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Dectection of microbial concentration in water

The impedance method

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Impurities in drinking water are a major source of concern. These factors that contribute to low drinking water quality can have consequences ranging from minimal to mild to life threatening. Microbial presence in water is one such factor (source) that can become life threatening if not properly dealt with. Therefore detecting not only the presence but of the concentration level of microbes in water is a crucial step in assessing the quality of drinking water.

The proposed detection system for the thesis is based on the impedance microbiology method, a rapid microbiological technique used to measure the microbial concentration of a sample by monitoring the electrical parameters of the growth medium. The prototype operates based on the integrated circuit AD9533 which is interfaced with a microcontroller (32 bit Arduino Due) and a pair of electrodes for detecting changes in the sample under test. Various tests were conducted to obtain a proof of concept. Since acquiring the strain of bacteria that are lengthily discussed in the thesis is challenging for many reason, the proposed measurement system instead attempts to measure the changes in impedance for a group of nitrogen fixing bacteria called Rhizobium, which was acquired from the Helsinki University microbiology laboratory. The results from the measurements were inconclusive about the level of concentration of the bacteria in the contaminated solution due to lack of the appropriate controlled environment but nevertheless provided results that could be attributed to the growth characteristic of the contaminating bacteria though there were also other possible explanations. Moreover, the developed measurement system is capable of assessing the gradual change in impedance that might occur in a solution for a specific range of impedance, frequency and excitation voltage.

Keywords	Impedance microbiology, SUT, I^2C, AD5933 , Detection time, Concentration threshold, CFU , Arduino due

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ISO	ORGANIZATION FOR STANDARDIZATION
ACK	ACKNOWLEDGE
ADC	ANALOG-TO-DIGITAL CONVERTER
CFU	COLONY-FORMING UNITS
DFT	DIGITAL FOURIER TRANSFORM
DDS	DIRECT DIGITAL SYNTHESISER
IDE	INTEGRATED DEVELPOMENT ENVIRONMENT
I^2C	INTER INTEGRATED CIRCUIT
LSB	LIST SIGINIFICANT BIT
MCLK	MASTER CLOCK
MPS	MILLION PER SECOND
MSB	MOST SIGNIFICANT BIT
PC	PERSONAL COMPUTER
PGA	PROGRAMMABLE GAIN AMPLIFIER
SUT	SOLUTION UNDER TEST
SCL	SERIAL CLOCK LINE
SDA	SERIAL DATA LINE
USB	UNIVERSAL SERIAL BUS
WHO	WORLD HEALTH ORGANIZATION
YMB	YEAST-MANNITOL BROTH

1 Introduction

The proposed prototype makes an attempt at measuring the concentration level of bacteria in water using the impedance microbiology method. Microbiological content in drinking water is one of the major reasons that affects the quality of drinking water and if not properly handled can have a serious impact on human health. This thesis focuses on the indicators measured for assessing the quality of drinking water and suggests a system for detecting one out of the many known indicators. Any system therefore that can assess the microbial content of drinking water is capable of having significant impact. Current popular methods that are employed for assessing the microbial content of water rely on experts and take more time to provide findings than the proposed prototype.

Assessing the safety of water is a major concern since inadequate drinking water supply contributes to a high level of preventable mortality. According to the World Health Organization, waterborne diseases outbreaks are a serious health risk. And this is not a problem localized to developing countries but is also a serious concern in the developed part of the world as well. Sterilization is one of the process that drinking water undergoes which allows it to meet the regulations set by international standards regarding the level of contaminants. [1]

The plate count method is one that is usually used to detect the level of microbial concentration in water. This process may take from 24-72 hours and is also dependant on many things like the proper laboratory and expert for proper detection. Moreover, in cases where the contamination happens after the distribution of the water from the main source the plate count method becomes more difficult to apply. A method that takes less time and is more independent in detecting microbial concentration in water is important and contributes towards maintaining human health.[1]

The impedance method is a rapid method that investigates the changes in the electrical parameters of the medium (water) in order to find out the concentration level of microbes. The microbial concentration changes the ionic content of the water and thus altering the conductivity.

The impedance method may take from 3-12 hrs and is a better alternative for carrying out tests on site as it is more independent.

This thesis proposes to employ this method using fairly available electronics components and tools.

2 Theorethical background of water quality and microbial detection

2.1 Water quality

Water is a substance that is crucial for the existence of all forms life that is known. The quality of water is a vast topic that examines the different attributes of water. The chemical, physical, biological and radiological aspects of water are all taken in to consideration when studying that quality of water. The type of assessment of the quality is very much dependent on the intended use (purpose) of water. The parameters considered when examining water intended for human consumption can be very different from another used for water intended for industrial purposes. As a result, water quality standards set are purpose driven. [1]

Water quality standards rely on various sampling and measurement techniques that seek out specific indicators. Different internal organizations have published regulations specific to their role. The International Organization for Standardization (ISO) has published regulations on general water quality and the World Health Organization (WHO) has set out guidelines for the quality of drinking-water. [1]

This thesis focuses on the indicators measured for assessing the quality of drinking water and suggests a system for detecting one out of the many known indicators.

