaxation was not located, it was observed that measurements of live biomass present a variation in the impedance modulus along a range of frequencies, while the impedance modulus of water remains constant. This particular behaviour was developed as the hypothesis of this work, for which biomass samples were analysed using bioimpedance and conventional wastewater analytics, in order confirm that bioimpedance is sensitive to different biomass concentrations. Then, samples of activated sludge in different concentrations were analysed using bioimpedance and compared with their Chemical Oxygen Demand (COD) and Volatile Suspended Solids (VSS).

More tests were performed to decide the measurement range and the position and number of the cell electrodes.

After analysing the obtained results with different biomass concentration, another objective was to determine whether bioimpedance measurement can distinguish active and non-active biomass. For this, 3 samples of activated sludge were kept in the oven at 80 ºC during 1 hour.

During these experiments it was observed that turbulences in the medium produced by mechanical agitation and sedimentation caused distortions in the impedance readings. This effect was more evident in 25% AS diluted samples, were there was a great visual difference between when the sample was mixed or let settled. To study this effect, other experiments were performed, in whose a 25% AS diluted sample was measured in mixed state and after determined periods of time, when biomass was settled down. A chronometer was used to stablish the periods of time. All the results are all detailed in the results section.

3.3. Bioimpedance measurements

Measurements of Bioimpedance have been performed using the Agilent 4296A Impedance Analyser, located at the EETAC (Escola d'Enginyeria de Telecomunicació i Aeroespacial de Castelldefels) of the UPC.

3.3.1. Measuring cell

Prior assays were performed to develop the measuring cell. Those experiments used a measuring cell consisting of a 100ml PET measuring cylinder, with 4 stainless steel electrodes linearly distributed horizontally on its wall. Another measuring cell of the same kind but with 2 electrodes was used, both provided by Dr Carles Aliau from his work (Aliau, 2015). Figure 13 shows a picture of impedance measurements with the 4-electrode cell, using a standard solution of known electrical conductivity.

After this, some design criteria were established in order to develop the final measuring cell. The final cell was made using a 500ml PET measuring cylinder and placing 4 electrodes on its walls in a radial distribution, as shown in Figure 14-c.

After studying the distribution of equipotential lines (see chapter 2.3.) in both vertical and horizontal arrangements, it was decided to make measurements with the cell vertically oriented.

This way, biomass can be detected in a more specific region, which is required to simulate bioreactor conditions.

Although this distribution can be used either with 2 or 4 electrodes, in the end 2 electrodes were used, so no additional electronics (front end) were required. Some construction details can be seen in Figure 15.

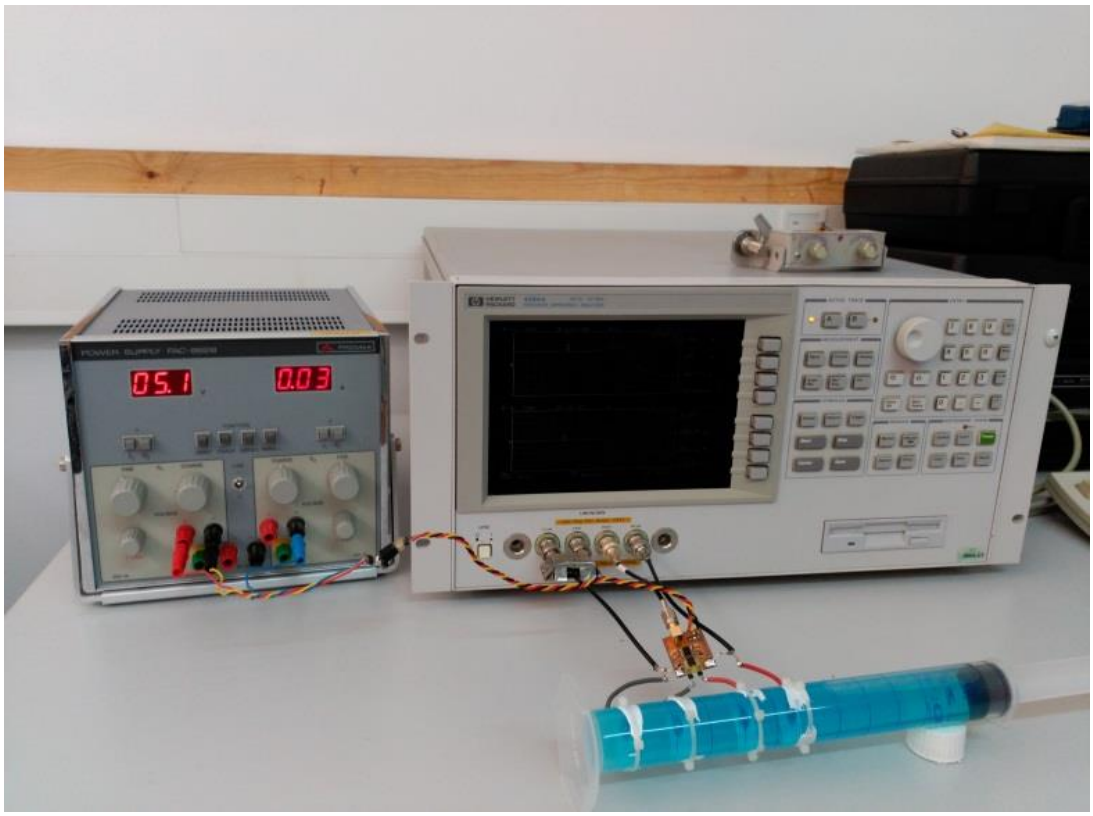
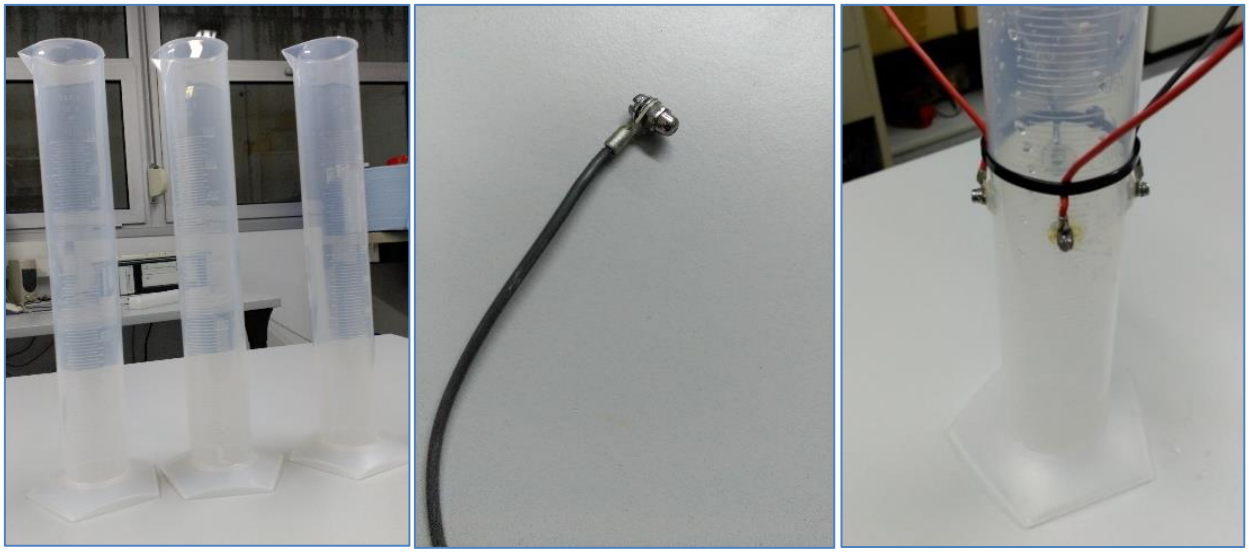


Figure 13 Image of the impedance analyser (right) using the 4-electrode cell and a standard water solution (σ=1412µS/cm) for prior assays**.** On the left there is a power supply which is required for the front end used for the 4-electrode measurements.



(a) (b) (c)

Figure 14 Details of the construction of the cell. In picture (a) there are three 500ml PET cylinders; (b) shows an electrode before insertion into the cylinder wall and (c) the final measurement cell. A flange was used to hold the wires.

3.3.2. Impedance Analyser configuration

The impedance analyser must be configured to obtain the desired reading. Some of the configuration criteria were developed during the current essays, so they are detailed in Results. The setup protocol used was as follows:

- Display: mode SPLIT ON
- Scale: mode AUTOSCALE
- Sweep: type LOG
- Set the frequency range: START at 100KHz and STOP at 1MHz
- Source/Level: 500mV (default value)
- Bandwidth: recommended 2 or 3

Data is stored on flash memory.

A screenshot of the impedance analyser during activated sludge measurement is shown in figure 15. There, the upper plot represents the impedance modulus (|Z|) and represented in blue, the impedance phase shift (θ).

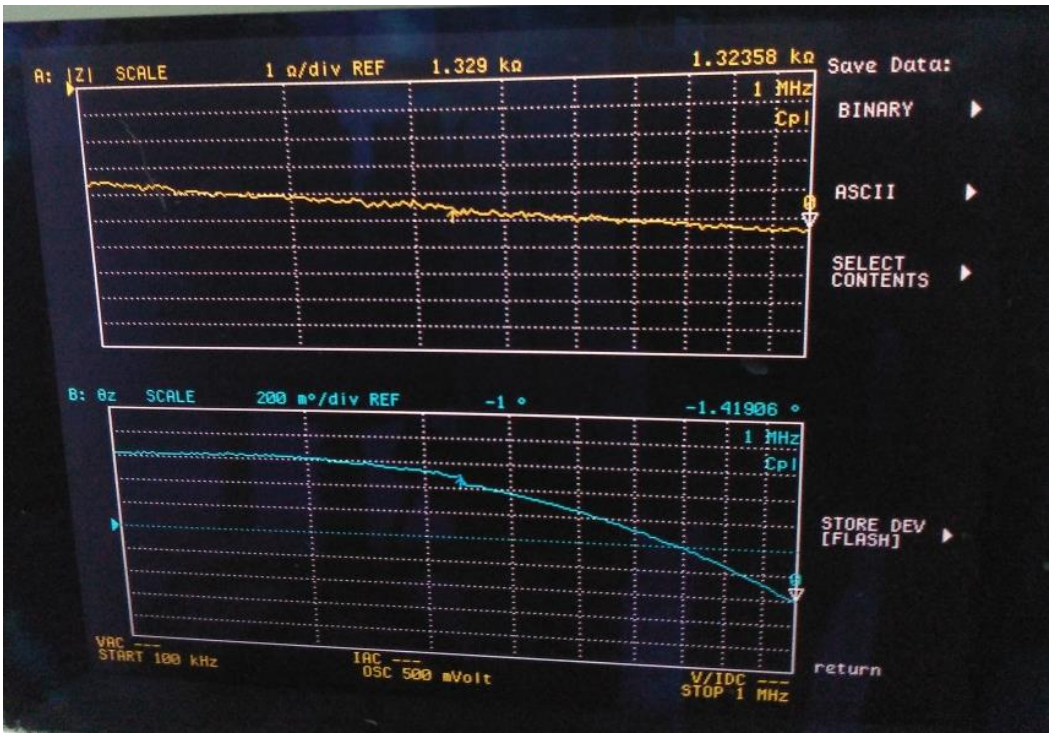


Figure 15 Screenshot of the Agilent 4294A during activated sludge measurements. The upper plot it is measured the impedance modulus variation while the bottom one depicts the phase shift.

It was observed that real part of impedance or either impedance modulus plots gave very similar results, so it can be said that both can be representative.

However, this work measures the impedance modulus so that it would be easier to develop an automatized system with the instrumentation used (Agilent 4294A) rather than if the real part is measured.

3.3.3. Measuring bioimpedance of Activated Sludge

Since it takes some time for the impedance analyser to get started, it has to be switched on about 15 minutes before starting.

Once the equipment is ready, cell wires can be connected to the impedance analyser. To avoid undesired effects, the cell must be always the same distance from the device. For this reason, the cell location was marked with tape, as can be observed in Figure 16. The distance between the connection leads was made to be always the same using perforated plastic pieces as shown in Figure 17.

Temperature and electrical conductivity were measured for all samples before and after readings.

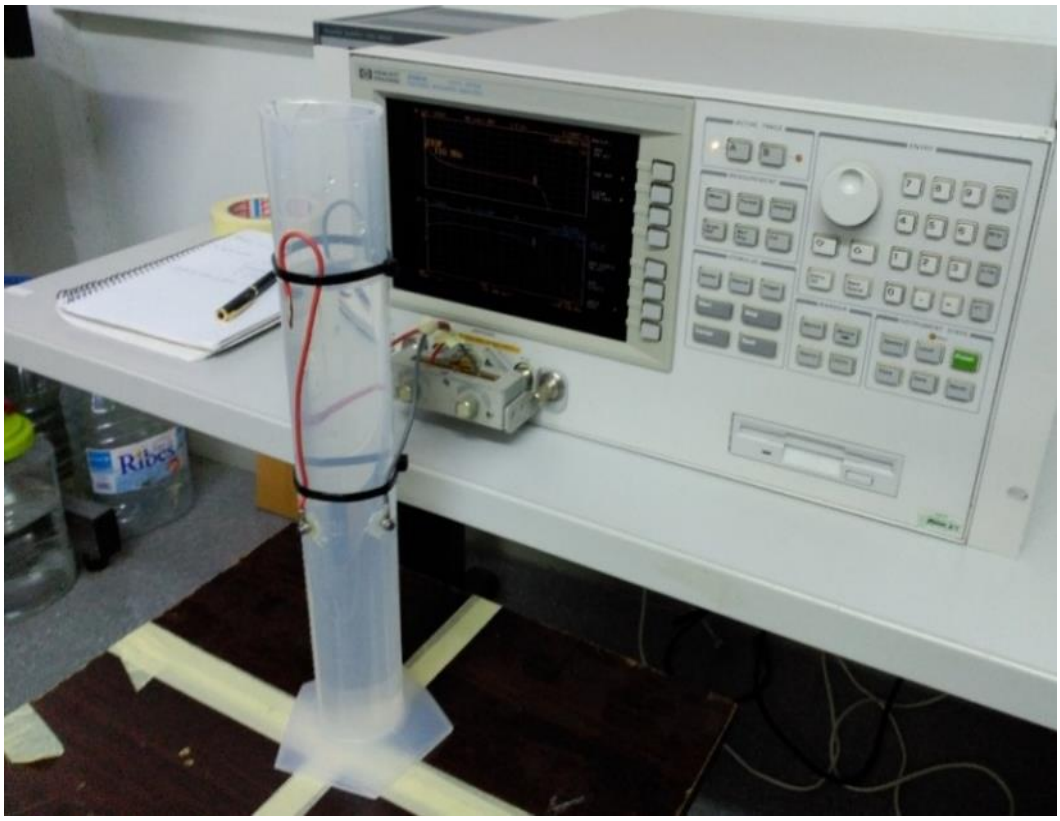


Figure 16 Picture of the cell connected to the impedance analyser before starting measurements. The tape cross to mark the position of the cell during measurements can be observed.

