MELiSSA

TECHNICAL NOTE

Departament d'Enginyeria Química
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TECHNICAL NOTE 78.101

BIOMASS SENSOR PROTOTYPE DESIGN AND CIII INTERFACE ASSESSMENT

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ACRONYMS

COTS	Commercial Of The Shelf
EIS	Electrical Impedance Spectroscopy
I2C	Type of serial bus protocol
HC	High Current
HP	High Potential
HF	High Frequency
LC	Low Current
LF	Low Frequency
LP	Low Potential
MELiSSA	Micro-Ecological Life Support System Alternative
PCB	Printed Circuit Board
SPI	Serial Peripheral Interface
UAB	*Universitat Autònoma de* Barcelona

1. Scope

The present document reports the system specifications and design of the BIOMEL CIII Sensor for its use in the MELiSSA's Compartment 3 bioreactor.

The activities herein described have been done in accordance to WP 78.10, as defined in AD 1. This work package's objective is to conceive a prototype sensor for measuring viable immobilised biomass as present in MELiSSA's Compartment 3 using a test reactor available at ESTEC and assessing its performance. This document presents the electrical, mechanical and software design adaptations of NTE's Electrical Impedance Spectroscopy biomass measuring system required to obtain this prototype biomass sensor.

This document also highlights the main interface requirements discussed between UAB and NTE (RD 2) so that the biomass sensor design can be fitted eventually in a subsequent stage to the actual MELiSSA Pilot Plant CIII bioreactor, currently under definition / development.

2. APPLICABLE AND REFERENCE DOCUMENTS

2.1. APPLICABLE DOCUMENTS

AD 1 Subcontracting Agreement UAB-NTE, MELiSSA Pilot Plant activity. CCN7 to contract 13292/98/NL/MV, dd 28/10/05

2.2. REFERENCE DOCUMENTS

RD 1 Design Report: VIAMASS sensor for use on bioreactors – TN 2.1. Ref.: NTE-VSS-TN-009, issue 2, 29/06/06

RD 2 Minutes of Meeting. Interface meeting UAB-NTE. Ref.: NTE-BMC3-MN-002, 18/01/06

3. Introduction

The purpose of the measurement system under development is the determination of a direct viable biomass density estimator based on the frequency variation of the electrical impedance of the viable cells present in the medium. This measurement system is to be applied to the nitrifying bioreactor of MELiSSA (Compartment III). The expected microbiological strains in this compartment are Nitrosonomas Europae and Nitrobacter Winogradskyi. Cells are immobilised in beads of ca. 4 mm diameter.

3.3. ELECTRICAL IMPEDANCE SPECTROSCOPY

The biomass estimator relies on performing electrical impedance measurements of the medium, performed at several frequencies (Electrical Impedance Spectroscopy, EIS). The method is widely described in previous documents (LBC-1000-001-NTE). Briefly, the impedance spectrum of a cellular suspension presents a decay (relaxation) with the frequency that depends on the cell characteristics, as shown in Figure 3-1.

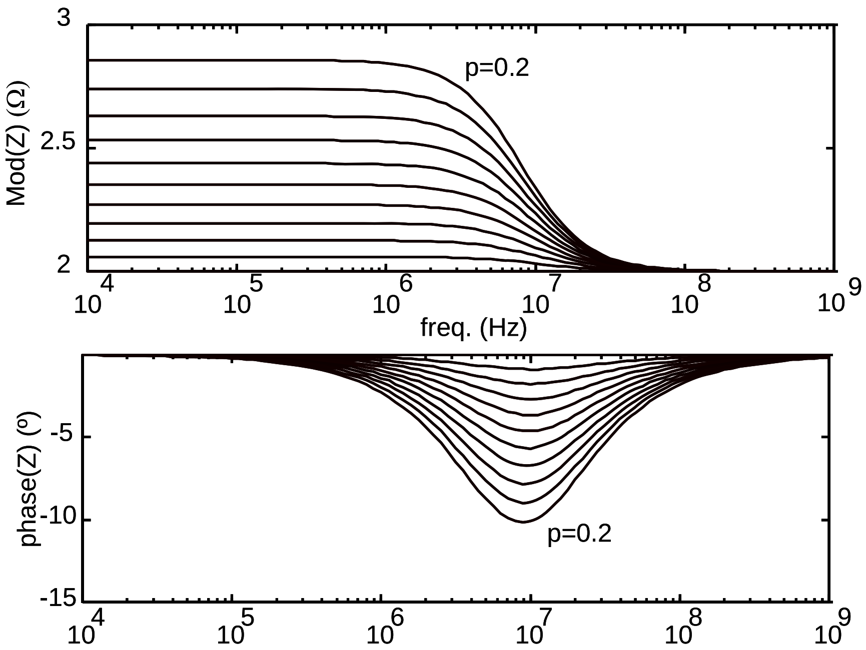


Figure 3-1: Electrical Impedance Spectroscopy of a cellular medium

The location of the decay in the frequency axis is known as *central relaxation frequency* (f_c) and depends inversely with the cell size. The slope of the decay is higher if there is dispersion in size and shape of the cells in the medium (e.g. due to the presence of diverse type of micro-organisms). The depth of the decay depends on the viable cell density p, where p is defined as the ratio between intracellular volume and total volume.

Figure 3-1 displays the modulus and phase variation with frequency of the electrical impedance measured in a medium with different cell concentrations ($p \leq 0.2$).

Several biomass density estimators can be derived from the EIS measurements. The estimator used in the biomass sensor is E_2. It is defined as the relative variation of the impedance magnitude between two frequencies. One impedance module is measured at low frequency (LF) placed below f_c and the other impedance module is measured at high frequency (HF), taken above f_c:

$$E2(\%) = 100 \cdot \left(1 - \frac{|Z(HF)|}{|Z(LF)|} \right) \alpha \ p$$

- Eq. 1

If biomass density is high the impedance ratio in the formula will be small, therefore the estimator will be high. If the density is small then the ratio will be close to 1 and the estimator will be small. Thus, this estimator is proportional to the cell concentration in the medium.

3.4. SPECIFIC ASPECTS FOR IMMOBILISED BIOMASS

When EIS is applied to specific fermentation processes, there is a single microorganism present. Thus, f_c is fixed and HF and LF frequencies can be also pre-determined. This aspect simplifies the measurement system design. Additionally, the chemical parameters of the suspension are usually well determined and kept constant.

Nevertheless, when a new set-up is prepared, even with single microorganisms, there is an initial uncertainty about the optimal frequencies to be chosen. Also practical aspects related with electrical paths to ground in the bioreactor make necessary to keep the ability of acquiring several frequency samples of the impedance spectrum. Usually a logarithmic frequency sweep with 3 to 8 frequency points per decade is performed. With this raw data, frequencies which present a specific noise could be neglected and groups of close frequencies could be averaged to improve the signal to noise ratio. Additionally, if enough biomass is present in order to ensure a detectable impedance relaxation, the acquired spectrum could be fitted to the Cole-Cole impedance model (Eq. 2) and the model parameters could employed to characterize the suspension:

$$Z(f) = R_\infty + \frac{R_0 - R_\infty}{1 + \left(j\frac{f}{f_c}\right)^{1-\alpha}}$$

- Eq. 2

Where:

R_∞: Impedance measured at high frequency
R_0: Impedance measured at low frequency
f_C: Central relaxation frequency
α: Adjusting parameter

According to measurements of different types of biomass with different characteristics it is observed that f_c provides information about the average cell size and the parameter α is related to the dispersion of the biomass' cells size and shape. The viable biomass estimator is now:

$$E2_m(\%) = 100 \cdot \left(1 - \frac{R_\infty}{R_0}\right) \alpha \ \ p$$

- Eq. 3

For typical biological wastewater treatment plants f_c is between 2 to 8 MHz, and for pure bacterial suspensions (*E.Coli*) achieves 14 MHz. The dispersion parameter α ranges from 0.1 to 0.5. The relaxation depth goes from 0.3% to 2% with a phase minimum value of -0.1° to -1°.

In the specific case of biomass immobilised in the surface of microcarriers, the mentioned considerations are still valid. A reduction in the sensitivity could be expected due to the concentration of the biomass in discrete volumes, which provide broad conductive paths to the electrical current in the extracellular space, and also due to a probable masking effect of the external cell layers respect to the inner cell layers on the carrier surface. Previous experiments have led to an apparent sensitivity reduction in a factor between 2 and 3.