2.2 The quality of drinking water

Water is one of the most important and basic needs for human survival. The average adult man and woman need to drink about three litres and about two and half litres a day respectively. Therefore access to safe drinking water play a major role in maintaining the health of human beings. In order to be deemed safe, drinking-water is expected to meet many guidelines that are set by organization like the WHO. These guidelines are a way

to assure that the water meant for drinking doesn't pose any immediate or gradual serious health risks regardless of the age of the consumer. Waterborne diseases become more adverse to those whose physical condition is weaker than normal. Therefore children and the elderly are the first to bear the consequences of consuming unsafe drinking-water. [1]

The World health organization (WHO) has found that nearly two third of all water related diseases are a result of diarrhoea, which is a result of a microbiologically contaminated water consumption. Such a phenomenon is more prevalent in the developing part of the world since access to safe drinking water is highly correlated with the level of infrastructure that a country has in order to assist the availability of this basic need. Though the consequences are not as catastrophic, such problems are also a source of concern for the developed part of the world. This part of the world is mainly preoccupied with impurities in drinking water that result from industrial contaminants. From the perspective of human health, the quality of drinking water can be examined from the following four major aspects.[1]

2.2.1 Chemical aspects

One of the major sources for the decline in the quality of water is one that results from chemicals. These chemicals can come from varied sources. Not all the chemicals occurring in drinking water affect human health and the ones that do range in their potency. Manufacturing plants of many kinds that don't have a proper disposing system for the residues of the productions process will contribute to the pollution of the environment. Sources of drinking water might get affected as a result and depending on the type of chemical exposure the effects on health might be different in the level of adversity. Some contaminants (chemicals) can have potent and immediate damaging capabilities while others might take a prolonged exposure to take effect. Chemical contaminants might require costly clean up efforts to have the drinking water meet the standards set. Therefore it is important to find a solution that eradicates the problem at the source. [1]

2.2.2 Radiological aspects

Radioactive substances that are found in drinking water can pose a serious risk to human health. The degree of risk is tied to the level of radiation found in the water. Though the contamination from radioactive substances is not as frequent as the once resulting from

microorganisms, the necessary measures must be taken in order to keep this from happening. Once a radiation contamination occurs getting the contaminant out of the water is a hefty and complicated task. That's why it is important to critically employ all of the safety measures when working with radioactive substances.[1]

2.2.3 Acceptability aspects: taste, odour and appearance

Human beings rely on their senses to assess the quality of water they consume. The appearance, taste and odour can provide relevant information about the chemical and microbial constituents of water. Not all of these impurities can necessarily affect human health, nevertheless it should be dealt with because odour, taste and appearance are by themselves important factors for water quality. Also important to note is that not every contaminant has easily obvious attributes that can be picked up by human senses. In fact an aesthetically pleasing water can lead one to make unwarranted conclusions about its safety aspect and consequently be regarded better than an unpleasant looking but otherwise safe to drink water.[1]

2.2.4 Microbial aspects

Microbial contamination of water can have serious and catastrophic consequences. The contamination can be a result of pathogenic protozoa, viruses and bacteria. Human and animal faeces serves as a source of these microbes and become a problem when they contaminate drinking water. It is therefore why pathogen that come from faeces are considered a major factor in assessing the microbial safety of drinking water and setting proper guidelines so that contamination doesn't not take place. These pathogens are called faecal indicators. "Faecal indicator bacteria are organisms such as total coliforms, faecal coliforms, and indicator *E. coli*. Indicator *E. coli* are a type of faecal coliforms and both are a type of total coliforms." [1]

The presence of faecal indicators can vary rapidly and at times when the concentration level is above the standards set by the sectors that regulate water quality waterborne disease outbreaks may be triggered. Therefore detecting the presence and concentration of these indicators can make a huge difference. "Total Coliforms (including faecal coliform and *E. coli*) should not be above 5% mg/L and there should be no detectable

amount in a 100 ml sample.” [1] Current methods that have been used traditionally are time consuming and the information acquired from using them might not as consequential as many people might have already been exposed to the contaminated water. This is adequate reason for why faster and better methods of detection should be sought.