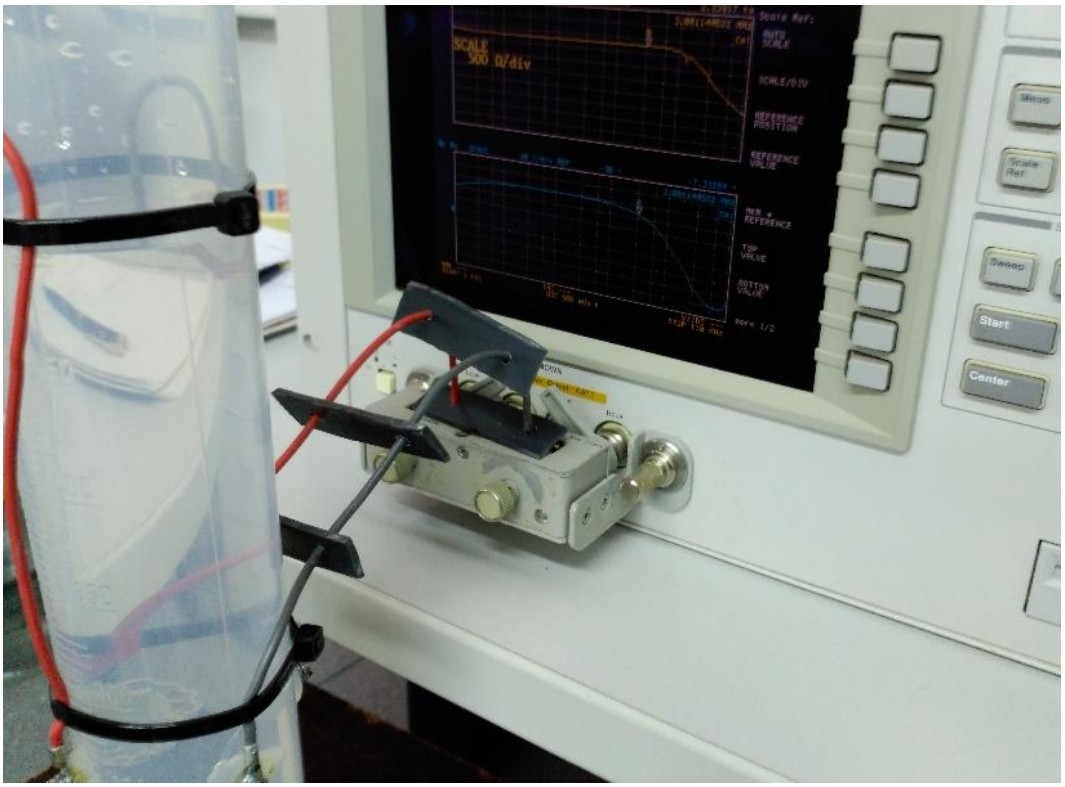


Figure 17 Image of the plastic pieces between the leads to maintain the same distance during measurements.

Measurements of activated sludge have always been carried out measuring 4 samples:

- Clarified water (CW)
- Outflow water (OW)
- Activated sludge dissolved 25% in volume with clarified water (AS 25%)
- Activated sludge dissolved 50 % in volume with clarified water (AS 50%)
- Activated sludge directly obtained from the water treatment plant (AS)

In those cases that CW and OW gave almost same behaviour, only one of them was analysed. These samples have all been put into the measurement cell filling it up to the 500 ml line on the cylinder. First of all, clarified water was measured and then the other samples in the order listed above, to avoid remaining biomass accumulation. During the measurement process, interfering actions were observed and noted down. In the case of the observation of the effect of sedimentation, an agitator was also used to mix samples and a chronometer to measure settling time.

3.4. Analythic methods

Temperature and Electrical Conductivity (EC) were measured at the same time as the bioimpedance readings. Other analytic methods (Chemical Oxygen Demand, COD; Total Solids, TS; Volatile Solids, VS; Volatile Suspended Solids, VSS and Total Suspended Solids, TSS) were carried out at ESAB (Escola Superior d'Agricultura de Barcelona), at UPC (Universitat Politècnica de Catalunya).

In order to establish a relationship between impedance variation and biomass, COD and VSS were taken into account: COD to measure organic matter and VSS as an estimator of microorganisms. To measure VSS, also TS, VS and TSS must be determined.

3.4.1. Temperature and EC

Temperature and EC have been determined with a conductivity meter with automatic temperature compensation. The instrument is automatically calibrated at 25 °C.

It has to be placed inside the sample before waiting for the reading, which gives the temperature and EC.

3.4.2. COD (Chemical Oxygen Demand)

Previous steps:

It is important to homogenise the samples before starting the analytics to ensure that they are representative. Three repetitions were done for each sample and they had to present minimum variation (less than or equal to 5%).

The COD digester takes some time to reach 150 °C, so it can be switched on before starting to prepare the tubes.

Basis:

Chemical Oxygen Demand (COD) is defined as the required amount of oxygen to chemically oxidize organic matter and oxidisable compounds of a sample. It is expressed in mgO_2/L or mgO_2/kg.

This theoretical quantity of oxygen is determined by putting the sample in contact with potassium dichromate into an acid medium at high temperature (150 °C) for two hours, and measuring the remain oxidizer by titration.

In these experiments, prepared COD reagents (Hanna Instruments ©) were used instead of preparing the potassium dichromate and sulfuric acid mixture, thus avoiding the need to prepare and handle corrosive and toxic chemicals. All that is required is to add the sample to the COD vial, digest and measure using a control sample.

Materials:

- COD regents (*HI 94754C-25, HANNA Instruments©*)
- Test tubes
- Graduated pipette and pipette tips
- Beakers 250ml (one for each analysed sample)
- Glass rod
- COD digester (*HI 839800 COD Reactor and Test Tube Heater 2008 Series, HANNA Instruments©*)
- Spectrophotometer (*HI 83224 Wastewater Treatment Photometer, HANNA Instruments ©*)

Procedure:

- First of all it must be decided if any samples will need to be diluted, if a high COD value is expected.
- A standard tube is also required to calibrate the spectrophotometer, therefore 3 COD reagents are filled with 200µL of distilled water.

- With a graduated pipette, put 200µL of each sample into the COD reagent (3 copies).
- Properly cap all the test tubes. It is important to tape all the tubes so that they will not open during digestion.
- Then, shake all the tubes well with up and down movements to ensure that the reactants are homogenized.
- When the digester has reached a temperature of 150 °C, insert all the tubes and start the 2-hour countdown.
- After that time, remove the tubes. They will be hot, so a cooling time is needed.
- Finally, COD values can be read with the spectrophotometer using the COD program. Before this is done, it has to be calibrated with the standard tubes.

3.4.3. Total Solids and Volatile Solids

Previous steps:

It is important to homogenise the samples before starting the analytics so that it can be ensured that they are representative. Therefore, 2 repetitions are done for each sample and they have to present a minimum variation (less than or equal to 5%).

Basis:

Total Solids: The term applied to the material left in a dish after evaporation of a sample and its subsequent drying in an oven at a temperature 105 °C for 24 hours non-stop. Total solids include “Total Suspended Solids” and “Total Dissolved Solids”.

Volatile solids: Those solids that are lost during ignition (by burning) for 15-20 minutes at 550 °C in a muffle oven. In general, volatile solids are representative of organic material.

Materials:

- Drying oven
- Desiccators
- Analytical Balance (SCALTEC with 0.0001g precision)
- Measuring cylinder
- Muffle oven
- Porcelain crucibles
- Glass agitator

Procedure:

1. Preparation:

- First of all, porcelain crucibles have to be cleaned and dried for 1 hour in the muffle oven at 550 °C and then allowed to cool down into a desiccator.
- The oven and muffle oven will have to be switched on about 20 minutes before starting to guarantee that they reach a constant temperature.
- Before starting the solids determination, samples must be identified in each crucible that will be used.

2. Total Solids Determination

- Weigh the empty but dried crucibles, and record the value as weight **A**.
- Then, 1ml of sample must be put in the crucible and weigh it all again. This will be weight **B**.
- The samples have to be carefully put into the oven at 105 °C for 24 hours.
- After that, the samples will be hot, so before weighing again they must be kept in the desiccators for almost 30 minutes. The weight value obtained must be recorded as weight **C**.
- The final value of Total Solids, expressed in % in fresh weight is obtained using the following equation:

$$TS(\%) = \frac{C-A}{B-A} \cdot 100 \quad . \quad (10)$$

3. Volatile Solids Determination

- The samples obtained at the end of TS determination are put into the muffle oven at 550 °C for 3 hours.
- After being in the muffle oven for this long, samples will be very hot and they will have to be kept in the desiccators for 45 minutes.
- Finally, weigh the samples and record the value as weight **D**.
- Value of Volatile Solids, expressed in % in fresh weight is obtained using the following equation:

$$VS(\%) = \frac{C-D}{B-A} \cdot 100 \quad . \quad (11)$$

3.4.4. Total Suspended Solids and Volatile Suspended Solids

Basis:

Total Suspended Solids: Those solids that will not pass through a standard glass fibre filter. This includes both those solids that will settle or float in the clarifier and the lighter non-settleable (colloidal) solids.

Volatile Suspended Solids: Those volatile solids that will not pass through a standard glass fibre filter.

Materials:

- Drying Oven
- Desiccators
- Analytical Balance (SCALTEC © SBA 31 with 0.0001g precision)

- Forceps
- Fiber glass filter paper
- Vacuum pump
- Büncher funnel
- Kitasato
- Muffle oven
- Porcelain crucibles
- Glass agitator
- Beaker
- Pipette
- Measuring cylinder

Procedure:

1. Preparation:

- First of all, the porcelain crucibles must be cleaned and dried for 1 hour in the muffle oven at 550 °C and then allowed to cool down into a desiccator.
- Filter papers must also to be prepared: using the Büncher funnel, they must be filtered with distilled water three times and allowed to dry in a clean crucible in the oven at 105 °C for 1 hour,
- After being allowed to cool down in the desiccator, the filter paper and the crucibles have to be weighed together and the weight recorded as weight **A**.
- Weigh 5mL of sample in a beaker and record it as weight **B** and filter that sample. Clean the beaker with deionized water to make sure the entire sample has been filtered.

2. Determination of Total Suspended Solids:

- Put the filter into the corresponding crucible (properly identified) and put them into the oven at 105 °C for 24 hours.
- After this time samples will be hot, so they must be cooled down in the desiccators for 30 minutes.
- Then, weigh them with the analytical balance and record the weight as **C**.

- Final value of Total Suspended Solids, expressed in % in fresh weight is obtained using the following equation:

$$TSS(\%) = \frac{C-A}{B} \cdot 100 \quad (12)$$

3. Determination of Volatile Suspended Solids:

- Put the crucibles into the muffle oven at ambient temperature and switch it on to 550 °C for 3.5 hours. The first half hour will be to reach the desired temperature avoiding spontaneous ignition.
- After this time, the samples will be very hot, so they must be cooled for 1 hour in the desiccators.
- Then weigh and record as weight **D**.
- Value of Volatile Suspended Solids, expressed in % in fresh weight is obtained using the following equation:

$$VSS\ (\%) = \frac{C-D}{B} \cdot 100 \quad (13)$$

3.5. Statistic methods

A simple linear regression model has been used to contrast the possible linear relationship in two cases: first, impedance slope vs. VSS and then, impedance slope vs. COD.

Absence or presence of relationship between the mentioned variables has been contrasted by a correlation test, with a confidence interval (CI) of 95%. Following, the linear regression model has been tested, again with a CI of 95%.

After knowing its significance, the correlation coefficient has been studied, in order to know how good the model is. Finally, a normality test has been done in order to contrast if there is normality or not. Obtained models with correlation coefficients higher than 90% and satisfy the normality presumptions and constant variance have been considered as good model.

4. Results and discussion

4.1. Previous assay

The results obtained from the previous assays, performed in order to design the measurement cell and the experimental procedure, are detailed here. It starts with the simple (and first) bioimpedance measurement of a sample of activated sludge (AS) and clarified water (CW), the results of which are shown in Figure 18.

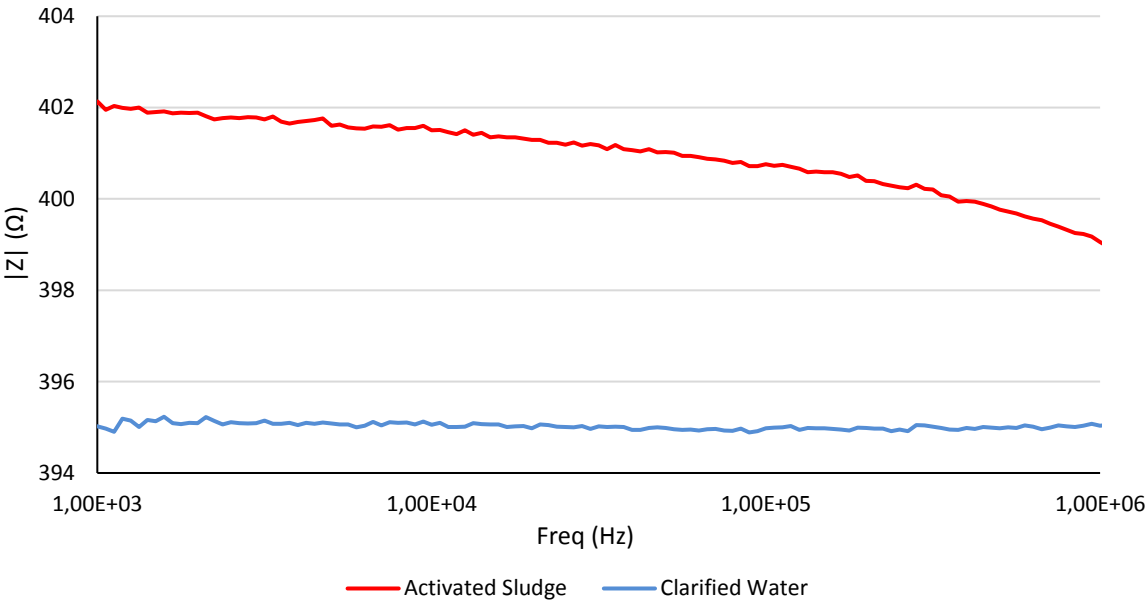


Figure 18 Impedance readings for activated sludge and clarified water.

Even though the β-relaxation was not located, another determining behaviour was observed and gave meaning to the work: measures of activated sludge present a variation in its impedance along a range of frequencies, while the value for water, without biomass, remains constant.

In other words, when measuring biomass through bioimpedance, the obtained curve presents a slope, while the slope is very close to zero when there is no biomass in the medium.

This explanation suggests that a simpler measuring system could be developed to detect biomass through bioimpedance by detecting a determined modulus variation.

4.2. Defining the measurement range

After knowing about the undesired effects of the parasitic capacities observed in the readings, it was necessary to set a measurement range of frequencies. For this, another assay was performed by measuring a patron solution with a known conductivity (σ = 1412µS/cm).It could then be observed how parasitic capacitances affect measurement. The result is shown in Figure 19.