4. SYSTEM design

4.5. GENERIC CONSIDERATIONS

The main error sources in the EIS measurement systems when applied to cellular biomass suspended on a medium are the changes in the physical and chemical conditions and the electrode impedances. The EIS method is also sensitive to temperature changes. By using a ratio-metric estimator (E_2), low theoretical temperature dependence can be achieved. Nevertheless, temperature changes can produce variations in the HF system error. Because of this, the temperature should be acquired and used to process the signal. High conductivity changes can also affect the biomass estimation, and should be compensated. Gas hold-up or the presence of solid particles produces noise. The solution to these problems is data processing (linear and non-linear filtering).

To acquire EIS measurements the medium is sensed using metallic electrodes. The interface between the electrodes surface and the ionic solution has its own impedance (Ze). The electrode impedance varies not only with frequency but also with temperature, time and physical-chemical characteristics of the medium. Ze is usually higher than the impedance under measurement and is the worst cause of error that drives to a slow measurement drift. The robustness against Ze should be provided by design. The conditions to minimize this error are:

- To implement a four electrode structure (two current drive, two voltage measurement), as shown in Figure 4-1
- To design the electrodes properly so that the Ze is kept as low as possible in absolute values
- Ze imbalance as low as possible respect to the input impedance of voltage measurement circuit

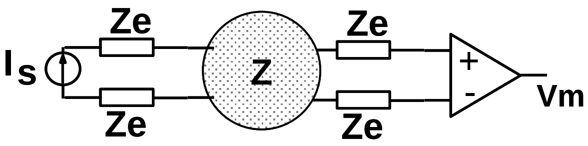


Figure 4-1: Four-electrode EIS system

These conditions imply the following generic system design considerations:

- Ze depends to great extent on the electrode's geometry. Low Ze can be achieved using large electrodes (large surface).

- Balanced electrode impedances, to be obtained by manufacture. (It must be noted that adhesion effects, e.g. biofouling, can lead to unbalanced impedances which can be compensated by periodical electrodes cleaning

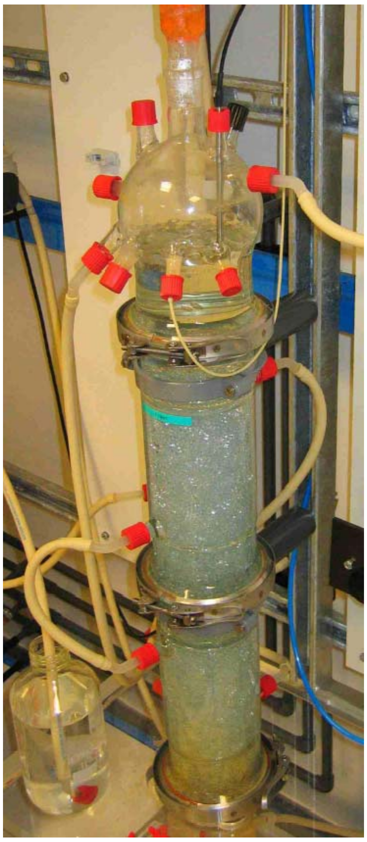
- Accurate electrical design, which implies:
  - Parasitic capacitances between the electrodes and the first stage electronics must be kept as low as possible
  - Coaxial cables cannot be used to connect the electrodes and the first-stage electronics as they exhibit large parasitic capacitances at the frequencies of use.
  - To avoid the crosstalk effect if normal cables would be used instead of coaxial cables the first-stage (or front-end) electronics must be very close to the electrodes
  - In this case the front-end electronics must feature a very high input impedance

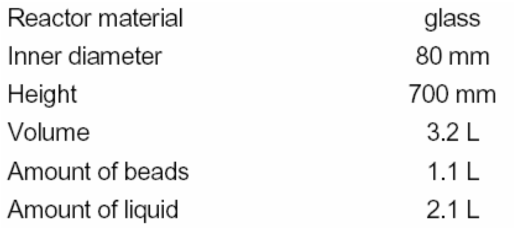
These generic system design considerations are to be implemented for the MELiSSA's CIII immobilised biomass sensor in two stages. Firstly, a prototype sensor is to be developed to assess its performance on a test reactor. This process is described in next par. 4.6 to 4.8.

Secondly, the design needs to be eventually upgraded so that the biomass sensor system can be used in the actual MELiSSA Pilot Plant CIII bioreactor, which is currently in a definition phase. Therefore, at this stage, only interface requirements between the biomass sensor and the bioreactor can be investigated. This is developed in chapter 5

4.6. PROTOTYPE BIOMASS SENSOR

The EIS measurement system needs to be adapted to fit into a test bioreactor where the feasibility of this method for the determination of the viable immobilised biomass is to be assessed. The test bioreactor proposed by ESA and its main physical characteristics are presented in Figure 4-2.





Reactor material	glass
Inner diameter	80 mm
Height	700 mm
Volume	3.2 L
Amount of beads	1.1 L
Amount of liquid	2.1 L

Figure 4-2: Test bioreactor with immobilised biomass

A first consideration when performing EIS measurements on immobilised biomass reactors is the need of measuring large bioreactor volumes to overcome the dispersion due to the discrete number of beads contained in the measurement region. For the proposed test bioreactor this can be achieved by performing four-electrode measurements between two-electrode pairs placed at both sides of the bioreactor, at three different heights. Figure 4-3 displays the two-electrode pairs arrangement over a plane perpendicular to the longitudinal axis of the bioreactor, with the two-electrode structures diametrically opposed. Because the electrodes structure is split in two halves the front-end electronics concept presented in Figure 4-1 is also split in two and each part is directly connected to the electrode structure

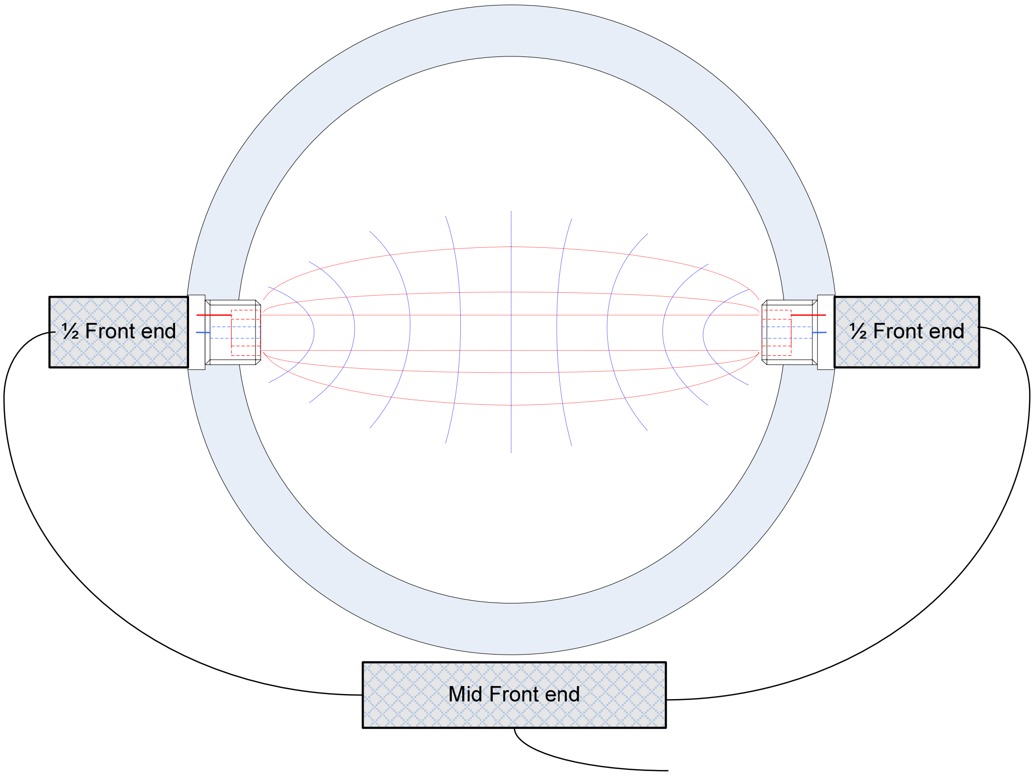


Figure 4-3: The front-end is split in two ½ front-end. Differential measurement and multiplexing is performed in the mid front-end.