2.3 Detection of microbial concentraion

As discussed in the previous subtopic, detecting the microbial concentration in water is crucial as it provides necessary information regarding the quality if drinking-water. The traditional methods used for detecting concentration levels of bacteria can vary in their degree of accuracy. This inaccuracy can result from the characteristic and age of the sampled water. Another source for this inaccuracy is the type of growth medium and the state of the environment in which concentration detection tests are taking place. The count and type of species detected are influenced by these factors.

“The detection of pathogenic microbes in water typically involves concentration, enrichment, detection and quantification. Since in many cases the amount of microbes is very low in drinking water, the detection requires some enrichment.” [3] Standardisation of the procedures used is important so that the findings can lead to the same conclusion about the water quality wherever the experiments are being carried out. There are numerous methods that are used in detecting the presence and concentration of microbes. The following are two methods that relate most to this project.

2.3.1 Standard plate count method

This method is widely used around the world and relies on the flourishing of bacteria in water to the extent it becomes visible to the naked eye and the colonies can be counted. “Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term “colony-forming units” (CFU). The final count also depends on the interaction among the developing colonies.’’ [4] There are different kinds of plate count methods and each may employ a media that is suitable from the different types available.

The sample under test is diluted using a chosen media (nutrients for the bacteria). The dilutions are done in a series of 1:10, 1:100, 1:1000 etc. It is important that the sample in a series is diluted in a way that would allow for colonies to form with in a range of 30

to 300. The diluted sample then is sealed and incubated to let the bacteria flourish. The types of dilution medium i.e. agar used influences the incubation temperature and time. The agar is made out of nutrients that serve as nourishment for the target bacteria and discriminate against other organisms that might be present in the sample water. The constituting nutrients allow the target organisms to be easily visible, perhaps by a change in the colour of the agar. After the allotted time in the incubation chamber the colonies visible to naked eye are counted. Such a procedure requires a trained eye, perhaps a laboratory technician in order to have better accuracy. [4]

2.3.2 Defined substrate technology

The Defined substrate technology relies on the production of Beta-glucuronidase (enzymes that breakdown carbohydrate) from the metabolic activity of the coliforms. This method requires a bit less time than the standard plate count method for identifying coliforms and is done in a laboratory setting. A dehydrated medium and the water sample are placed in well that is 100 ml. After an incubation of 18-22 hours at 37 °C (degree Celsius) the wells that exhibit a yellow color or fluorescent yellow are said to have microbial presence and the concentration level is deduced by statistical tables. Instruments that use the defined substrate technology are made by IDEXX, a company that works on instruments for innovative diagnostics.[2]

2.4 Impedance microbiology

Impedance microbiology is a much more efficient way of determining the amount of microbial concentration in a solution. Microorganisms exhibit certain electric properties when they exist in a solution and the impedance microbiology method examines these properties and their changes in order to draw conclusions about the microbial content of the solution via a pair of electrodes as shown in figure 1. [5]

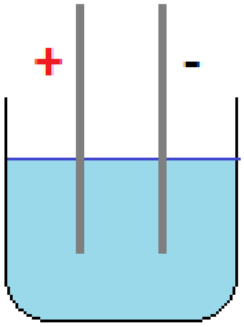

Figure 1. Electrodes immersed in a medium

If a pair of electrodes are introduce to a solution, at the contact point of the solution and the electrode, a dc (direct current) boundary will be formed. To adjust the polarization that occurs between the electrodes and the solution an AC (alternating current) is applied. This adjustment creates a capacitance polarization effect (C pol) or captive double layer(C dl) as in Figure 2, at the interface of the solution and the electrode.

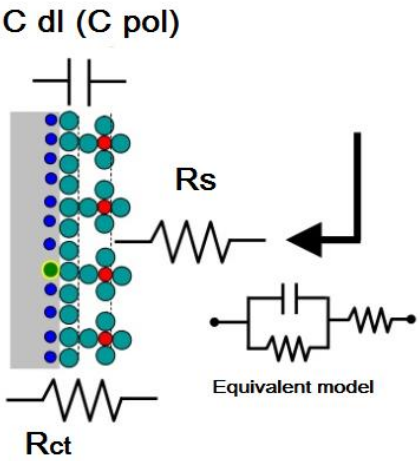

Figure 2. Equivalent electrical circuit to model an electrode in direct contact with a solution[6]

As found by Warburg (1899, 1901), when a pair of electrodes are introduced in to a solution the resulting system can be equivalently modelled as either a capacitor Cs (total capacitance of the electrode electrolyte interface) and a resistor Rs (total resistance of the solution) in series as in Figure 3 and Figure 4, or a resistor (Rct) and capacitor (Cdl) in parallel forming a series arrangement with the resistance of the solution, Rs as in Figure 2. Depending on the level of frequency and ac (alternating current) applied, the

electrode electrolyte interface can be thought of as a series combination of the capacitance and the conductance (resistance) of the electrode, which is the model that will be used for the proposed system in this thesis. For low level frequencies (less than 1 MHz) the capacitance of the solution can be neglected and the equivalent model of the system will be as shown in Figure 4. [5]