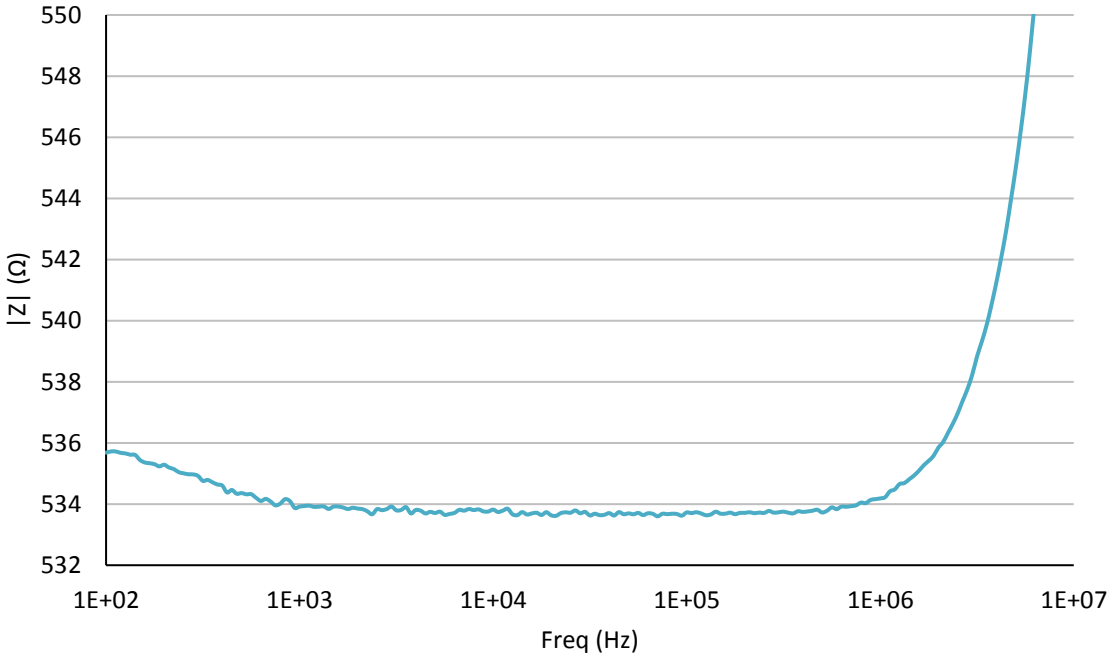


Figure 19 Impedance readings for measuring patron solution with conductivity σ = 1412µS/cm.

Knowing that impedance for no-biomass remains constant, it can be observed that only from 10^4Hz to 10^6Hz is there the flat tendency which corresponds to the true impedance of the medium. Therefore, it can be said that there are two undesired effects at the lowest and highest frequencies: from 10^2Hz to 10^4Hz the capacity of the electrodes prevails, while going up to 1 GHz the polarization of free water molecules produces another capacitive effect (see chapter 2.3).

From this result, a measurement range of frequencies was approximately established, at which the effects of high frequencies and capacitive artefacts can be negligible. First, it was accepted a measurement range from 10^4 to 10^6 Hz, but considering the disposition of the β-dispersion and

the noise affecting measurements at 10^4 Hz, the definitive frequency range for the readings was set at between 10^5 and 10^6 Hz (100kHz and 1MHz).

4.3. Establishing number and position of the electrodes

Another design criterion for the measurement cell was defining whether it would require 4 or 2 electrodes and its distribution along the cell. As is known, with 4 electrodes a more sensitive reading can be obtained, but the setup is more complex and it requires designing specific electronics (front end). On the other hand, using 2 electrodes would enable a simpler and cheaper instrumentation, but it must be ensured that readings will be as representative as with 4 electrodes.

Table 1 and Table 2 summarize the impedance slopes for 2 and 4-electrode configuration measurements, respectively. It can be observed that the slopes for activated sludge samples are higher in both cases. Moreover, the differences are even clearer in the 2-electrode configuration. The impedance readings are shown in figures 20 and 21.

Table 1 Impedance slopes and temperatures for 2 electrodes measurements of activated sludge, outflow water and clarified water.

2 el. measurement	Activated sludge	Outflow water	Clarified water
Slope [100kHz, 1MHz] (Ω/MHz)	-8.1978	-0.7900	-0.8867
Temperature (°C)	22.6	22.7	22.5

Table 2 Impedance slopes and temperatures for 4 electrodes measurements of Activated sludge, outflow water and clarified water.

4 el. measurement	Activated sludge	Outflow water	Clarified water
Slope [100kHz, 1MHz] (Ω/MHz)	-2.2113	0.0731	0.1894
temperature (°C)	23.2	23.0	22.9

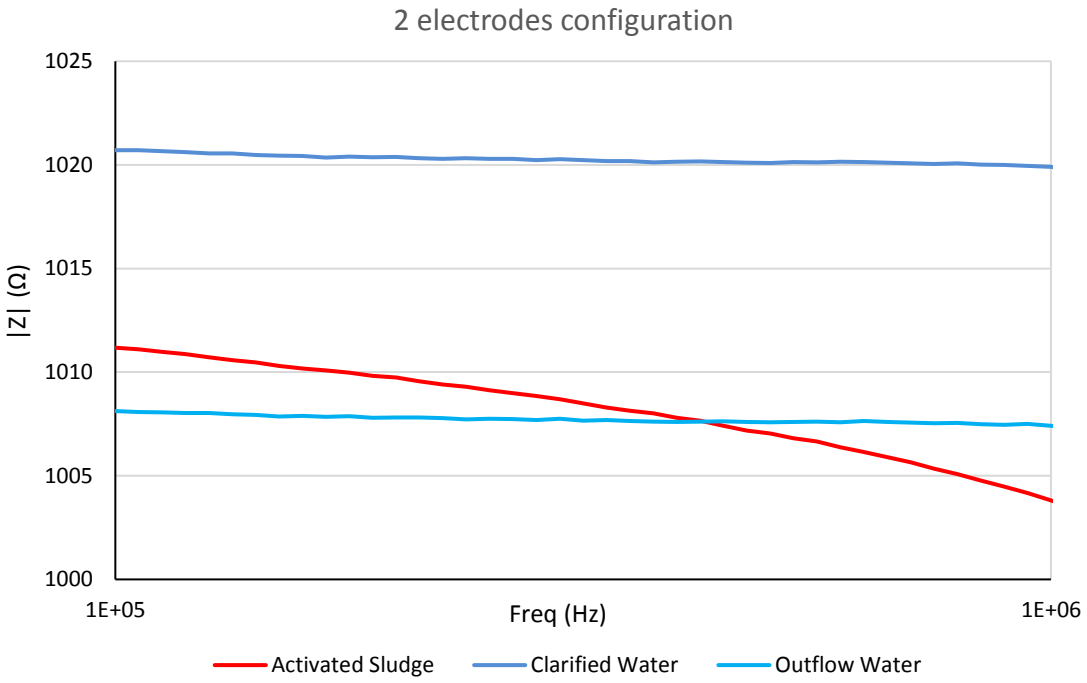


Figure 20 Bioimpedance measurement of activated sludge, clarified water and outflow water with 2 electrodes

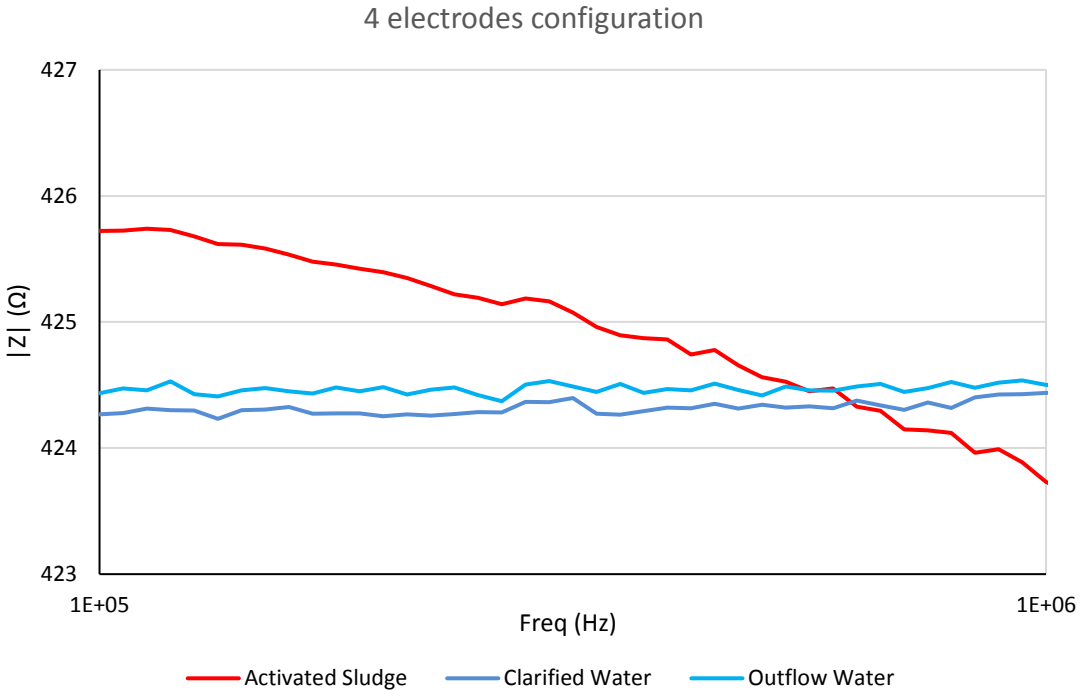


Figure 21 Bioimpedance measurement of activated sludge, clarified water and outflow water with 4 electrodes

Looking at Figures 20 and 21, it can be confirmed in both 2 and 4-electrode measurements that there is an impedance gradient in the activated sludge samples, while the impedance value for water remains almost constant. So, it is confirmed that both systems can be used to detect biomass in a frequency range from 100 kHz to 1MHz.

However, the 2-electrode design was chosen. Firstly, because it would enable easier handling of data as well as a generally cheaper instrumentation, but also because the results have less noise in the readings and the slope difference is more evident (see Tables 1 and 2). Therefore, is not just that using 2 electrodes is enough to detect the presence or absence of biomass, but also that it will give a clearer result and it will become easier to automatize.

Note that temperatures after 4 electrodes measurements had increased respect the previous 2 electrodes measurement, suggesting that this measurement device produces a heating of about 0.3 ºC ± 0.1. This effect will be discussed in chapter 4.6.

4.4. Measures of different biomass concentrations

After establishing measurement characteristics and defining that there is an impedance slope when biomass is measured, the aim was to find out if bioimpedance measures are sensitive to different biomass concentrations. For that, samples with different biomass concentration (100%, 50% and 25%) were measured as well as clarified water samples. Furthermore, VSS and COD analytics, water electrical conductivity (EC) and water temperature were measured for each sample as control.

These measurements were performed 3 times in order to have 3 experimental replicates. Samples were taken from the water treatment plant on 3 different days, so biomass concentration, EC and temperature were different in each case. The 3 impedance readings obtained from the impedance analyser are shown in Figures 22, 23 and 24. Moreover, Tables 3, 4 and 5 summarize their impedance variation along a range of frequencies (slope) and the analytic results.

Table 3 Impedance variations (slope), temperatures, EC, COD and VSS results of 1st run biomass concentration measurements.

	Activated sludge	AS diluted 50%	AS diluted 25%	Clarified water
Slope [100KHz, 1MHz] (Ω/MHz)	-5.9556	-3.7289	-2.5589	-1.8889
temperature (°C)	19.7	19.5	19.5	19.4
EC (dS/cm)	2.03	2.03	2.04	2.04
COD (g O_2/l)	10010	5989	3402	55
VSS (g VSS/l)	0.6220	0.4350	0.2114	0.1000

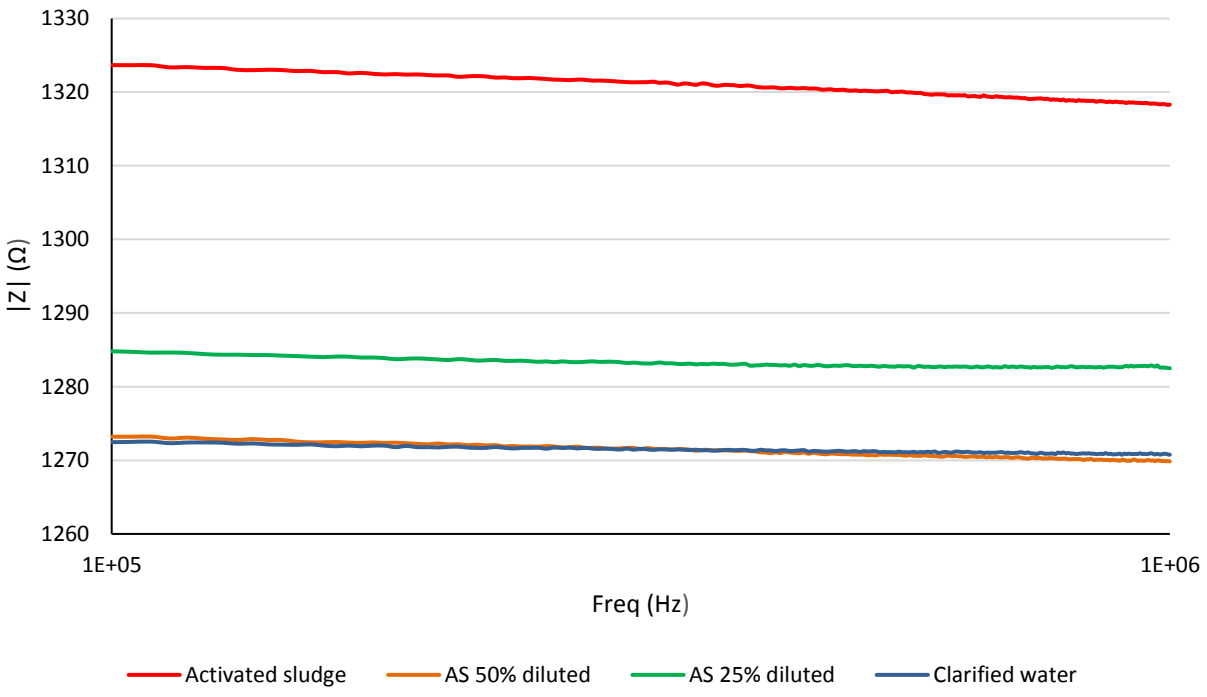


Figure 22 Impedance readings of activated sludge, diluted activated sludge and water samples of 1st run of biomass concentration measurements.