Given that the front-end should perform not only the current injection and measurement but also the side-to-side voltage difference between electrodes, a differential amplifier should be placed between both ½ front-ends. In addition, the complete system features three electrode pairs structures at three different heights, with a total of six two-electrode elements. As a measurement involves the selection of two out of these six elements a multiplexing function is introduced in the system for the selection of the active measurement pairs for the various possible measurement configurations. Therefore a mid front-end element is introduced which houses the above-described differential amplifiers and the multiplexing function. The mid front-end (also referred to as multiplexer box) is connected to the main system box, which includes all the electronics required for signal acquisition and processing. Finally, the main system box is connected to a PC, which contains a specific SW application for measurement control. The system overview is depicted in Figure 4-4.

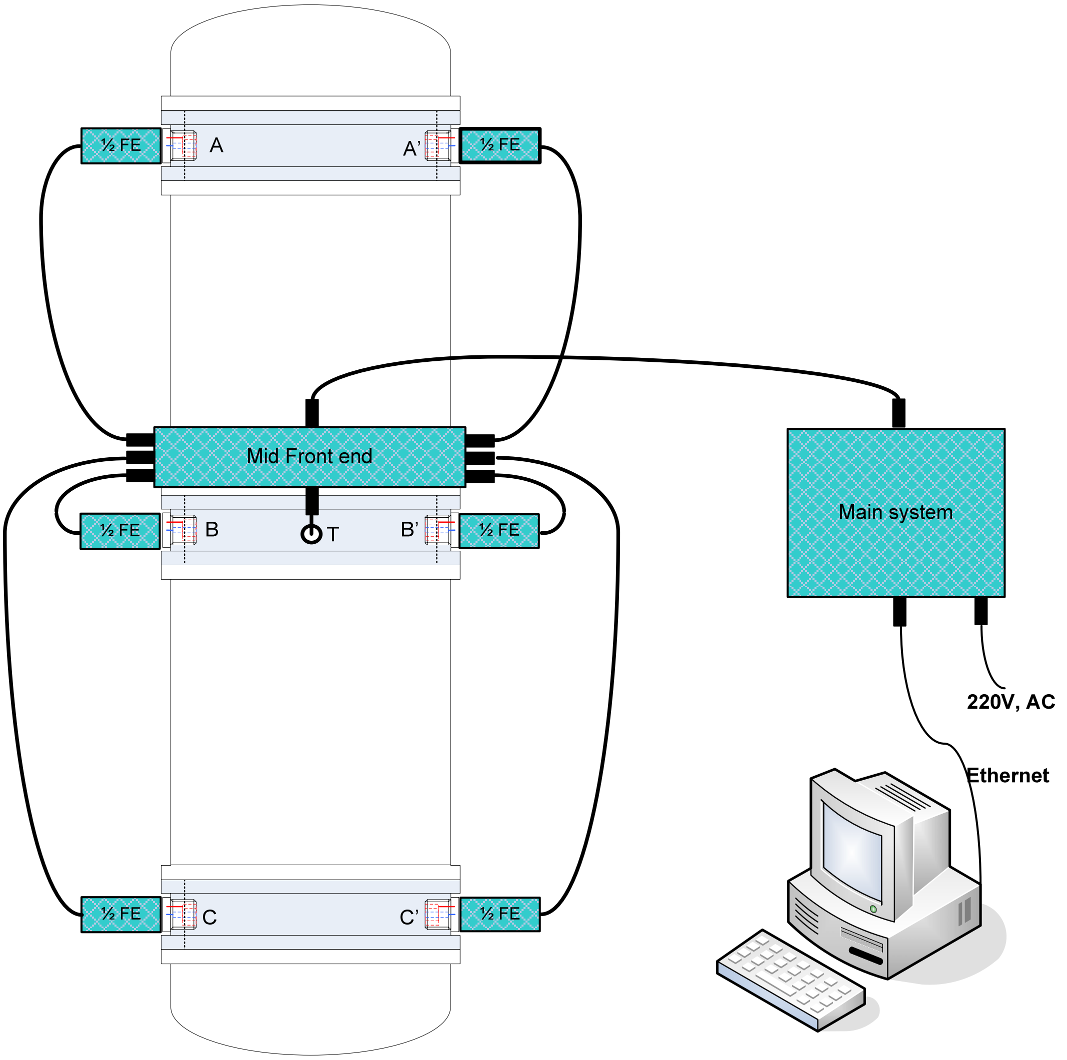


Figure 4-4: System concept

The proposed structure allows the selection of several electrode-pair arrangements in order to perform different measurements through the reactor column. Thus, transversal measurements can be done by selecting electrodes placed at the same level but also vertical ones, between electrodes placed at different levels. Due to the cylindrical symmetry some of these arrangements lead to redundant measurements. This characteristic allows certain simplification of the system. Thus the current injecting electrode elements can be placed in one side (e.g. elements A, B and C) and the current draining and voltage measuring electrode elements at the other side (e.g. A', B', C'). All of them perform voltage measurement at the inner electrodes.

The non-redundant measurement configurations are the following:

- Transversal: A-A', B-B', C-C' (in red in Figure 4-5) for detecting concentration gradients along the bioreactor vertical axis.
- Vertical, half-length: A-B', B-C' (in blue in Figure 4-5) which allows the integration of half bioreactor volume and detecting differences between lower and upper section.
- Vertical, full length: A-C' (in green in Figure 4-5) which allows the integration of the entire bioreactor volume.

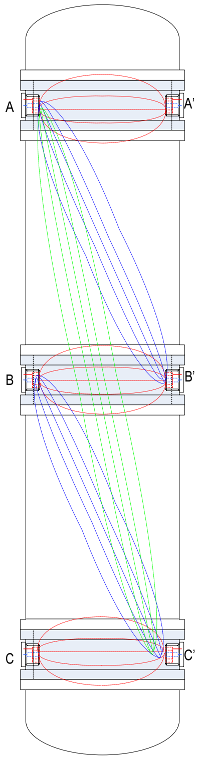


Figure 4-5: Non-redundant measurement configurations.

Then, the switching structure contained in the mid-front-end should be able to select these six configurations and convey the resulting differential voltage and measured current to the main system box. Additionally, a temperature probe is placed at the middle ring and connected to the mid-front-end. The cables that carry the analogue signals and the power supply from the electrode elements to the mid front-end box are kept as short as possible. The main system box contains the signal demodulator and the power supply and the digital parts of the system: signal generation, signal acquisition and controller. A digital link (a network connection) provides the acquired information to a local or remote computer.

Based on these considerations prototype biomass sensor system consists basically in five main components, as shown in Figure 4-6:

1. Support ring
2. Sensor element (half-front-end sensors)
3. Multiplexer box (mid front-end)
4. Main system box
5. PC

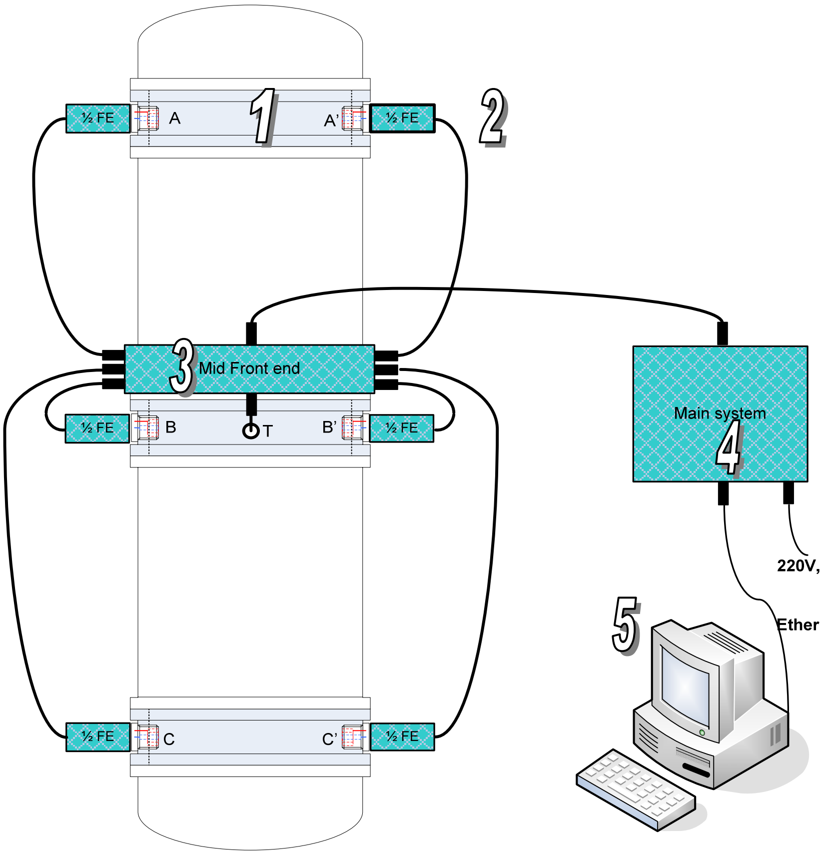


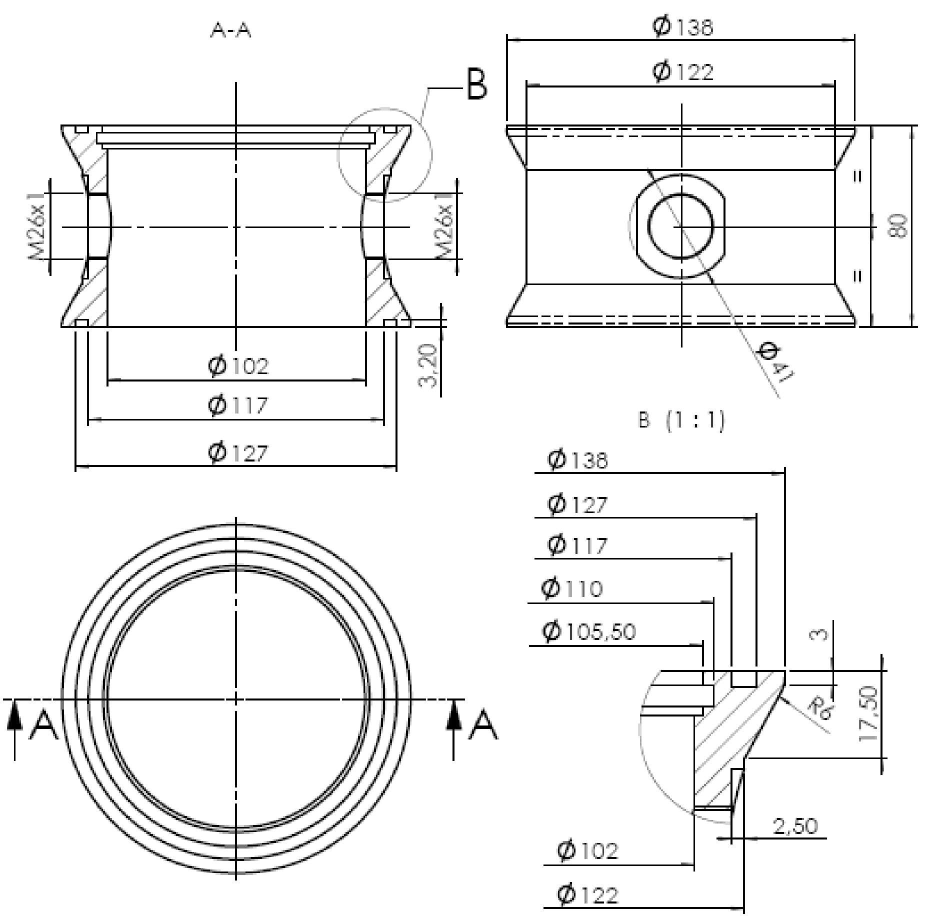
Figure 4-6: Prototype biomass sensor elements

4.6.1. Support ring

The support ring has been designed so that the sensor elements can be mechanically fitted into the test reactor's glass body.

This adaptor consists of two delrin pieces:

- Interface ring (Figure 4-7): structure that interfaces with the test reactor’s glass body and provides mechanical support for two sensor elements. Three adaptor elements are fitted into the test reactor’s lower, middle and upper parts, respectively.
- Grid (Figure 4-8): two grids are fitted in the upper and bottom adaptors. The size of the holes is small enough so that the immobilised biomass is contained within the volume defined by these adaptors



Biomel-CIII Sensor	**NTE-BMC3-DR-001**			
TITULO / TITLE:	Nº DIBUJO / DRAWING Nº:			
ENSAMBLADO EN / ASSEMBLED IN :				
		1.0	**First Issue**	**29/09/06**
		ED/ISS:	MODIFICACIONES / MODIFICATIONS :	FECHA/DAT

	Medidas Nominales Nominal Dimensions	hasta 6 to 6	más de 6 a 30 more than 6 to 30	30 a 100 30 to 100	100 a 300 100 to 300	300 a 1000 300 to 1000
	Basto / Gross	±0.2	±0.5	±0.8	±1.2	±2
X	Medio / Medium	±0.1	±0.2	±0.3	±0.5	±0.8
	Fino / Fine	±0.05	±0.1	±0.15	±0.2	±0.3

Medidas Angulares / Angular Dimensions : 1º DIN 7168 Teil 1 / ISO 2768

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AUT./AUTHORISED:		**J.Mas**

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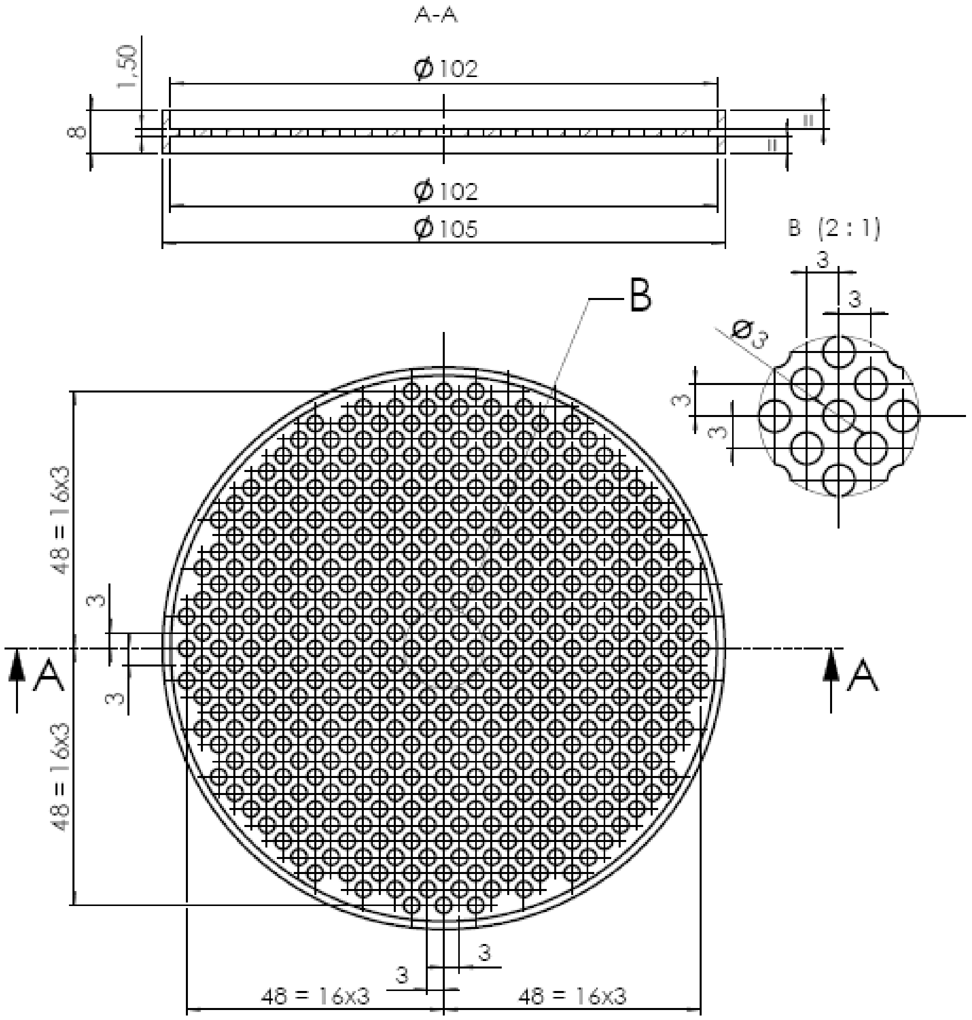
MATERIAL / MATERIAL :	**Delrin**	FORMATO / FORMAT :	**DIN A4**
ESPECIFICACIONES / SPEC :	-	HOJA / SHEET 1 DE / OF : 1	
ACABADO / FINISH :	-	ESCALA / ESCALE :	**1:2**
RUGOSIDAD / ROUGHNESS :	**3.2 µm**	UNIDADES / UNITS :	**mm**
MASA / MASS :	**440.7 gr.**	ARCH. CAD :	**Interface Ring**