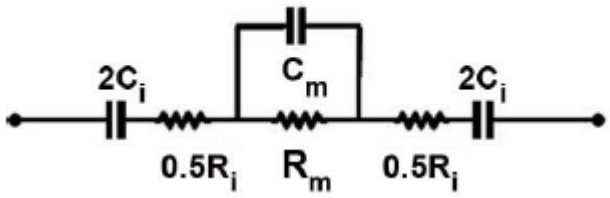

Figure 3. Electrical model for the system composed of a couple of electrodes immersed in a liquid medium [7]

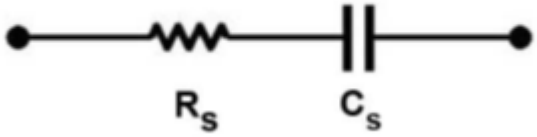

Figure 4.Simplified model when a test signal of relatively low-frequency of 1 MHz is applied [7]

The impedance to the current applied to the solution might have different source within the system. When the solution is excited by higher frequencies of alternating current the impedance that results is largely attributed to the resistance of the solution (R_S), but at lower frequencies the resistances to the electron flow that exists / R_{ct} at the electrode interface dominates the total impedance. [7]

Impedance Microbiology analysis

1. The SUT (solution under test) is maintained in temperature range that is between 37 to 42 °C, which is a range that is suitable for the type of bacteria the proposed project is interested in. The components of the impedance Z_S, X_S and R_S are measured every couple of minutes via a pair of electrodes submerged in the solution. The reactive and resistive components of the impedance vs time is

plotted to illustrate the change that occurs when the concentration level varies significantly. [2]

2. The electrode electrolyte system stabilizes and a steady state of electrical parameters of the solution is observed. When the bacterial concentration level reaches a threshold (C_{TH}) of 10^7 cfu/ml (colony forming units), the impedance level starts to deviate from the baseline value. The microbial concentration level that results in impedance change primarily depends on the species of bacteria and the SUT (solution under test). [2]

3. Detection time (DT) is the time it takes for Zs (impedance of the solution under test) to decrease significantly, and it is used to calculate C_O, the initial bacterial concentration level. [2]

As bacteria propagation is determinable, the time required to reach the threshold concentration level C_{TH} is informative of the initial bacterial concentration level. The amount of time that it takes to reach the critical threshold level is the DT or detection time. Detection time is dependent on the initial concentration level. The higher the initial concentration level the less time it will take to reach the threshold concentration level and therefore lowering the detection time DT. The opposite is true for low levels of initial concentration as referenced in Figure 5.

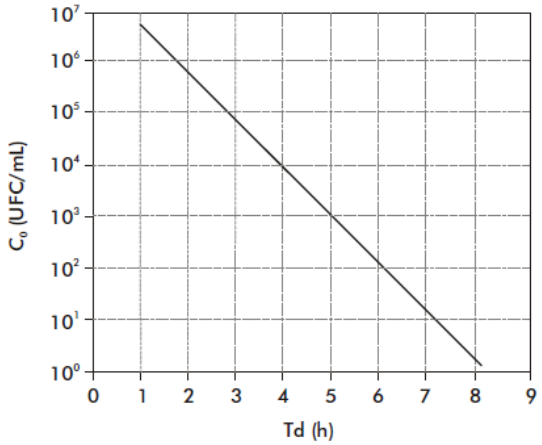

Figure 5. Relationship between detection time (DT) and initial cell concentration (C_O) [8]

It is important to examine why the change in electrical parameters (decrease in impedance) takes place when the microbial concentrating in the solution increase. To do this, understanding the nature of bacterial growth in as solution as a function of time is necessary. As seen in Figure 6, at the initial phase of the growth the bacteria experiences no change in growth and then is followed by fast growth and then a steady state. The first two phases are called the “lag phase” (which is where the bacteria cells get used to the solution media and metabolizes without binary fission) and the “logarithmic phase”(when the adapted cells start to propagate via binary fission at a constant speedy rate) respectively. The last phase is characterized by the cooling of the logarithmic phase as a result of the depreciation of the nutrients in the solution.
To obtain the bacterial concentration level during the lag phase C_B, the following is applied since there is no growth.[E] The initial concentration level is C_{BO} and t_l

$$C_B(t) = C_O \qquad where\ t \leq t_l \qquad (1)$$

During the logarithmic phase the bacterial concentration increases as a result of propagation by binary fission, a biological process whereby every cell divides to become two. Generation time is known as the duration of the division. To obtain the bacterial concentration level after n generations the C_O (initial concentration level) is taken into consideration. [5] Therefore the C_B level is shown in the equation below as follows

$$C_B = C_O * 2^n \qquad (2)$$

The generation number n can be calculated by equation (3).