Table 4 Impedance slopes, temperatures, EC, COD and VSS for different concentration biomass samples and clarified water of 2nd run of biomass concentration measurements

	Activated sludge	AS diluetd 50%	AS diluted 25%	Clarified Water
Slope [100KHz, 1MHz] (Ω/MHz)	-6.8744	-3.0722	-2.3856	-2.0067
temperature (°C)	22.8	22.4	22.3	22.3
EC (dS/cm)	1.56	1.56	1.57	1.56
COD (g O_2/l)	4929	2768	1612	167
VSS (g VSS/l)	0.5047	0.3143	0.1726	0.1516

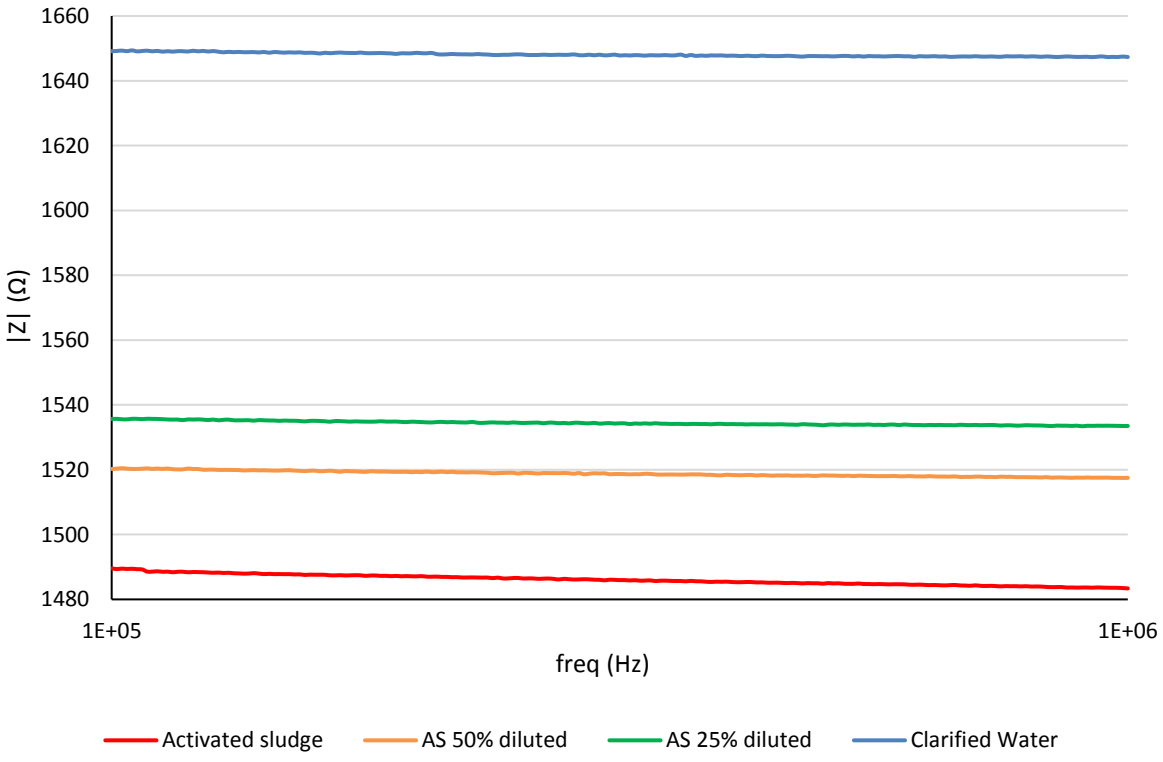


Figure 23 Impedance readings of activated sludge, diluted activated sludge and water samples of 2nd run of biomass concentration measurements.

Table 5 Impedance slopes, temperatures, EC, COD and VSS for different concentration biomass samples and clarified water of 3rd run of biomass concentration measurements

	Activated sludge	AS diluted 50%	AS diluted 25%	Clarified water
Slope [100KHz, 1MHz] (Ω/MHz)	-9.5478	-6.8778	-7.1189	-1.8889
temperature (°C)	27.7	27.7	27.7	27.5
EC (dS/cm)	1.69	1.69	1.69	1.69
COD (g O_2/l)	11808	4896	2412	40
VSS (g VSS/l)	0.8714	0.4782	0.2752	0.1343

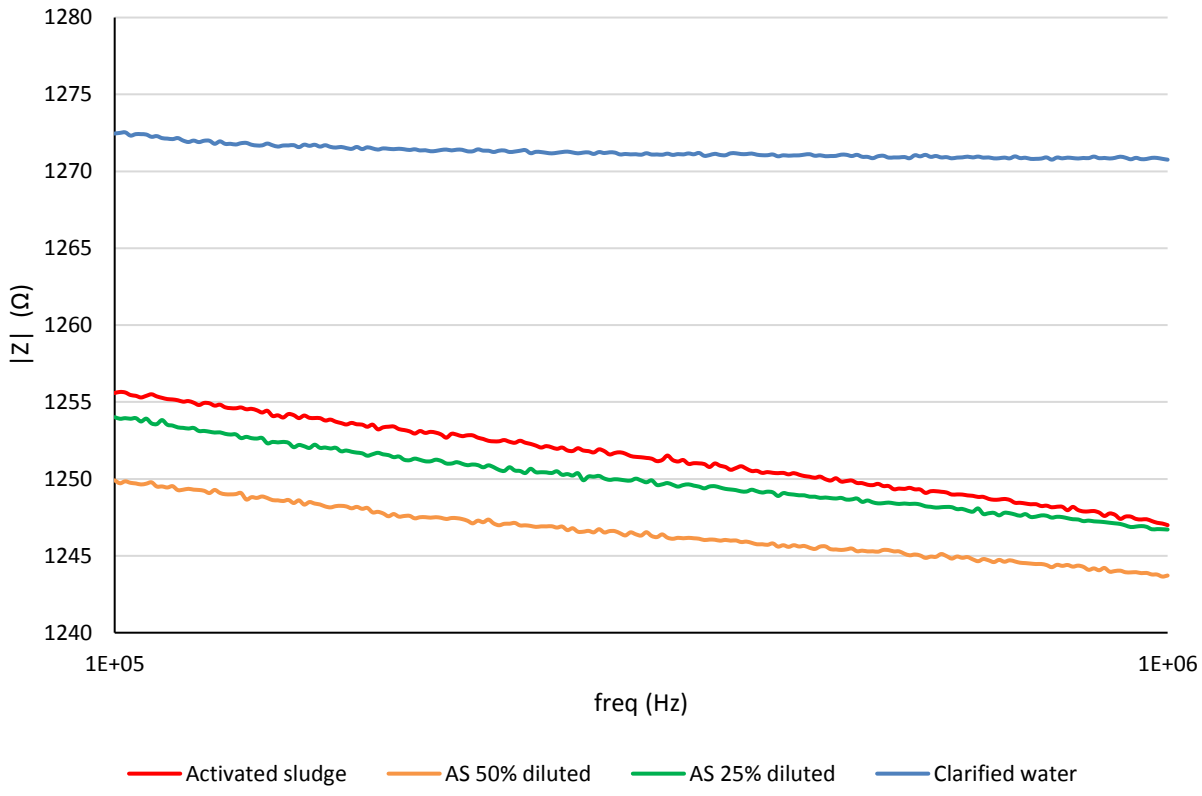


Figure 24 Impedance readings of activated sludge, diluted activated sludge and water samples of 3rd run of biomass concentration measurements

In all the observed cases, there are different slopes for different biomass concentrations. Observing the results from each day, it can be seen that for higher biomass concentration, the slope is more pronounced, with clarified water samples having the lowest slope values.

There is one anomaly which can be observed in Table 5: the AS 75% diluted sample of the 3rd test presents a high slope variation (-7.1189Ω/MHz) but biomass concentration was low (0.2752 gVSS/l). It is thought that this exception could have been caused by the settling of the biomass during the measurement. Thus, biomass was accumulated in the zone around the electrodes, giving an impedance reading of a higher biomass concentration.

Comparing all the samples from each day, it can be observed that there are samples with lower biomass concentrations that have higher slopes, and there are samples with higher biomass concentration that have lower slopes. This implies that there are more factors altering bioimpedance measurements that have not been taken into account.

However, it is thought that biomass concentration (VSS) is related to impedance slope (from 100kHz to 1MHz) and even with the organic matter content (COD). To find out if there is a relationship between them and moreover, if it is a linear relationship, statistical tests were done. All the followed steps and complete results can be found in the annexes.

The statistic test for the linear regression model between VSS and slope has resulted significant. With an adjusted R^2 of 0.928, it can be said that the linear model fits to the experimental data and that it is statistically valid. The obtained model can be observed in Figure 25.

The statistic test for the linear regression model between COD and slope has also resulted significant. With an adjusted R^2 of 0.403, the model is significant. However, this low R^2 shows that it is not a good model and the residuals-analysis indicated that it is not reliable. The obtained model can be observed in Figure 26.

Hence, there can be a linear relationship between biomass concentration and impedance slope, whereas it cannot be confirmed for organic matter content.

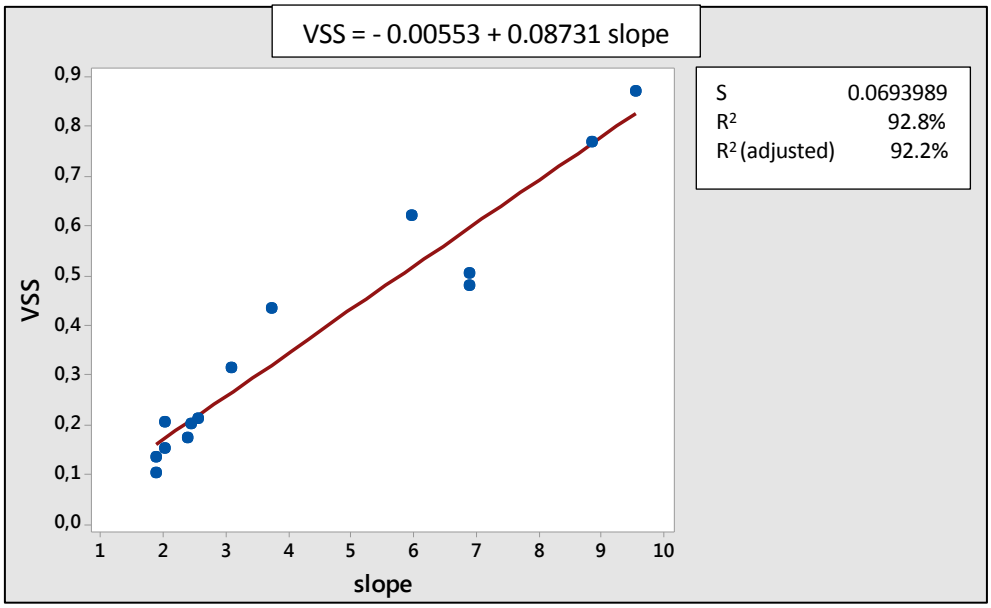


Figure 25 Linear regression of VSS vs. slope

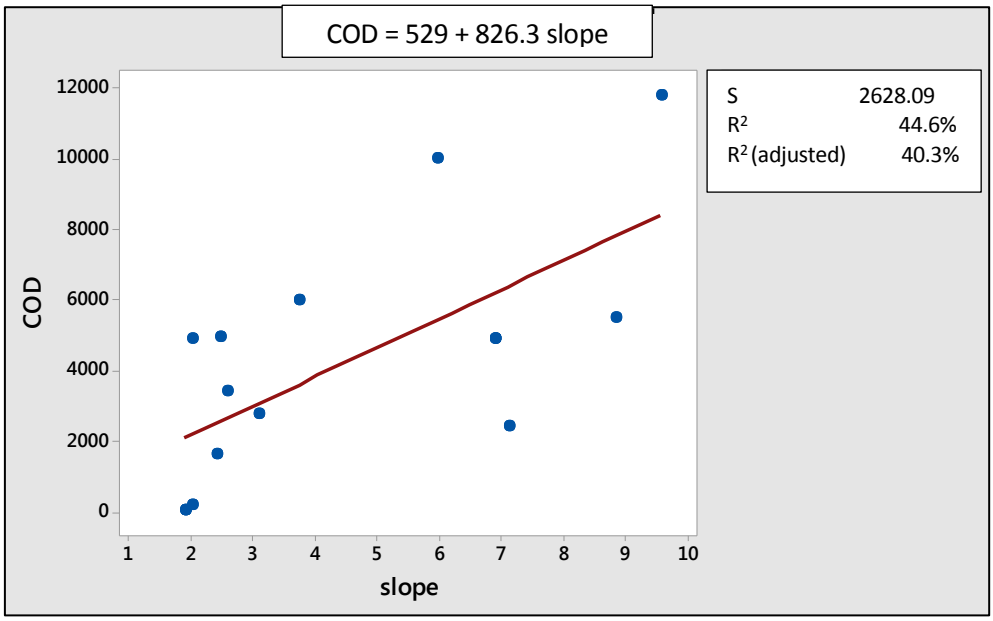


Figure 26 Linear regression of COD vs. slope

4.5. Measures of non-active biomass

Whereas VSS can be used as biomass estimator, COD measures the organic matter of the sample, but not necessarily biomass. As conclusions of the statistical tests of the last chapter (4.4.), it was imperative to contrast experimentally that bioimpedance is sensitive to biomass concentration and the organic matter content does not interfere in the result. To prove that, 3 samples of activated sludge were thermally treated in order to inactivate biomass and then measured through bioimpedance. One of the bioimpedance reading is not shown due to technical problems during the data acquisition. Results are shown in figure 27 and table 6.

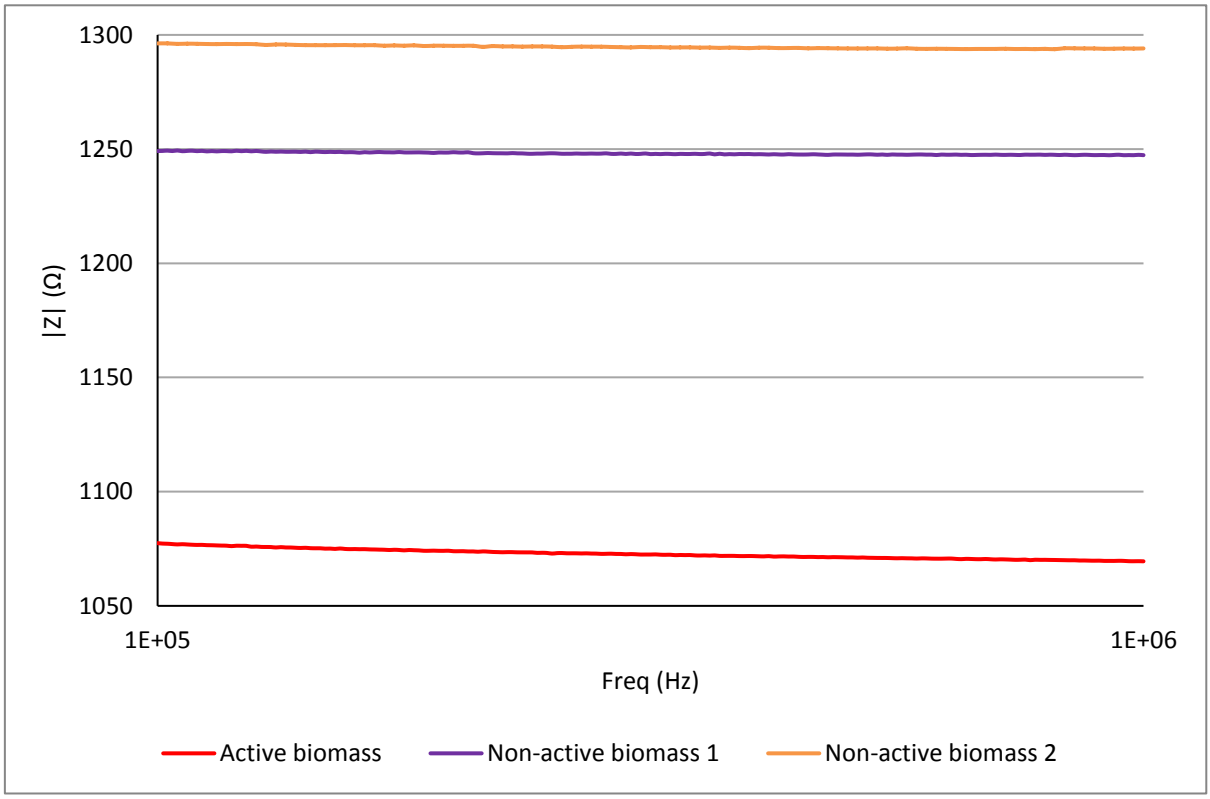


Figure 27 Impedance readings of 2 samples of non-active biomass.