PROYECTO / PROJECT : **Biomel-CIII**

TITULO / TITLE :	Nº DIBUJO / DRAWING Nº :	ED / ISS :
Interface Ring	**NTE-BMC3-DR-009**	**1.0**

Figure 4-7: sensor adaptor – Interface ring



Biomel-CIII Sensor	NTE-BMC3-DR-001
TITULO / TITLE:	Nº DIBUJO / DRAWING Nº:

ENSAMBLADO EN / ASSEMBLED IN :

	Medidas Nominales Nominal Dimensions	hasta 6 to 6	más de 6 a 30 more than 6 to 30	30 a 100 30 to 100	100 a 300 100 to 300	300 a 1000 300 to 1000
	Basto / Gross	±0.2	±0.5	±0.8	±1.2	±2
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Medidas Angulares / Angular Dimensions : 1° — DIN 7168 Teil 1 / ISO 2768

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MATERIAL / MATERIAL :	Delrin	FORMATO / FORMAT :	DIN A4
ESPECIFICACIONES / SPEC :	-	HOJA / SHEET	1 DE / OF : 1
ACABADO / FINISH :	-	ESCALA / ESCALE :	1:1
RUGOSIDAD / ROUGHNESS :	3.2 µm	UNIDADES / UNITS :	mm
MASA / MASS :	16.4 gr.	ARCH. CAD :	Grid

PROYECTO / PROJECT :
Biomel-CIII

TITULO / TITLE :
Grid

Nº DIBUJO / DRAWING Nº :
NTE-BMC3-DR-010

ED / ISS :
1.0

Figure 4-8: Sensor adaptor - grid

4.6.2.Sensor element (Half front-end sensors)

The sensor element is designed with a coaxial symmetry and houses two electrodes. The outer electrode performs the current injection or draining and the inner one performs the voltage measurement. This arrangement is shown in Figure 4-9.

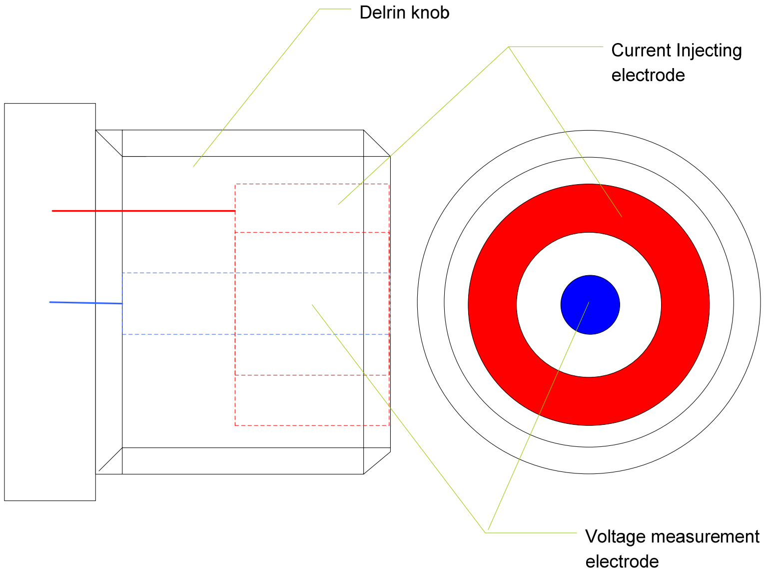

Figure 4-9: Sensor element concept and electrodes mechanical arrangement

4.6.2.1. *Electrical design*

Figure 4-10 shows the electrical block diagram corresponding to the front-end associated to a 4-electrodes sensor. As in this application the sensor element contains two electrodes only, the corresponding front-end must also be halved. Figure 4-11 shows how the half front-ends are derived from the original full front-end. Temperature measurement and differential voltage measurement are implemented in the multiplexer box (see par.4.6.3)

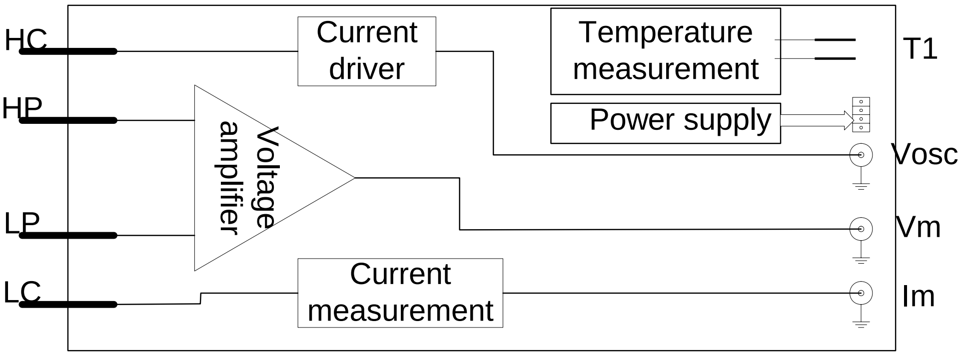

Figure 4-10: four-electrode front-end

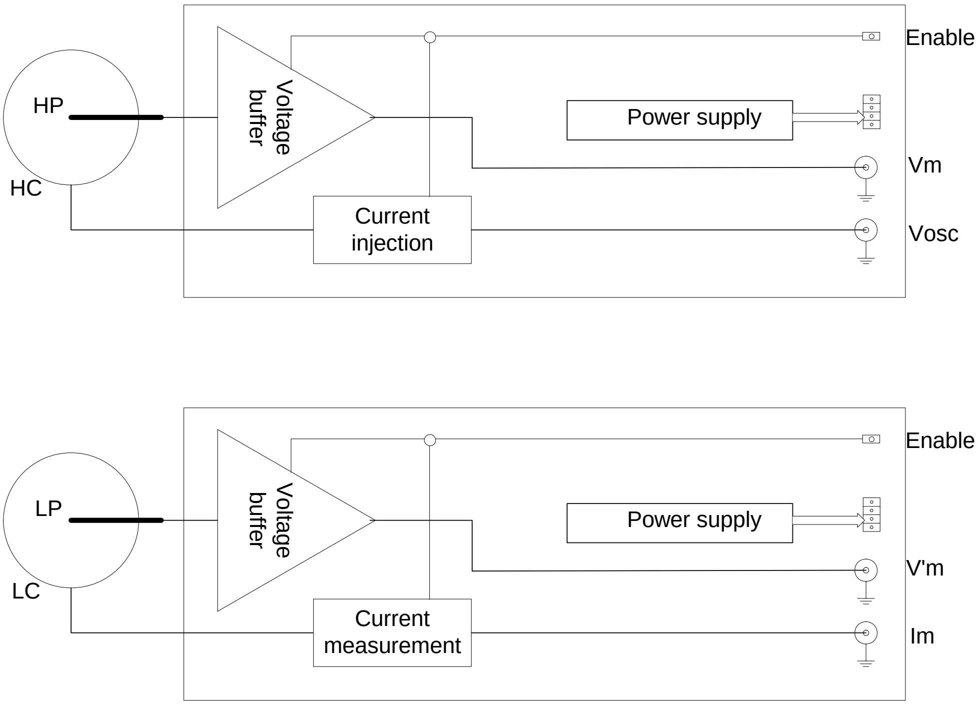

Figure 4-11: Half front-ends. Injecting (upper), A, B, C and detecting (lower), A', B', C'.

The half front-end circuits contain the current driver and measurement electronics, which are connected to the high and low current electrodes (HC and LC). The current driver is excited by the V_{osc} signal, which comes from the signal generator circuit and provides the I_m signal to the acquisition circuit. The voltage buffers provide high input impedance to the LP and HP voltage electrodes, then allowing the use of coaxial cables between front-ends and the system. They also provide AC decoupling to prevent electrode polarization due to the amplifier bias currents. Local power supply stabilization components should also be provided to minimize resistive and inductive modulations induced through connection cables. In order to optimize the signal integrity, the signals V_m, V'_m, I_m and V_{osc} should be conveyed from the front-ends to the mid-front-end connector using coaxial cables.