$$n = (ln\ C_B - ln\ C_O) / ln\ 2 \qquad (3)$$

If t_g is taken to be the generation time then it follows that

$$n = (t - t_l) / t_g$$

$$t - t_l / t_g = ln\ C_B - ln\ C_O / ln\ 2$$

$$C_B(t) = C_O\ e^{(t - t_l) \ln 2 / t_g} \qquad where\ t \geq tl \qquad (4)$$

Where C_O is the concentration level when $t = t_l$.

The duration of the lag phase, t_l and the generation time t_g can be calculated from measuring the electrical characteristics of the SUT (solution under test). From various literature it is understood that DT (detection time) is the linear function of the logarithm of C_O, which is considered as the unknown initial level of concentration [5]. And so it follows that

$$DT = A * log(C_O) + B \quad (5)$$

$$C_{TH} = C_O * 2^{(DT + T - t_l) / t_g} \quad then \quad (6)$$

$$DT = log_2 (C_{TH} / C_O)\, t_g - (T - t_l)$$

Where C_{TH}, is concentration threshold when the impedance of the solution starts to change and T is the time it takes for the electrode electrolyte system to stabilize then equation (6) follows

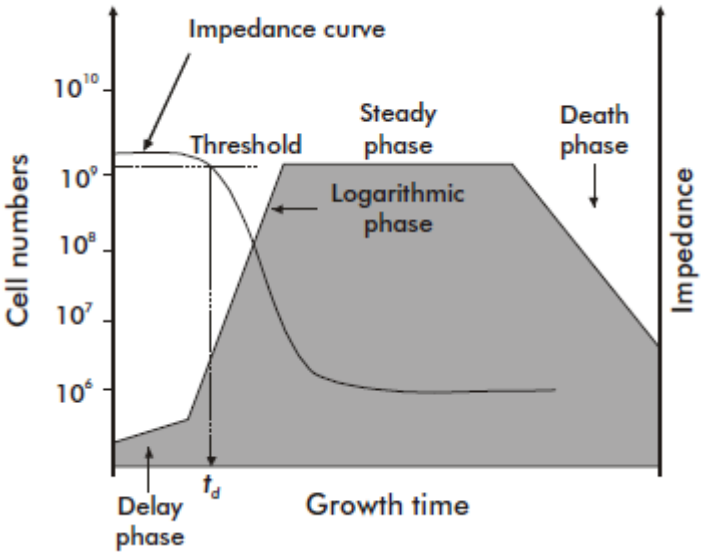

Figure 6. Impedance growth curve with the threshold and detection time together with the two typical phases of bacterial growth [8]

The increase in the impedance of the SUT (solution under test) is a result of the increase in ions. When binary fission taken place, neutral and weakly changed compounds of the solution become strongly charged compounds. The charged compounds then proceed to alter the conductivity of the solution.[8]

Bacteria in the solution release ions during the logarithm phase. This happens by either of these two mechanisms, energy metabolism (catabolism) or active transportation (ion exchange through cell membrane). During catabolism the oxygen and carbohydrate that is absorbed by the bacteria is processed to become carbon dioxide and organic acids as can be seen from the chemical reaction in equation 7. The solution exhibits an increase in conductivity when non ionized glucose substrate is converted to two lactic acid molecules. The bacterial metabolism will then go on to produce carbonic acid by combining lactic acid with oxygen molecules. Lactic ions are not as good a conductor as the small mobile bicarbonate ions and hydrogen ions are more effective ionic conductors than sodium ions. All of these chemical reaction help the rise of conductivity of the solution. [8]