Table 6 Impedance slopes, temperatures, EC, COD and VSS for active and of non-active biomass

	Active biomass	Non-active biomass 1	Non-active biomass 2
Slope [100KHz, 1MHz] (Ω/MHz)	-8.8372	-2.0067	-2.4467
temperature (°C)	25.5	23.3	23.1
EC (dS/cm)	1.80	1.79	1.79
COD (g O2/l)	5510	4829	4837
VSS (g VSS/l)	0.7675	0.2047	0.1998

Again, results shows that bioimpedance is sensitive to biomass concentration. Although all the measured samples of Figure 27 present a similar organic matter content (expressed in COD), their impedance variation (slope) is different according to the biomass concentration (VSS), as it can be observed in table 6. This result confirms the sensitivity of the slope to biomass concentration, but does not ensure that can distinguish active form non active biomass.

It must be said that the thermal treatment applied to inactivate biomass was not effective, and for some technical reason the VSS content decreased. However, the obtained slopes for non-active biomass shows similarity to those values obtained in measuring clarified water or 25% diluted samples (see tables 3, 4 and 5), which indicates that there is the same biomass-slope tendency as discussed in previous chapter (4.4.).

4.6. Effect of sedimentation

Biomass sedimentation and agitation during bioimpedance measurements induced variations in the results. It is thought that the interphase phenomenon which occurs during sedimentation can be related.

Sedimentation plays an important role in wastewater treatment processes. It can be used as a separation process in itself, but also as indicator of the end of a biological phase. In Figure 28 a scheme of the process is shown. During sedimentation, biggest particles tends to settle first, concentrating at the bottom (in Figure 28 represented as compression zone). In the upper zone, smallest particles start settling, producing a clear water zone. Between these two zones, there are particles of different sizes and densities (which can be attached to bubbles, to other particles or forming floccules etc.). When concentration increases, particles start interacting between, preventing its downwards movement due to sedimentation. This behaviour results in the form of an interphase or transition zone, as it can be observed in Figure 28.

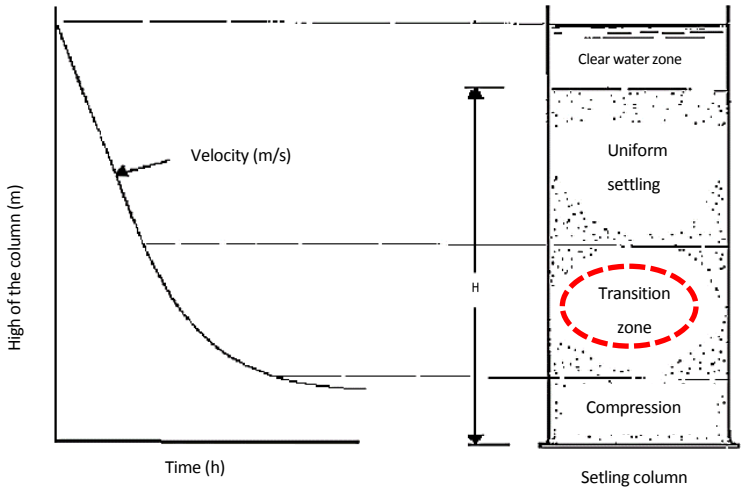


Figure 28 Scheme of the sedimentation process. A red circle indicates the interphase zone (Medina, 2007).

To observe how this behaviour interferes to impedance measurements, one sample of activated sludge diluted at 25% was measured during different moments of the biomass sedimentation. Impedance readings are shown in Figure 28 and slopes between 100 kHz and 1 MHz are summarized in table 6. Pictures of the measurement cell, taken during the experiment, have been added in order to show the settling state of each measurement.

Control 1 and 3 readings were taken with the sample in completely mixed state, as it can be observed in Figure 30. Then, biomass started to settle down. Observing bioimpedance results in Figure 28 and with the aid of table 6, there can be distinguished three remarkable patrons. First, control lectures gives the slopes of the sample homogeneously mixed, which value should correspond to the real biomass concentration. It must be said that control 2 was mixed more energetically, and turbulences inside the cell were more perceived. This could be the reason for having a higher slope value than Control 1.

After 30 seconds, turbulences stopped and the sample became more stabilized. In table 6 it can be observed that impedance slope at second 30 changes to an intermediate value between the two controls. As long as biomass is settled, slope becomes more pronounced, because sludge is concentrating at the zone of the electrodes, giving an impedance value for a higher biomass concentration than for the sample in completely mixed state.

Second patron is described by the measured samples at seconds 120 and 180, and it can be appreciated in Figure 29. Hence, it can be observed that they do not present a continuous behaviour. Impedance reading after 120 seconds decrease at the beginning and gets stabilised at the end, whereas impedance reading after 180 seconds does the opposite effect. This behaviour is of great importance because these lectures correspond to the moment represented in Figure 31, when the interface zone is crossing the zone of the electrodes, producing impedance variations. Readings at 120 and 140 seconds show these fluctuations, while at 290 seconds measures the maximum concentration of biomass.

From this moment on, slope goes increasing as long as biomass is settling around the zone of the electrodes, till a maximum value of -4,0844(Ω/MHz), almost double than the initial slope.

Finally, the third behaviour is obtained after 405 seconds, when biomass has settled below the zone of the electrodes, giving the impedance value of the clarified water or an intermediate impedance value between activated sludge and clarified water. Pictures of the cell taken during seconds 405 and 2580 (43 minutes) are shown in Figures 32 and 33, respectively.

Table 7 Summary of impedance slopes of 25% diluted AS sample at different moments of the biomass

time of settling (seconds)	slope [100KHz, 1MHz] (Ω/MHz)	notes
0	-2,9756	Control 1
0	-3,2911	Control 2, more turbulences during the measurement
30	-3,1022	
60	-3,2511	
120	-3,4689	Interface zone around the electrodes. Impedance fluctuates measuring either biomass or water.
180	-3,1722	
290	-4,0844	
405	-2,3467	
2580	-2,2400	

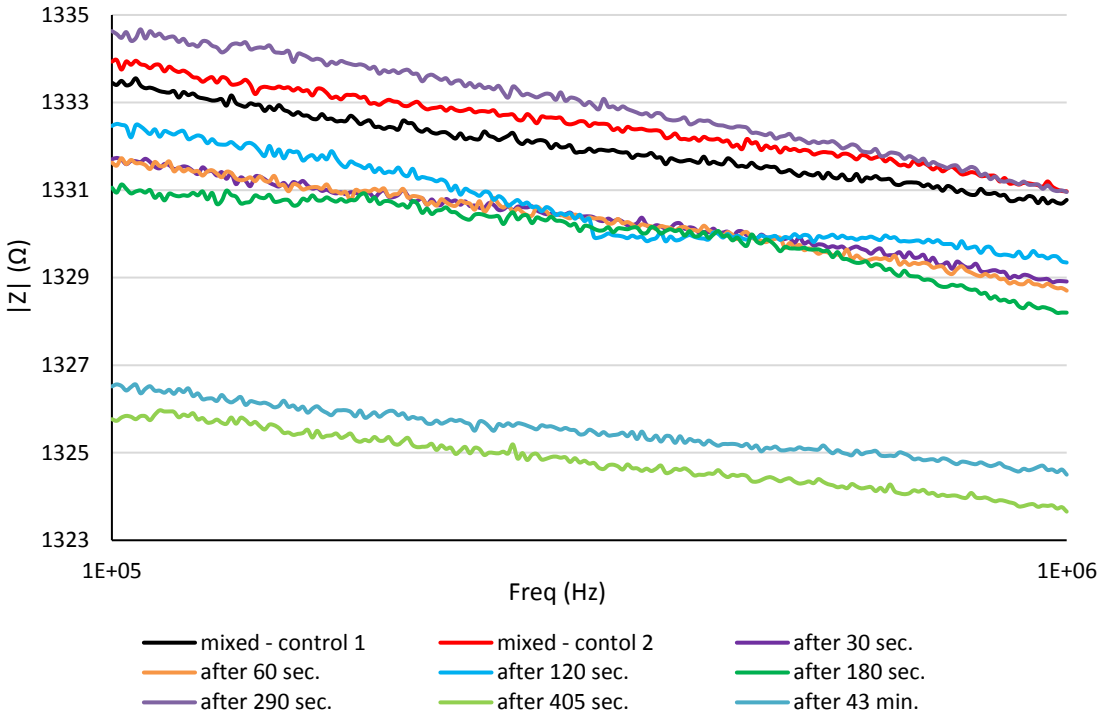


Figure 29 Impedance readings of a 25% diluted AS sample at different moments of the biomass settling.

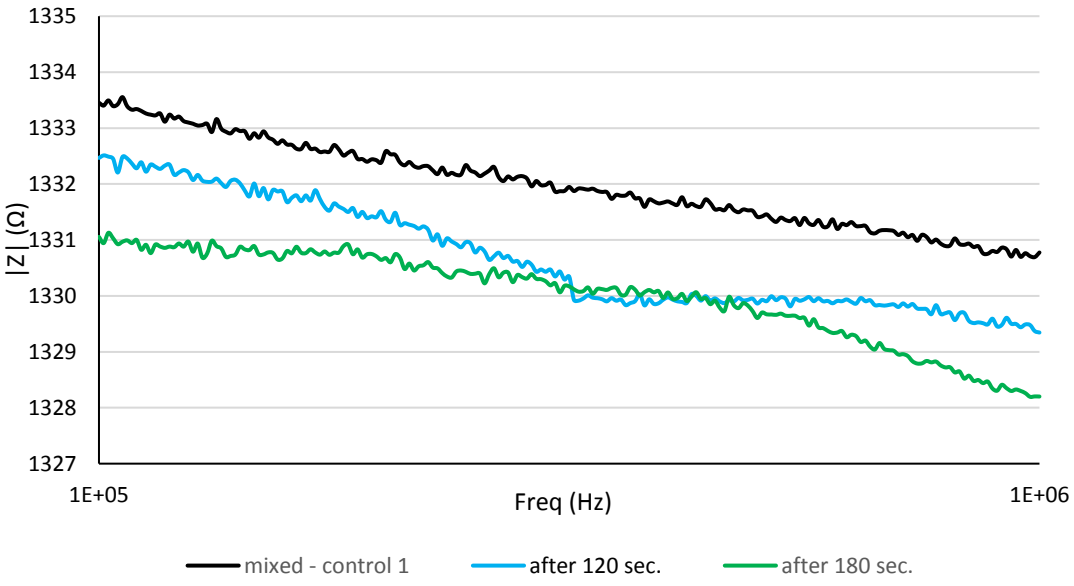


Figure 30 Impedance readings of 25% diluted AS sample at seconds 120 and 180, compared to Control 1

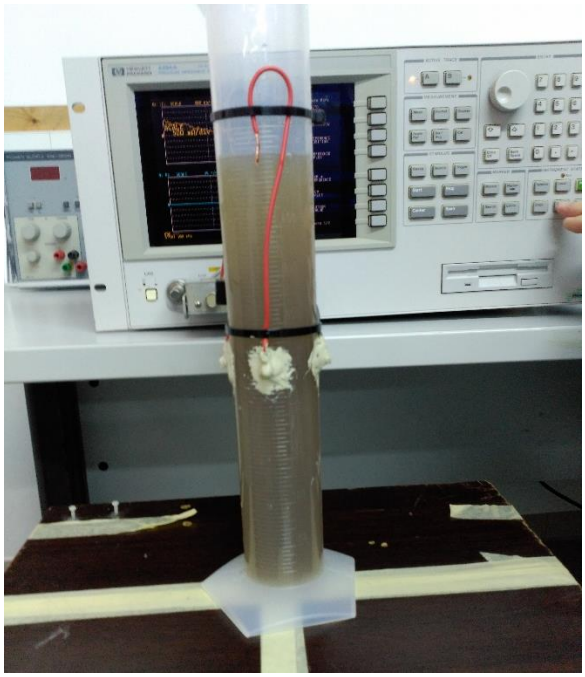


Figure 31 Picture of the cell during in completely mixed state

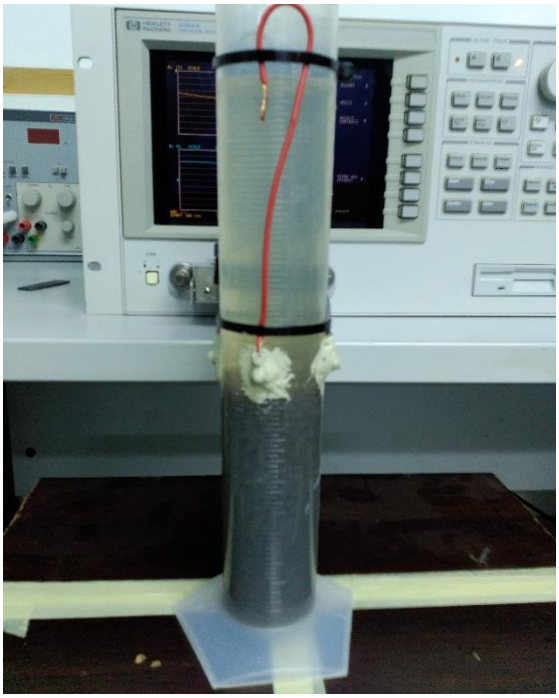


Figure 32 Picture of the cell after 290 seconds of sedimentation.