4.6.2.2. *Mechanical arrangement*

Figure 4-12 shows a section view of the sensor element attached to the interface ring. The container on the left houses the half front-end electronics PCB (not shown in the drawing) and the central structure holds the two coaxial electrodes. Figure 4-13 illustrates the assembly of the sensor element and the support ring.

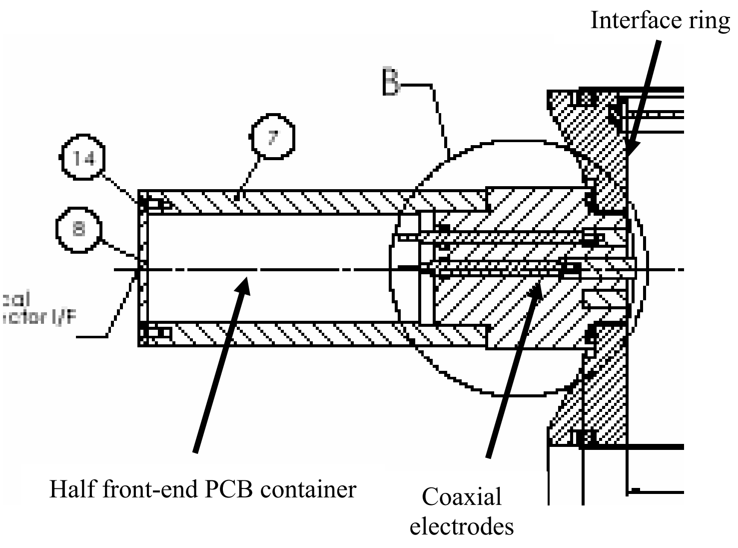

Figure 4-12: Sensor element attached to the interface ring

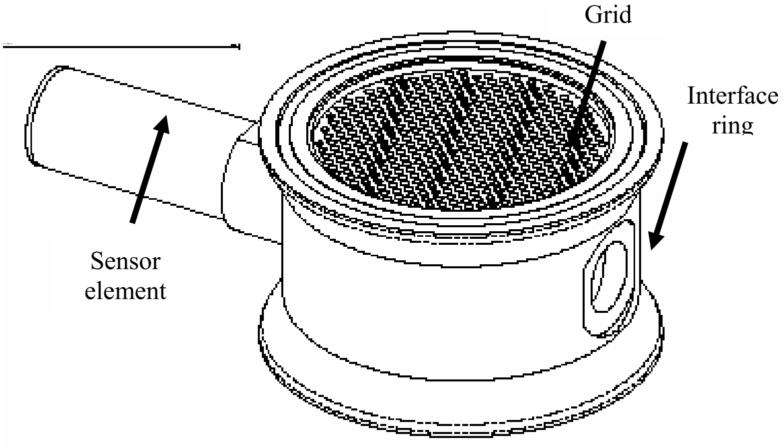

Figure 4-13: Assembly sensor element and support ring – 3D view

4.6.3.Multiplexer box

The two main functions of the multiplexer are to perform the signals multiplexing (i.e. selection of the sensor elements pairs) and to detect the voltage difference between the two selected voltage channels.

4.6.3.1. *Electrical design*

The classical methods to implement the multiplexing function are by using switching matrices or analogue multiplexers. Both of them present two problems:

- The blocks related with electrical current that are not used remain connected if a multiplexer is used. Then, the injected current finds several paths to return to the circuit ground and the measured current shows large errors. The unused blocks should be not only unselected but also switched-off, presenting high impedance to the electrodes.
- The parasitic components of analogue switches induce some noise into the signals which, given the frequency range and low errors required, make them inadequate.

Due to that, the proposed structure for the whole front-end uses very high impedance operational amplifiers with disable function. They should be carefully selected because some of them include these function only for energy saving purposes and their isolation when disabled is not good enough. Several operational amplifiers have been evaluated. The final choice is the Burr-Brown (Texas Instruments) OPA355 family. In order to guarantee their performance, a test board has been built and the voltage and current measurement structures have been tested both enabled and disabled. Thus, using a double amplifier structure, an isolation better than -60 dB at 10 MHz can be ensured with for both voltage and current (Figure 4-14) parameters.

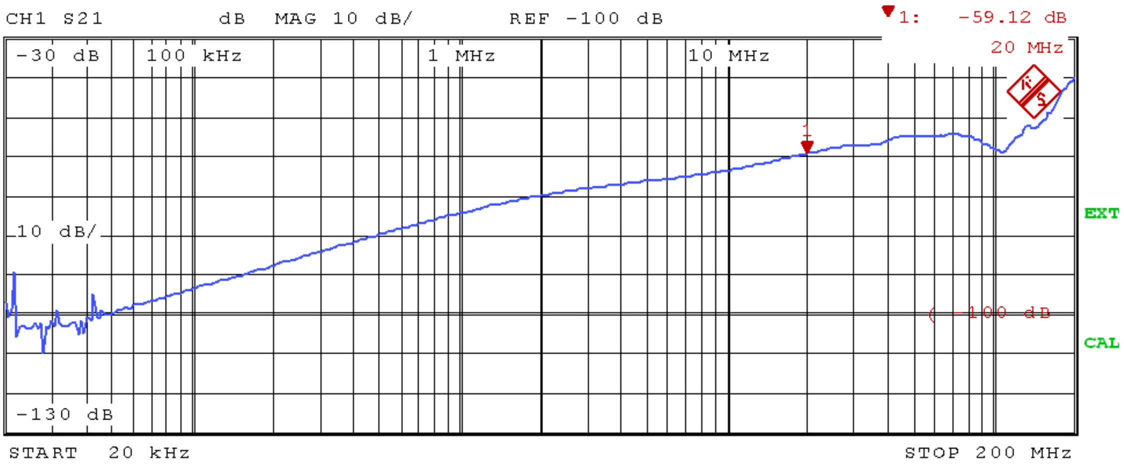

Figure 4-14: Isolation of a disabled current-measurement channel

Then, the three amplifiers present in the three half front-end circuits involved in each measurement (A-B-C or A'-B'-C') can be connected in parallel given that only one of them will be active at a time. The channel selection tree based on paralleled amplifiers has the problem that the output cable capacitances are added. Then, the active amplifier should stand the three cable capacitances. This fact produces high frequency variations of the amplifier's frequency response that would result in small but not negligible errors at the higher frequency end of the measurement range. Because of this, a double amplifier is used for each channel. One of them is placed at the half front-end of the sensor element and the second one is located at the multiplexer box. Both are enabled or disabled simultaneously. Then, the active amplifier should only stand the capacitive load of its own output cable.

The resulting structure of the multiplexing unit is displayed in the Figure 4-15. There are three signal selection trees:

- Voltage measurement (left)
- Signal injection (upper central)
- Current measurement (lower central).

Labels "I_{mx}", where x equals A, B or C of this latter block refer to measured currents but in fact the actual signals are voltages that are proportional to the measured currents. There is a power supply level adaptor due to the different power supply levels employed by the front-end amplifiers (±2.5 V) and the isolated analogue part of the main system (±5 V). There is a control signal-decoding block. The six measurement configurations can be coded with three bits (x, y, z). Then only three control signals should be conveyed from the microcontroller (located at the main system box). Given that the microcontroller is electrically placed in the non-isolated section, a signal isolator should be placed between both systems. The truth table of the decoder is shown in Table 4-1, showing the six non-redundant measurements identified in Figure 4-5. The control words 000 and 111 do not correspond to any valid configuration and can be used to disable all the amplifiers.

Conf.	Meas.	XYZ	Ea	Eb	Ec	Ea'	Eb'	Ec'
1	A-A'	001	1	0	0	1	0	0
2	B-B'	010	0	1	0	0	1	0
3	C-C'	011	0	0	1	0	0	1
4	A-B'	100	1	0	0	0	1	0
5	B-C'	101	0	1	0	0	0	1
6	A-C'	110	1	0	0	0	0	1

Table 4-1: Truth table

4.6.3.2. *Mechanical arrangement*

The multiplexer electronics is housed on a metallic enclosure with IP65 (IEC529) protection level, with rounded edges. It is to be fixed to the reactor’s central interface ring so that the cables connecting this unit to the six sensor elements can be kept as short as possible.