$$C_6H_{12}O_6 + 6O_2 ------> 6CO_2 + 6H_2O + 38\,ATP \qquad (7)$$

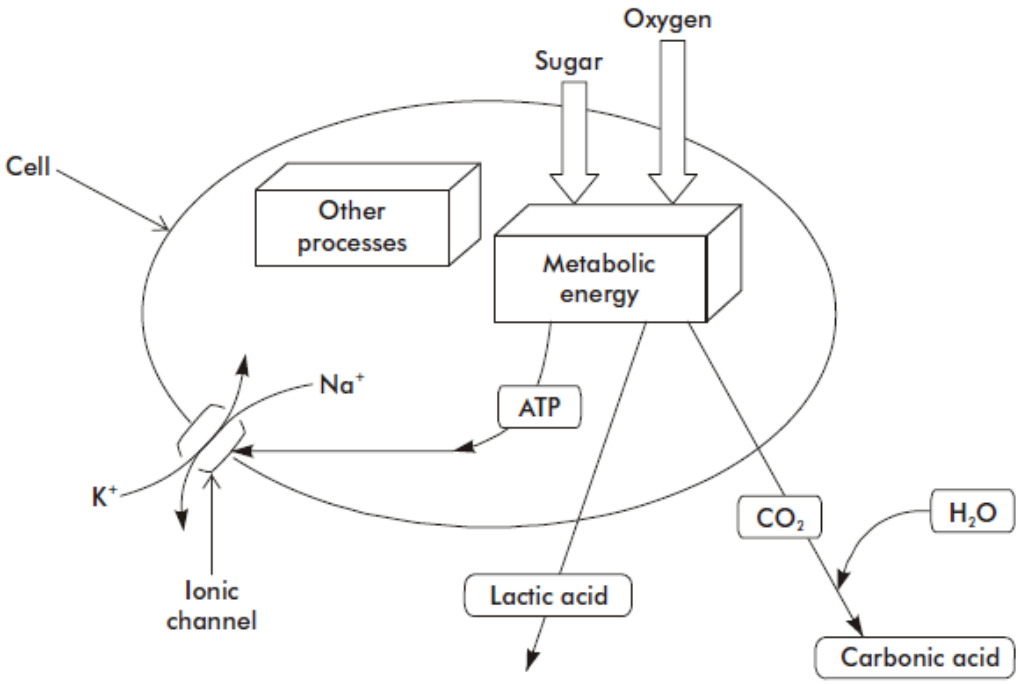

Figure 7. Diagram representing the two main mechanisms responsible for the release of ionic species by living cells: energy metabolism (catabolism) and ion exchange through the cell membrane. [8]

Though catabolism is the dominant contributor to the change in the electrical conductivity of the solution under test, active transportation also plays some role as is demonstrated in the Figure 7. During active transportation sodium and potassium ions are transported via ionic channels of the cell membrane which is known as the double lipid layer. The double lipid layer's role is to control the membrane potential and regulate the osmotic pressure between the exterior and interior parts of the cell.

3 Method and materials

3.1 The proposed system

In the previous chapter, different ways of detecting concentration level of microbes in solution were discussed. The current methodologies used for detection have serious drawbacks, detection time being of primary concern. The plate count method is one that is usually used to detect the level of microbial concentration in water. This process may take from 24-72 hours and is also dependent on many things like the proper laboratory and expert for reliable detection.

The impedance method may take from 3-12 hrs(which is significantly faster) and is a better alternative for carrying out tests on site as it is more independent and less costly as compared to other popular methods.

The proposed system is a detection mechanism consisting of a pair of electrodes(to be immersed in the solution under test) connected to AD5933 (an impedance analyser) integrated circuit, a microcontroller (Arduino Due that has a 32 bit processor) that communicates with the integrated circuit and a PC communicating with the microcontroller via an IDE (Integrated Development Environment) .

The SUT is excited via a pair of electrodes with a sinusoidal voltage from the AD5933 circuit at a magnitude of 200 mV peak-to-peak and at a frequency of 5 kHz. At test signal frequencies lower than 1 MHz, the electrodes electrolyte system can be modelled as the

series of a resistance R_s (accounting for the resistance of both the sample and the electrode-electrolyte interface) and a capacitance C*s* (related to the formation of a double layer region at the electrode electrolyte interface). AD5933 is an integrated circuit that has a built in programmable waveform generator that excites the SUT with an AC signal. It also has a built in ADC (analog -to-digital- converter) that converts the AC current (signal) that is generated as a result of the excitation. The converted signal is then sent to the microcontroller via an I^2C interface with the AD9533 as shown in figure 8. The program developed on the IDE of the microcontroller will give the measured impedance of the SUT and if several standard solutions are used to calibrate properly then the developed software can assess the approximate concentration level of microbes in the SUT.