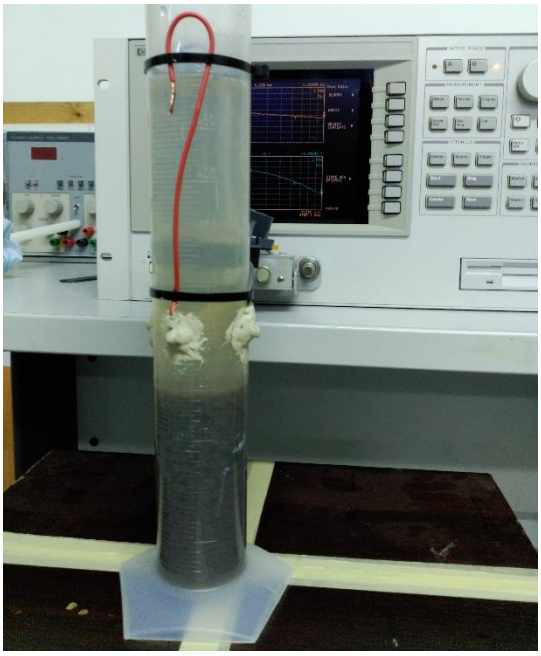


Figure 33 Picture of the cell after 405 seconds of sedimentation.

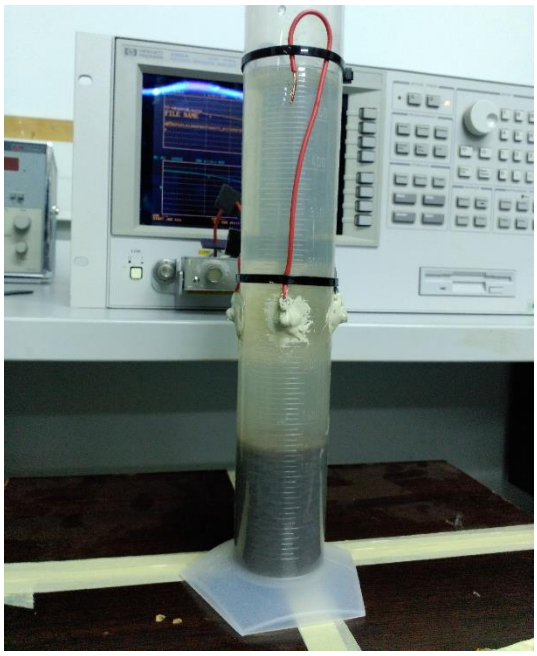


Figure 34 Picture of the cell after 43 minutes of sedimentation

4.7. Effect of temperature and electrical conductivity

Aside from observing that there is a relationship between impedance slope (in the range between 100kHz and 1MHz) and active-biomass concentration, it has been observed that, the impedance level can be different in each case. The parameters whose are known to be responsible of this phenomenon are temperature and electrical conductivity (EC).

The measurement of electrical conductivity is affected by the temperature of the solution, and it increases by about 2 percent per degree rise in temperature in the range of 15 to 35 °C (Hillel, 2000). The results obtained at higher or lower temperature are normalized by means of the following empirical equation (Hillel, 2000):

$$\sigma(T) = \sigma_{25}(1 + 0.02 \cdot (T - 25)) \quad , \quad (14)$$

where σ_{25} is conductivity at 25 °C, 0.02 (2%/°C) is the temperature coefficient for water solutions and T is the ambient temperature.

Hence, temperature and conductivity have a directly proportional relationship. Besides, impedance is indirectly proportional to conductivity and consequently to temperature.

Although temperature influences the impedance value, it does not modify its variation (slope) in different active biomass concentration. In Figure 34 a dot plot of slope with temperature is represented, showing that there is no relation between them. Dots looks dispersed with no tendency. Finally, in Figure 35 it can be observed the same tendency, but with electrical conductivity.

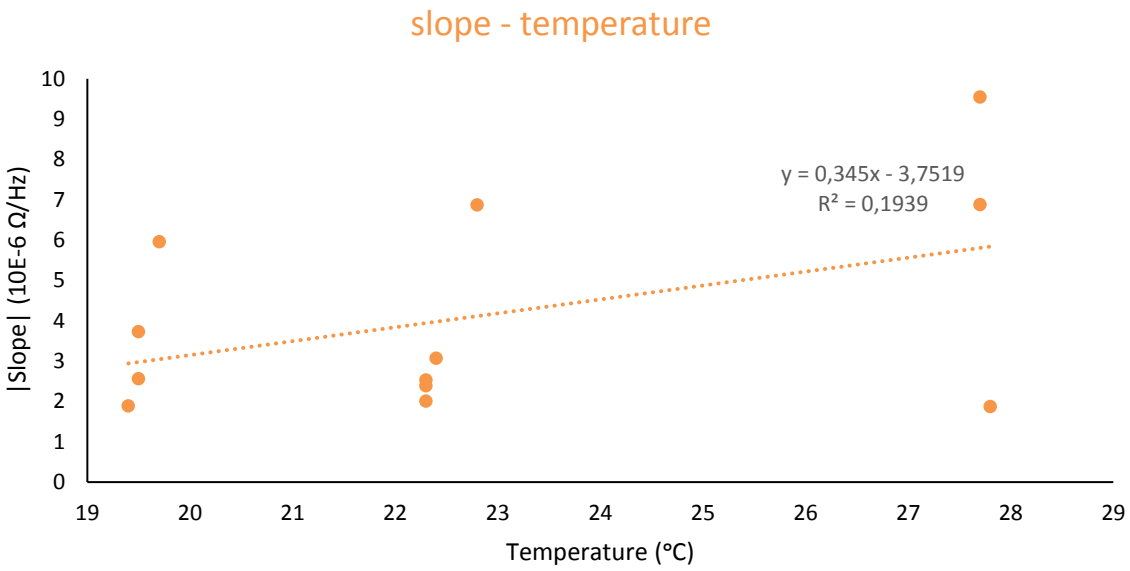


Figure 35 Dot plot of impedance slope and temperature.

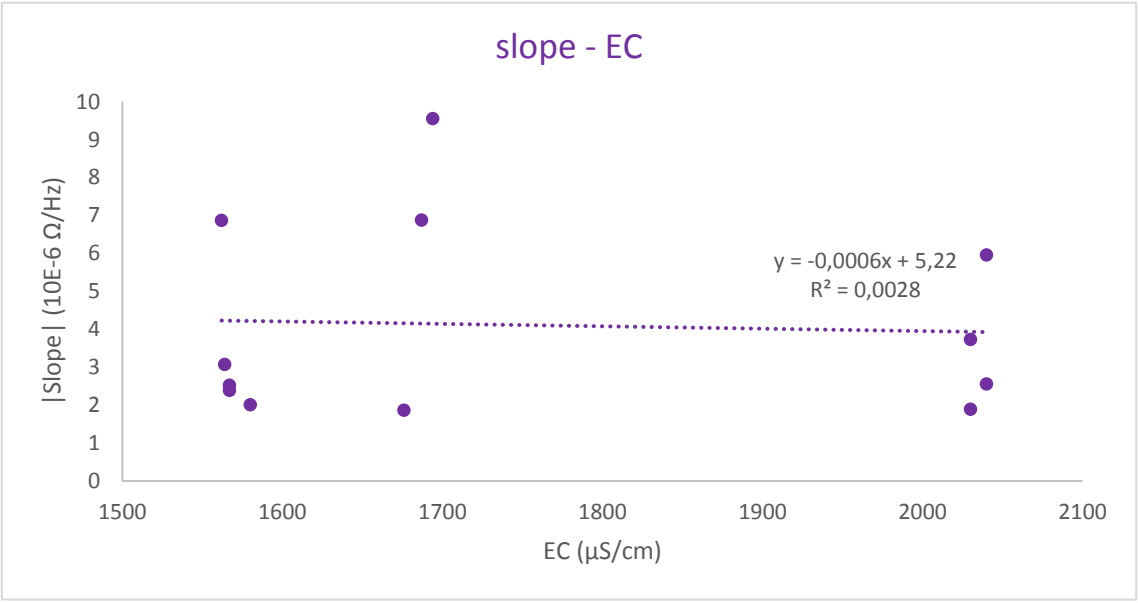


Figure 36 Dot plot of impedance slope and electrical conductivity (EC).

5. Conclusions and recommendations

5.1. Conclusions

From this work, it is concluded that impedance slope has a direct relationship with biomass concentration. Hence, a simple interpretation of the bioimpedance technique is suggested, for which the variation of impedance (or slope) can be correlated with the biomass concentration, expressed in VSS.

This new bioimpedance methodology could be used as biomass measurement in bioreactors for wastewater treatment. For that, slope impedance is measured in a range between 100kHz and 1MHz. Although the 4 electrodes configuration is known to be more precise, impedance slope can be well detected using 2 electrodes, enabling a simple electronic setup.

With regard to its application in bioreactors, there are some external interferences that need to be overcome. For example, magnetic agitation is not possible due to electrical interferences in the measurement. In addition, mechanical agitation has been demonstrated to interfere in the impedance readings. However, this behaviour could be studied in order to determine the amount of error that it introduces to the reading, in order to finally find the way to integrate the bioimpedance measurement into the bioreactor with other sensors (for example temperature, oxygen…). Moreover, although temperature and electrical conductivity influence the impedance value, they do not produce effect on the slope.

During sedimentation, different physic phenomena takes part which affect impedance readings. Among them, the interface zone has shown to produce alterations in the slope value. By this way, bioimpedance-slope technique could be used to follow the sedimentation process through a determined point of the bioreactor. Hence, interphase zone could be detected at the time that changes in the slope value were perceived.

Although this is a first contact work, there are optimistic reasons to confirm that the implementation of this technique for automatization purposes is possible. In wastewater treatment section, it would enable to design more efficient bioreactors and to have a better control of the process.

5.2. Suggestions for further research

It must be said that the used circuital model describes cellular suspensions of a single kind of bacteria into a homogenous medium. It is assumed it can be applied to all kinds of cellular suspensions, but they can appear undesired effects which are not taken into account in the model. A future object of study could be the proper electrical modelling for activated sludge or other biological waste products of interest.

In this work it is suggested that a 2-electrode system is used in order to simplify the detection system, which could be portable. However, most consulted publications recommends a 4-wire measurement method to cancel out the influence of the electrode-electrolyte impedance in the measurement. The possibility of using more specific electrodes such as large end-plate electrodes or microelectrodes could be studied. In this work, small electrodes have been used, which is said to provoke a non-uniform current density. Hence, another experimental line in the bioinstrumentation and sensors field could be to develop a portable system to measure bioimpedance that satisfies the mentioned design requirements.

Knowing that metabolic changes can be detected through bioimpedance, more experiments could be performed to observe how bioimpedance changes during biomass growing stages. Biomass samples could be forced to saturation of glucose or oxygen and their evolution followed. Furthermore, other kinds of biomass could be compared, such as anaerobic biomass.

However, this work encourages to keep developing this methodology, in order to find a simple and reliable biomass measuring technique to implement in the wastewater treatment sector.

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Annexes

Annex A. Statistical tests

Annex A - Statistic test

Relationships between bioimpedance slope and VSS and COD are studied.

Impedance slope is chosen as explicative variable due to its practical application in a possible model in which, knowing the impedance slope in a range from 100 kHz to 1 MHz could predict the biomass concentration expressed in VSS and COD.

1) VSS vs. bioimpedance slope

First, the behavior of these two variables is analyzed through the dispersion diagram:

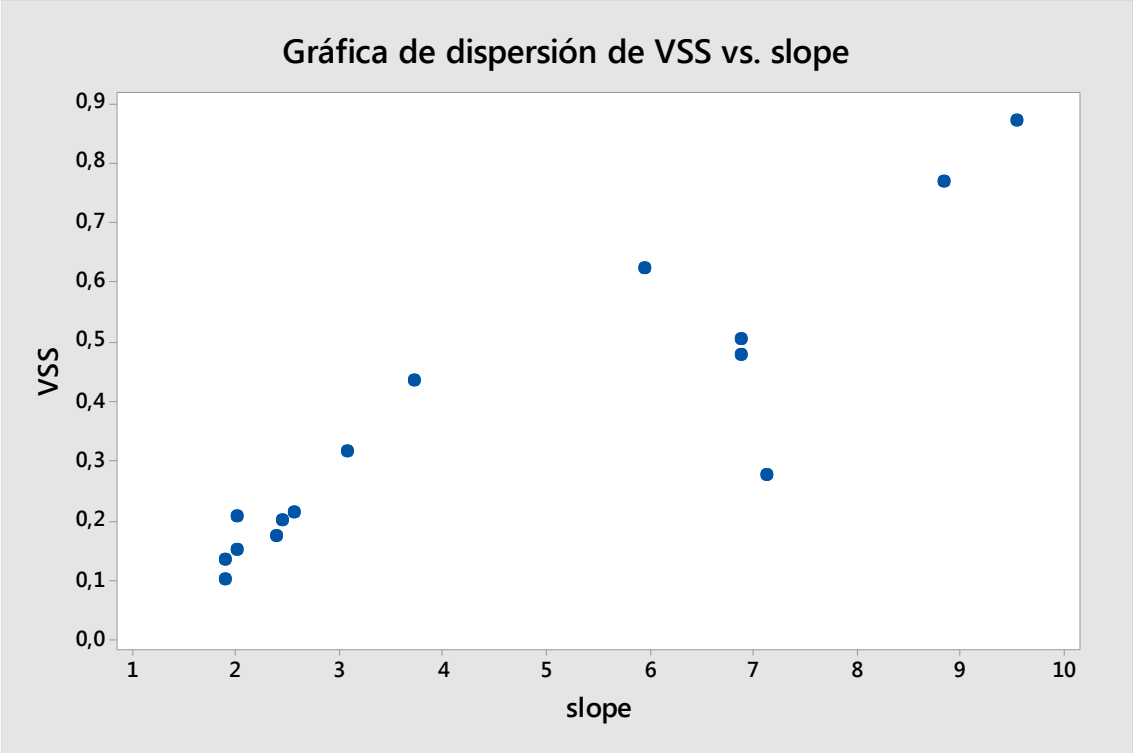


Figure 37 Scatterplot of VSS vs. slope

Seems that they present a linear tendency. To contrast their linear relationship, a correlation test is done between them.