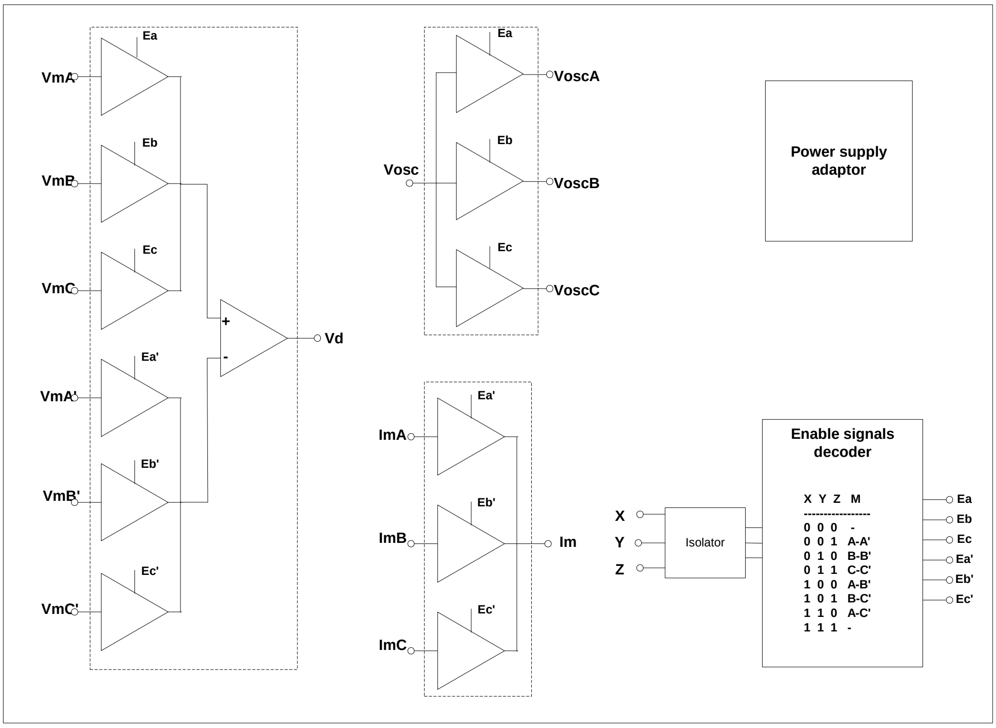

Figure 4-15: Multiplexer block diagram.

4.6.4.Main system box

The main system box contains the core of the measurement system, which includes the microcontroller, power adaptation, and signal interfaces.

4.6.4.1. *Electrical design*

The main system can be split in the non isolated and the isolated parts. The isolation of the measurement part of the circuits due to the need of minimization of the current leakage between the current electrodes and the electrical ground. Given that the bioreactor will have probes and metallic parts connected to ground, the measurement circuit should float respect to this reference voltage. Figure 4-16 shows the block diagram of the main system.

In the non-isolated part, there are the next blocks:

- Power supply circuit
  - 24 V input
  - 5V output for the non isolated digital circuits
  - ±5V with floating ground for the isolated circuits. This supply is generated by an alternative floating system composed by ±7.2 V regulator, a switched

ultracapacitor bank and two 5V linear regulators. Under the microcontroller supervision, the ±5V signal is provided during a time lapse to the isolated part.

- Microcontroller
  - Communications:
    - Ethernet (TCP-IP) to the external computer / network
    - I2C to circuit temperature sensor (T2) and to the remote probe temperature sensor (T1)
    - SPI (isolated through optocouplers) to the measurement circuits.
  - Control of the measurement process:
    - Three bits (x, y, z) that are used to select the active pair of electrodes.

In the isolated part, there are the next blocks:

- Measurement circuits
  - DDS: synthesized signal generator
  - A/D converter
  - Demodulator (detector), It gets the V_m and I_m signals from the mid-front-end and provides two voltages proportional to the amplitude ratio and phase shift between them to the A/D converter for each measurement configuration.
  - The mid-front-end and the 6 ½-front-ends are powered from the isolated part.

4.6.4.2. *Mechanical arrangement*

The measurement system electronics is housed on a metallic enclosure with IP65 (IEC529) protection level and dimensions 300x300x200 mm, with rounded edges.

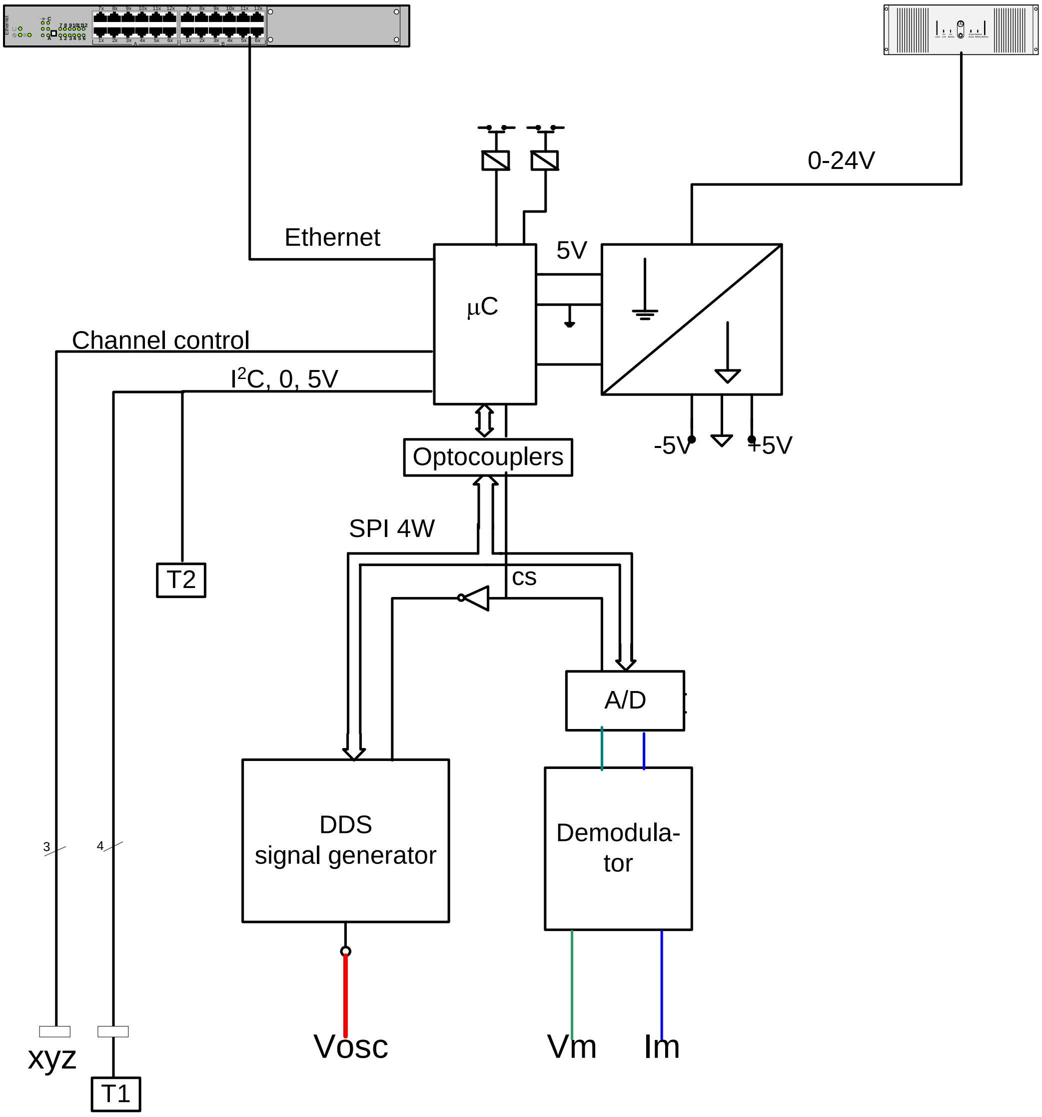

Figure 4-16: Block diagram of the main system

4.6.5.PC

The PC is connected to the main system box through an Ethernet link. A dedicated SW application runs on the PC to control the measurement process and to store the data.

4.7. SW DESIGN

There are two SW modules associated with the CIII biomass sensor, namely:

- CIII_MICRO.c
- CIII_PC.exe

The CIII_MICRO.c module is an embedded SW running in the sensor's microcontroller. This SW reads the commands sent by the PC, manages all the measurement process and sends to the PC the obtained results when they are requested. The user has no access to this SW.