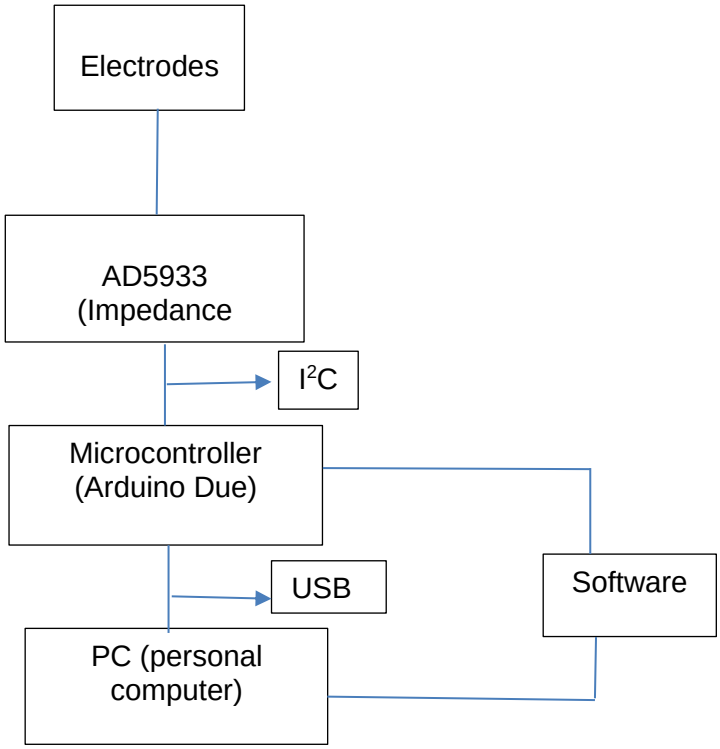

Figure 8. Communication scheme of the proposed system

3.2 The AD5933 integrated circuits

"The AD5933 is a high precision impedance converter system solution that combines an on-board frequency generator with a 12-bit, 1 MSPS, analog-to-digital converter (ADC)." [I]. It comprises of a programmable output peak-to-peak excitation voltage to a maximum

frequency of 100 kHz, a programmable frequency sweep capability with serial I^2C interface, a frequency resolution of 27 bits, impedance measurement range from 1 kΩ to 10 MΩ, an internal system clock of 16 MHz, a phase measurement capability system with an accuracy of 0.5% and a 2.7 V to 5.5 V power supply operation.

A known (programmable) frequency from the generator excites the unknown complex impedance. The built in ADC (Analog to digital converter) and DFT(Digital Fourier transform) samples the signal from the unknown impedance resulting from the excitation voltage. The converted signal is then processed by the on-board DSP (Digital Signal Processor). The magnitude and phase of the unknown impedance are therefore calculated from equation 8 and 9 respectively because R (real) and I(imaginary) data are the components of the unknown impedance. [9]

$$Magnitude = \sqrt{R^2 + I^2} \tag{8}$$

$$Phase = \tan^{-1}(\frac{I}{R}) \tag{9}$$

The AD5933 allows a frequency sweep, this aspect of the integrated circuit is not used because the experiment undertaken is interested in zeroing in on the change in impedance that occurs at one specific frequency and amplitude.

The amplitude (peak-to-peak) value of the output excitation signal can be programmed. There are four possible values, shown in the table 1, that are ratio metric with the supply voltage that powers the integrated circuit.

Table 1. Possible output voltage levels [9]

Range	Output excitation voltage amplitude
1	1.9 V p-p
2	0.97 V p-p
3	383 mV p-p
4	198 mV p-p

The chip has a transmit stage for providing an excitation signal, a receive stage for receiving the current response from the unknown impedance and a DFT for processing the response current as shown in figure 9.

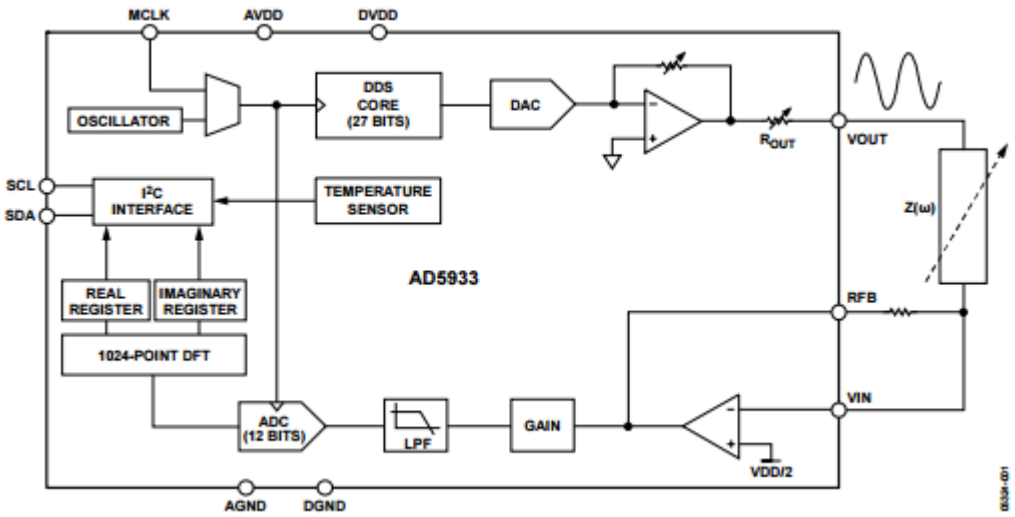

Figure 9. The Functional Block Diagram of AD5933 [9]

The transmit stage consists of a DDS (Direct Digital Synthesiser) core of a 27 bit phase accumulator which is responsible for the output excitation voltage and frequency as shown in the figure 10. The programmable start frequency register serves as an input to the accumulator. The digital signal from the accumulator is then converted to a sinusoidal excitation voltage. The peak-to-peak value of the voltage signal is programmed via the programmable gain.