Contrast statistic used: Pearson coefficient

H_0: Person coefficient = 0

H_1: Person coefficient ≠ 0

Alpha: 0.05

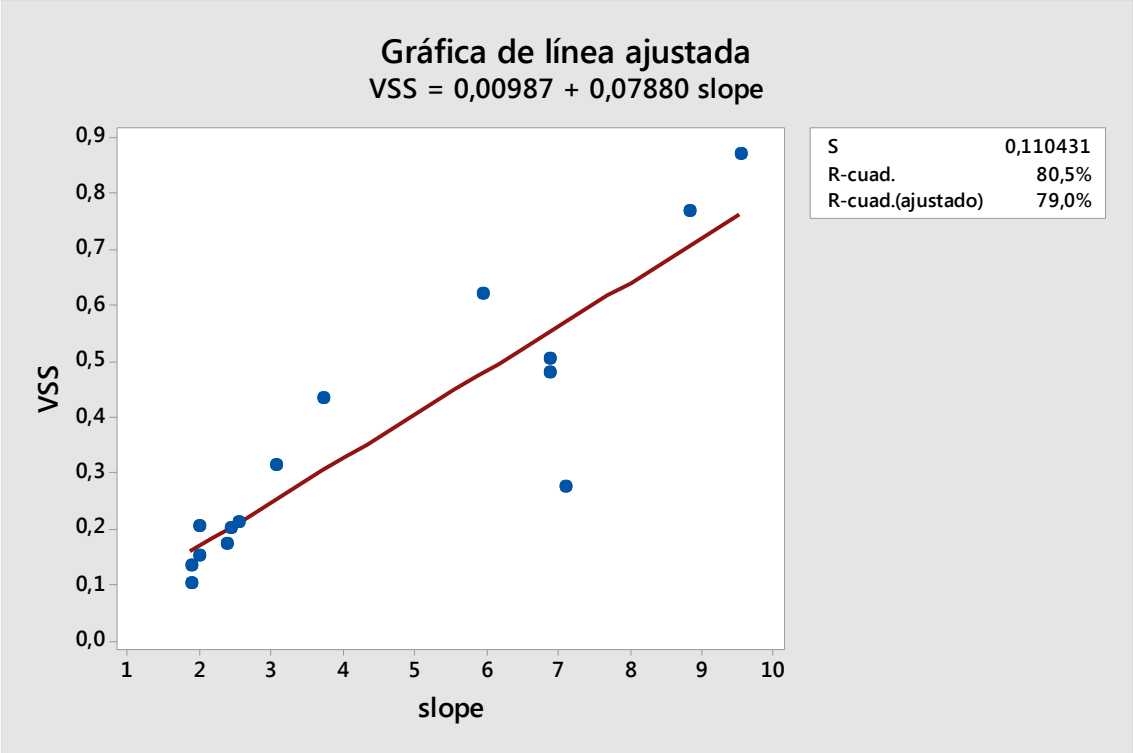


Figure 38 Linear regression of VSS and slope

The obtained value is 0.897, which indicates a strong relationship between both variables.

The obtained p-value is much lower than alpha, which indicates that the null hypothesis can be rejected. Hence, variables could be related.

The possibility of a linear model is studied. To statistically contrast this hypothesis, data is analyzed through a linear regression model.

Contrast statistic used: F of Fisher with 13 degrees of freedom

H_0: variables are not linearly related

H_1: variables are linearly related

Alpha = 0.05

The obtained p-value for this test is lower than alpha. Thus, null hypothesis is rejected. The model is well considered with an obtained R^2 of 79%.

To contrast the reliability of this regression test, the residuals are analyzed, assuming normality and constant variance.

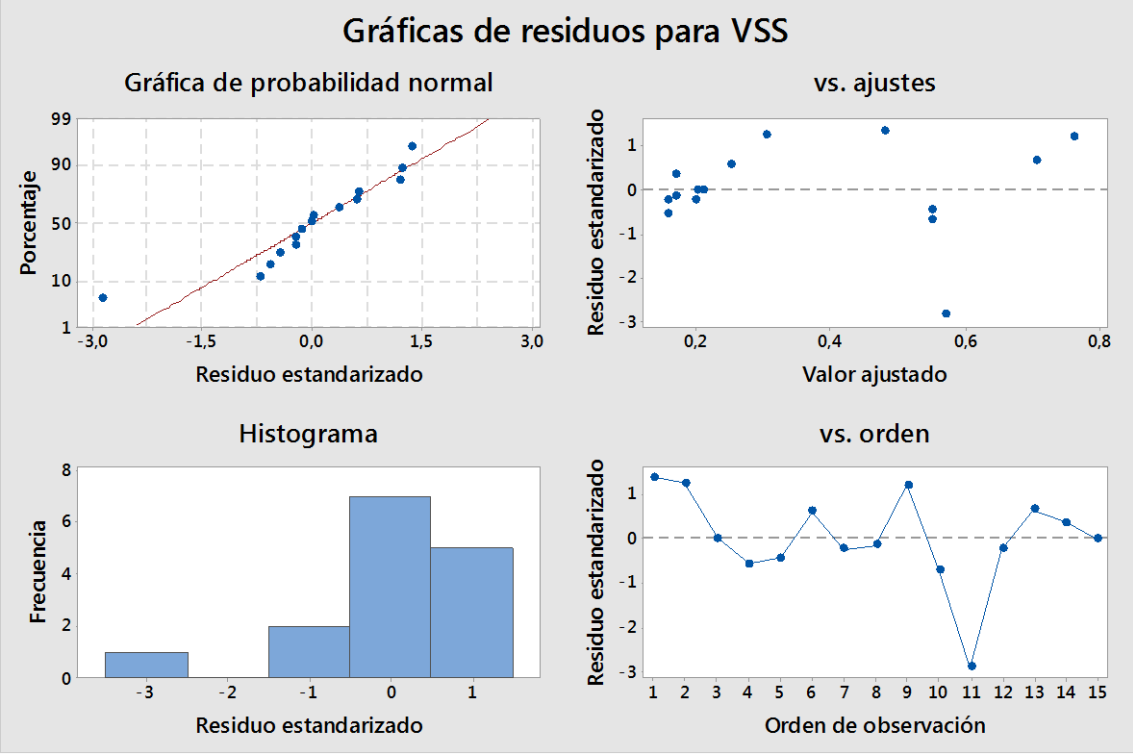


Figure 39 Residual plots of the regression of VSS vs. slope

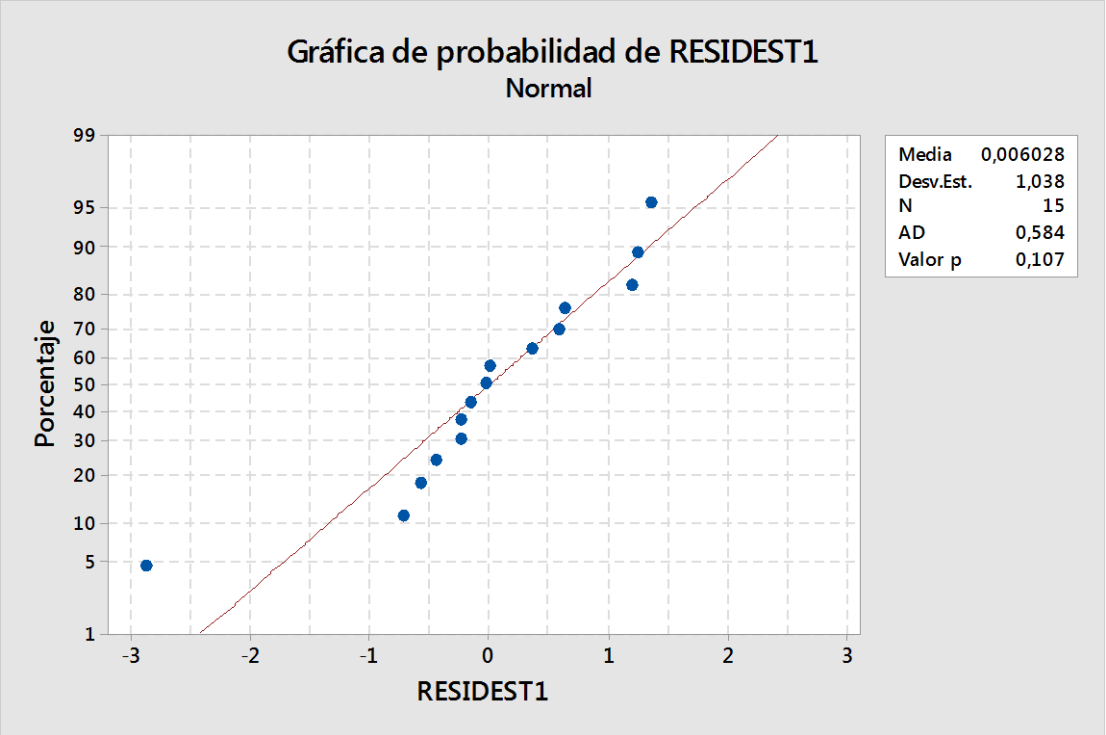


Figure 40 Normality test for residual plots of the regression of VSS vs. slope

Variance can be considered constant and residuals normal distributed. Finally, a normality test for residuals is done.

H_0: there is normal distribution

H_1: there is no normal distribution

Alpha = 0.05

The obtained p-value for this test is higher than alpha. Thus, null hypothesis cannot be rejected.

An outlier is perceived in number 11, which presents a deviation 3 times higher than standard deviation. The reason of this anomaly is explained in chapter 4.4. Hence, same statistical process is repeated but without this anomalous data.

Contrast statistic used: Pearson coefficient

H_0: Person coefficient = 0

H_1: Person coefficient ≠ 0

Alpha: 0.05

The obtained p-value is much lower than alpha. Hence, null hypothesis is rejected and it is assumed that the two variables could be related.

In this test the correlation coefficient is higher than the previous test, meaning a strong relationship between the two variables.

Following, the possibility of a linear regression model is analysed:

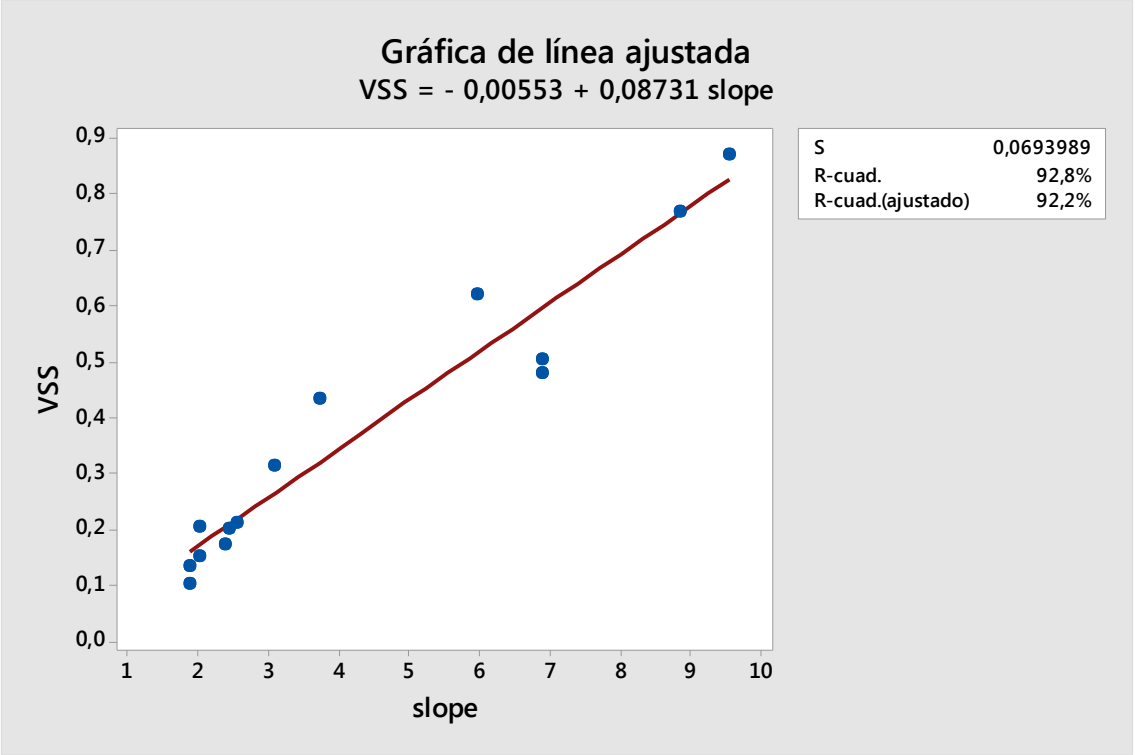


Figure 41 Corrected linear regression of VSS and slope

Contrast statistic used: F of Fisher with 12 degrees of freedom

H_0: variables are not linearly related

H_1: variables are linearly related

Alpha = 0.05

The obtained p-value is lower than alpha. Hence, null hypothesis is rejected. It can be observed that this model fits much better than the previously realized, with a R^2 of 92%

To check the reliability of this regression test, the residuals are contrasted, assuming normality and constant variance.

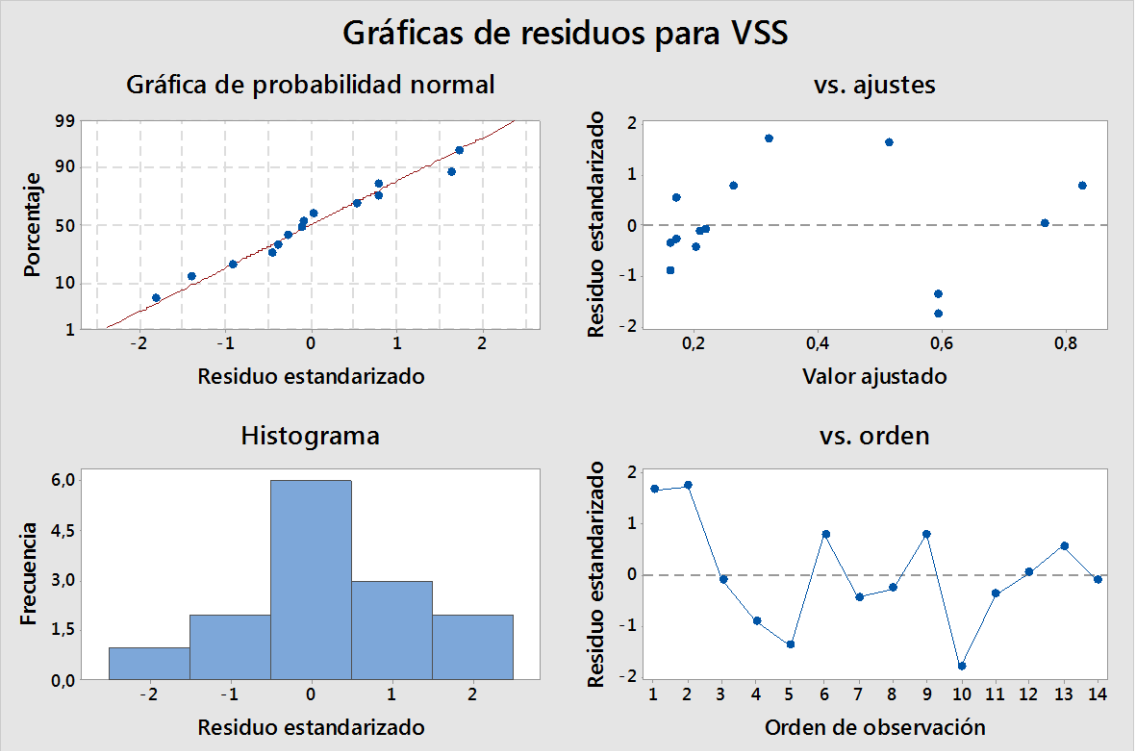


Figure 42 Residual plots of the corrected regression of VSS vs. slope

It can be said that this variance is constant and the provability distribution of the residuals fits into the normal distribution. The observation number 10 is an outlier, as it is twice deviated from the standard deviation.

To end with, residuals are analyzed through a normality test.