The CIII_PC.exe SW runs on the PC and it is the actual CIII SW manipulated by the user. It controls the system and manages the communication between the sensor and the PC through Ethernet. This program issues commands to measure the medium impedance at the commanded frequencies and channels and receives data packages with the measurement values obtained at these commanded frequencies and channels. One measurement consists of the injection of 32 signals at different frequencies in the range from 1kHz to 10 MHz, logarithmically spaced at 8 frequency points per decade. The lower frequency range has been extended from 10 kHz to 1 kHz to obtain information about the electrode surface impedance. The system returns the measured impedance spectrum samples plus two temperature readings, obtained from two different temperature sensors located at:

- The process zone
- The electronics box volume.

These measurement values are stored in a text file created by the SW.

4.7.1.Microcontroller firmware (CIII_MICRO)

The main features / functions of this SW are:

- Power supply management
  - Initial capacitor charge: 20minutes at below 250 mA
  - Measurement period: isolated position. Time lapse < 60 s for a load current of about 150 mA.
  - Charge position between measurements
- Periodic commanding and acquisition of frequency sweeps:
  - Single configuration measurement period: < 2 minutes
  - 6 configuration measurement period: < 12 minutes
  - Frequency sweep between low frequency (1 kHz to 100 kHz) and high frequency (5 MHz to 10 MHz). Frequency limits TBD from preliminary measurements.

  - 16 or 32 frequency points.
- Multiple measurements per frequency (noise averaging, adaptive)
- Robust acquisition / transmission

4.7.2.PC Software (CIII_PC)

The main features / functions of this SW are listed below:

- Management of frequency sweeps
- Storage of frequency sweeps (raw data)
- Calibration
- Model fitting
- Estimator calculation, conductivity correction
- Graphical representation, statistics
- Storage of processed data
- Possibility to remote access through Internet

4.8. VALIDATION / CALIBRATION

The following steps identify the steps to be performed on the prototype biomass sensor in order to validate it functionally and to calibrate it on the test reactor.

4.8.1.Functional validation

Initial measurements corresponding to a functional validation on a laboratory environment, to be performed with saline solution and yeast suspension. To determine:

- Ze (electrode – ionic solution interface impedance)
- Stability (with what?)
- Temperature dependence
- Appropriate calibration procedure

4.8.2.Bioreactor measurements

Set of measurements to be carried out in the test reactor. The very slow evolution of the biomass following a natural growth makes necessary to perform artificial manipulations to validate the system. The proposed approach includes two phases, the first one to be performed in a short time span (one-two days) and the second one to be performed remotely, leaving the bioreactor in its normal operation mode:

- System characterisation: the test reactor is consecutively filled with:

  - Medium (or saline solution with known conductivity close to the conductivity of the usually employed medium).
  - Medium (or saline solution) and empty beads
  - Medium (or saline solution) plus beads loaded with different biomass densities
    - By mixing different proportions of empty and filled beads
    - By filling the lower part of bioreactor with filled beads and the upper part with empty beads.
- Continuous measurements (normal operation of the bioreactor)

Off-line data processing in all cases

The manipulation of the bioreactor contents in phase one could cause artefacts in the measurements. This is because the test conditions could be altered when emptying or filling the reactor. One possible alternative to avoid this problem could be to fill the bioreactor with biomass-containing beads and performing some measurements for several hours. After that a change in the viable biomass contents can be provoked using a toxic agent (e.g. detergent like Triton X-100) without affecting the medium's conductivity and without modifying the reactor configuration. With this, it could be assured that changes detected by the sensor are only due to changes in the viable biomass contents.

5. FINAL BIOMASS SENSOR: EARLY INTERFACE ISSUES

The final biomass sensor system that could be included in the CIII bioreactor at the MELiSSA Pilot Plant will exhibit some differences with respect to the prototype design. It is expected that the main changes will be in the mechanical design and the selection of materials, whereas ideally the electrical and SW design should remain the same.

As the final biomass sensor system will be fitted into the CIII bioreactor that is currently under definition by the UAB, related interface issues that will have an impact on the final sensor design have been discussed (RD 2).

5.9. MECHANICAL INTERFACE

The sensor element containing the electrodes will to be fitted to the CIII bioreactor's wall through a standard fixed Ingold port, diameter TBC.

5.10. POSITION AND NUMBER OF SENSOR ELEMENTS

Assuming that the CIII bioreactor will have a cylindrical shape then the sensor elements are to be placed on the reactor's wall, in pairs, over planes perpendicular to the reactor's vertical axis, diametrically opposed.

The number of sensor elements is TBD, depending upon the overall reactor's dimensions (namely diameter and height).

5.11. CHOICE OF MATERIALS

The materials of the sensor element that will be in contact with the bioreactor's contents shall be compatible with the biochemical processes that take place in the bioreactor.

In addition, these materials shall withstand the sterilization procedures intended for this bioreactor:

- Sterilization with hot steam: at 121ºC for three hours
- Sterilization with pressurised steam: at 121ºC for less than one hour
- Chemical sterilization: Chlorohydric acid or bleach

5.12. PRESSURE

The mechanical design of the sensor element shall withstand up to 1.2 manometric bars.

5.13. MAINTENANCE

Provided that the sensor element is connected through a fixed interface, i.e. it cannot be removed or retracted while the bioreactor is in operation, the finishing of the electrode's surfaces in contact with the process shall be finely polished in order to avoid disturbing depositions.

Note: one possible procedure to clean the electrodes surface while the bioreactor is in operation is by applying a short electrical DC pulse (e.g. 15 V).

6. PROTOTYPE SYSTEM REQUIREMENTS

The measurement performance drives the specification of the prototype biomass sensor for its application to the test reactor and, eventually, to the MELiSSA's CIII bioreactor.

Due to the characteristics of the immobilised biomass and the fact that the prototype sensor system is to be used on a test reactor the objective is to assess the prototype's performance by obtaining relative indications of the biomass evolution within the reactor rather than an absolute value of the biomass density.

Based on this premise the below performance requirements can be set. These are derived from previous NTE's experience in the measurement of viable immobilised biomass material using EIS technology.

Taking into account the research nature of this application, requirements are worded in the form "should" rather than "shall".

6.14. SYSTEM BANDWIDTH

The system bandwidth should be 1 kHz to 10 MHz

Modulus and phase flatness are not important for this application as associated ripple drives to systematic errors that will be fully corrected by the calibration process.

The gain and phase flatness are not important for this application given that they drive to systematic errors and can be fully corrected by the calibration process. What should be ensured is the time and temperature stability of the frequency response relative variations, that is, the relative magnitude changes between low frequency (1 kHz) and high frequency (10 MHz) should be kept below 0.2%.

6.15. IMPEDANCE MEASUREMENT RANGE

The system should be able to respond properly in front of the impedance modulus under measurement, whose value may range between zero (for short circuit) and infinite (for open circuit).

6.16. ACCURACY

The accuracy in the measurement of relative impedance modulus changes should be better than 0.2%.

Note: the accuracy in the measurement of impedance values is not the main goal.

6.17. RESOLUTION

The system's resolution should permit the detection of

- 0.1% change in relative magnitude
- 0.02º change in phase angle

6.18. RESPONSE TIME

The system should be able to perform 6 frequency sweeps in less than 15 minutes in order to process them and provide a biomass density estimator value.

7. prototype sensor product Tree

Biomel CIII	1000	Support ring	1100	Mechanics	1110	Grid (x2)
					1120	Interface ring (3)
	2000	Sensor element (injector)	2100	Mechanics x3	2110	Electrodes Housing
					2120	PCB container
					2130	PCB container cap
			2200	Electronics x3	2210	Injector half front-end PCB
			2300	Harness x3 (Injector to Multiplexer)		
	3000	Sensor element (detector)	3100	Mechanics x3	3110	Electrodes Housing
					3120	PCB container
					3130	PCB container cap
			3200	Electronics x3	3210	Detector half front-end PCB
			3300	Harness x3 (Detector to Multiplexer)		
	4000	Multiplexer	4100	Mechanics	4110	Container
					4120	Clamps
			4200	Electronics	4210	Multiplexer Board
			4300	Internal Multiplexer Harness		
			4400	External Harness (Multiplexer To Main Box)		
	5000	Main Box	5100	Mechanics	5110	Container
			5200	Electronics	5121	Isolated Board

				5122	*Ultracapacitors Board*
				5123	*Non-isolated board*
				5124	*24 V Power Source*
		5300	*Internal Main Box Harness*		
6000	*Software*	6100	*CIII Micro*		
		6200	*CIII PC*		