H_0: there is normal distribution

H_1: there is no normal distribution

Alpha = 0.05

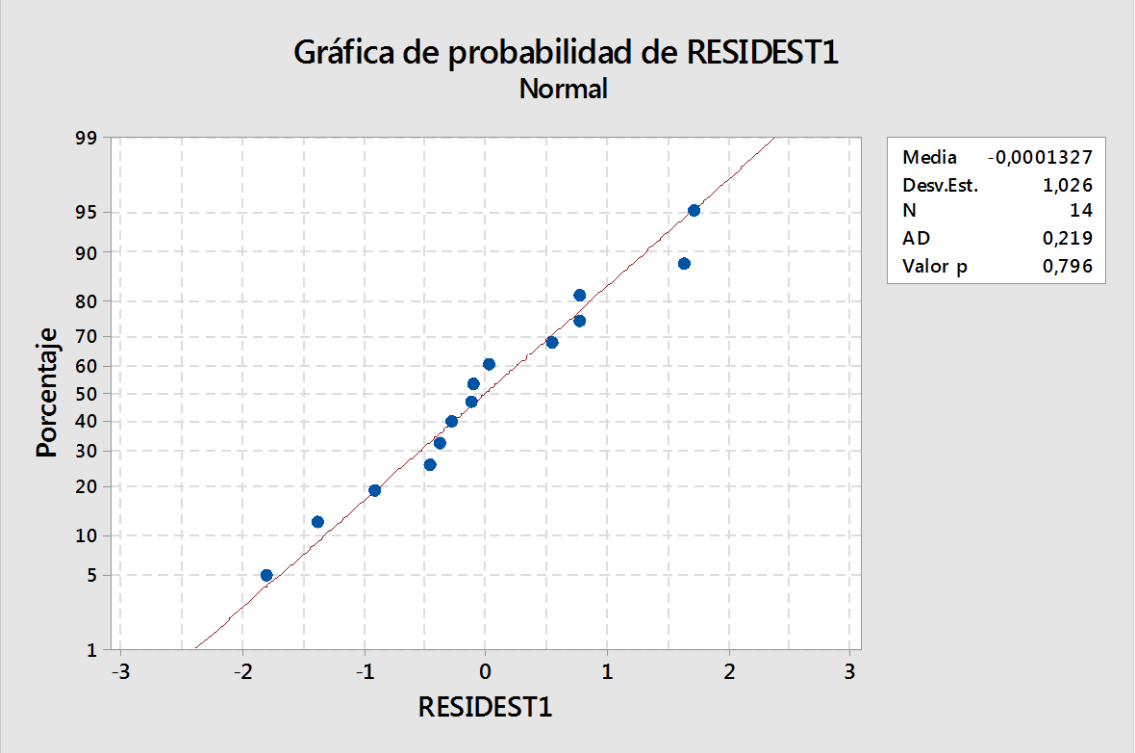


Figure 43 Normality test for residual plots of the corrected regression of VSS vs. slope

The obtained p-value for this test is higher than alpha. Hence, null hypothesis cannot be rejected. Residuals of this models could have a normal behaviour.

2) COD vs. bioimpedance slope

First, the behavior of these two variables is analyzed through the dispersion diagram:

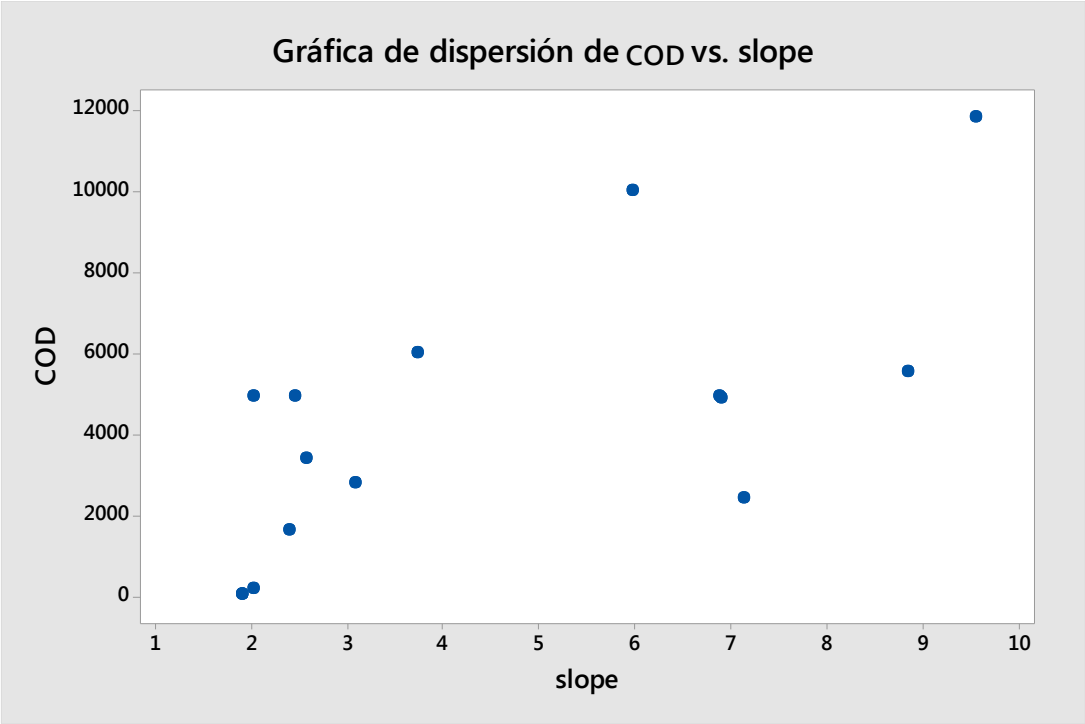


Figure 44 Scatterplot of COD vs. slope

Looks like that these variables have a linear tendency, even though it is diffuse. To check their relationship, a correlation test is done between them.

Contrast statistic used: Pearson coefficient

H_0: Person coefficient = 0

H_1: Person coefficient ≠ 0

Alpha: 0.05

The obtained p-value for this test is lower than alpha, which indicated that the null hypothesis can be rejected and that variables could be related.

The obtained Pearson coefficient is 0.668, which indicates a notable relationship between the two variables.

The possibility of a linear model which explains the relationship between the two variables is studied. To contrast statistically this hypothesis, data is analysed through a linear regression model.

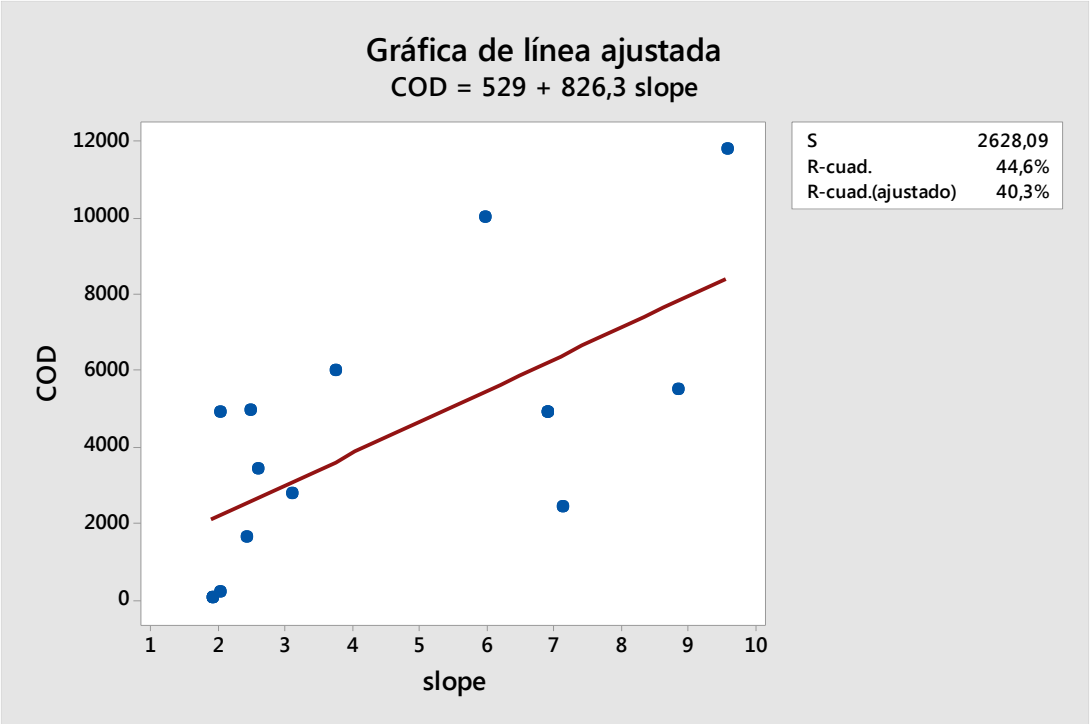
Contrast statistic used: F of Fisher with 13 degrees of freedom

H_0: variables are not linearly related

H_1: variables are linearly related

Alpha = 0.05

Figure 45 Linear regression of COD and slope



The obtained p-value for this test is lower than alpha. Hence, null hypothesis can be rejected. The obtained model is not considered reliable with a R^2 of 40.3%.

To validate this model, the behavior of the residuals is contrasted, assuming normality and constant variance.

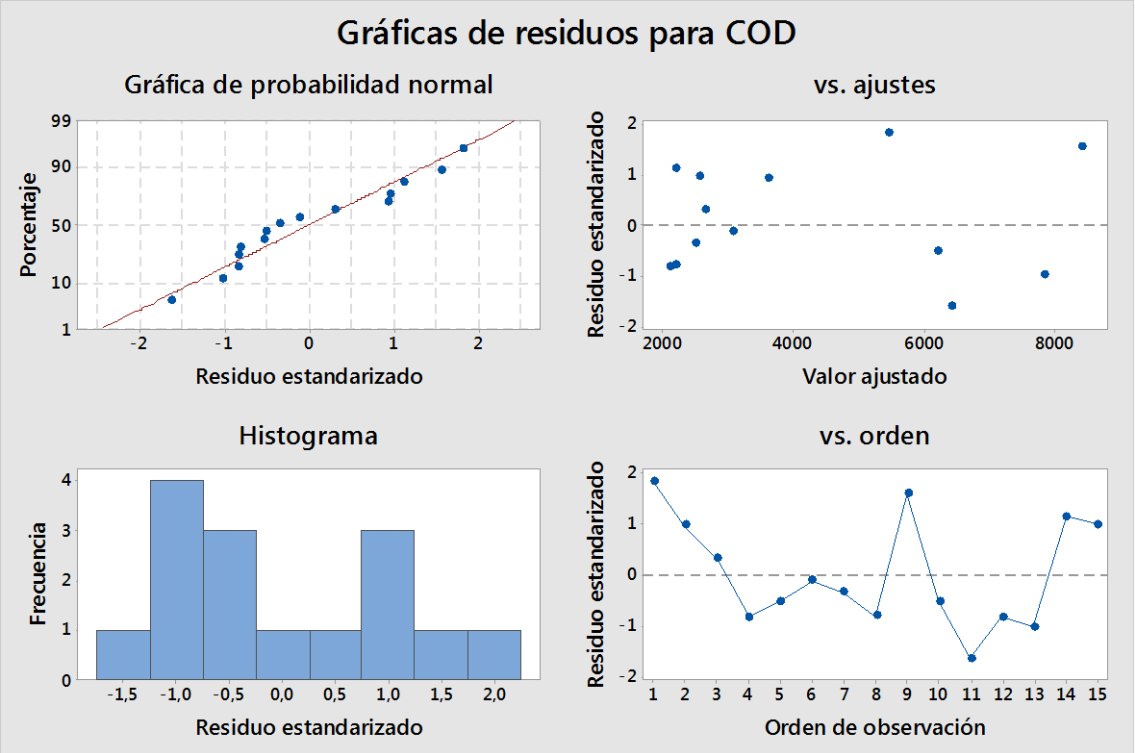


Figure 46 Residual plots of the regression of COD vs. slope

It can be said that variance is disperse and the probability distribution of the residuals does not adjust to the normal distribution.

To end with, residuals are analyzed through a normality test.

H_0: there is normal distribution

H_1: there is no normal distribution

Alpha = 0.05

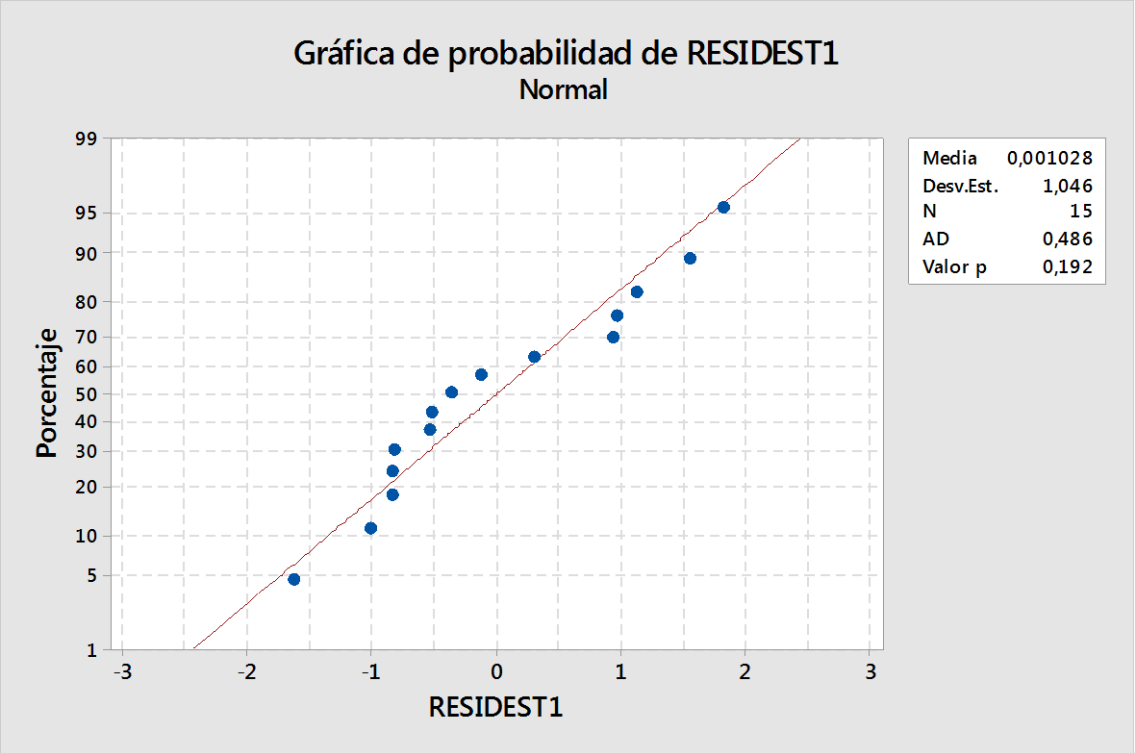


Figure 47 Normality test for residual plots of the regression of CODvs. slope

The obtained p-value for this test is higher than alpha. Hence, null hypothesis cannot be rejected and it is possible that residuals have a normal behaviour. Through the obtained results, the model cannot be rejected, although it is not considered a good model.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