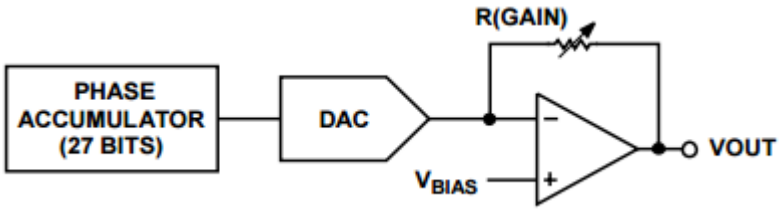

Figure 10. Transmit stage [9]

The receive stage as shown in figure 11 processes the response current of the unknown impedance (SUT). The current serves as an input to the current voltage amplifier of the receive stage. The gain of the amplifier depends on the value of the feedback resistor that is connected between V_{in} and R_{FB} pins of the chip. The chosen resistance value should be one that maintains the linear range of the ADC, which is from 0 V to

the V_{DD} (supply voltage) in accordance with the PGA (Programmable Gain Amplifier) gain.

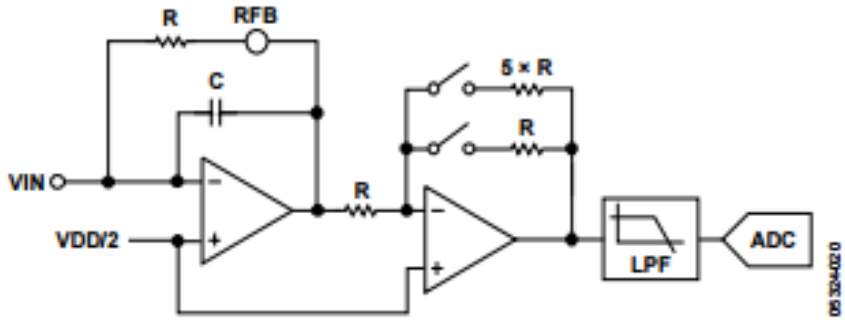

Figure 11. Receive stage [9]

The amplified signal is then passed through a PGA that has a gain option of either 1x or 5x. Then the signal is filtered via the low pass filter and sent to the ADC (12 bit, 1 MPS). The converted digital signal goes under a DFT by using 1024 samples for a specific frequency and employing equation 10.

$$X(f) = \sum_{n=0}^{1023}(x(n)\big(\cos(n) - jsin(n)\big)) \quad (10)$$

X(f) is the power in the signal at the frequency point f, x(n) is the ADC output, cos(n) and sin(n) are the sampled test vectors provided by the DDS core at the frequency point f.

Finally, the resulting data from the operation is stored in a two 16 bit-register, for the real(R) part and imaginary (I) part. The impedance of the SUT is calculated from these values by comparing the outstanding result with an already determined resistance value. The system clock for the chip can either be provided from an external oscillator connected to the clock pin (MCLK) or from the internal clock of the on-board oscillator with a typical frequency of 16.776 MHz. [9]

3.3 The microcontroller (Arduino Due)

The Arduino Due microcontroller is selected for the proposed system. As shown in figure 12, it has a 32 bit Atmel SAM3X8E ARM Cortex-M3 CPU. As shown in table 2, it has 54 digital input/output pins, 12 analog inputs, 4 UARTs , an 84 MHz clock, a USB OTG

capable connection, 2 DAC , 2 TWI(SDA and SCL), a power jack(which runs at 3.3 V), an SPI header and a JTAG header. The TWI consists of the SDA (serial data line) and SCL (serial clock line) pins used for the I^2C interface with the impedance analyser chip (AD5933). [10]

Table 2. AVR Arduino microcontroller specifications [10]

Microcontroller	AT91SAM3X8E
Operating Voltage	3.3V
Input Voltage (recommended)	7-12V
Input Voltage (limits)	6-16V
Digital I/O Pins	54 (of which 12 provide PWM output)
Analog Input Pins	12
Analog Output Pins	2 (DAC)
Total DC Output Current on all I/O lines	130 mA
DC Current for 3.3V Pin	800 mA
DC Current for 5V Pin	800 mA
Flash Memory	512 KB all available for the user applications
SRAM	96 KB (two banks: 64KB and 32KB)
Clock Speed	84 MHz
Length	101.52 mm
Width	53.3 mm
Weight	36 g

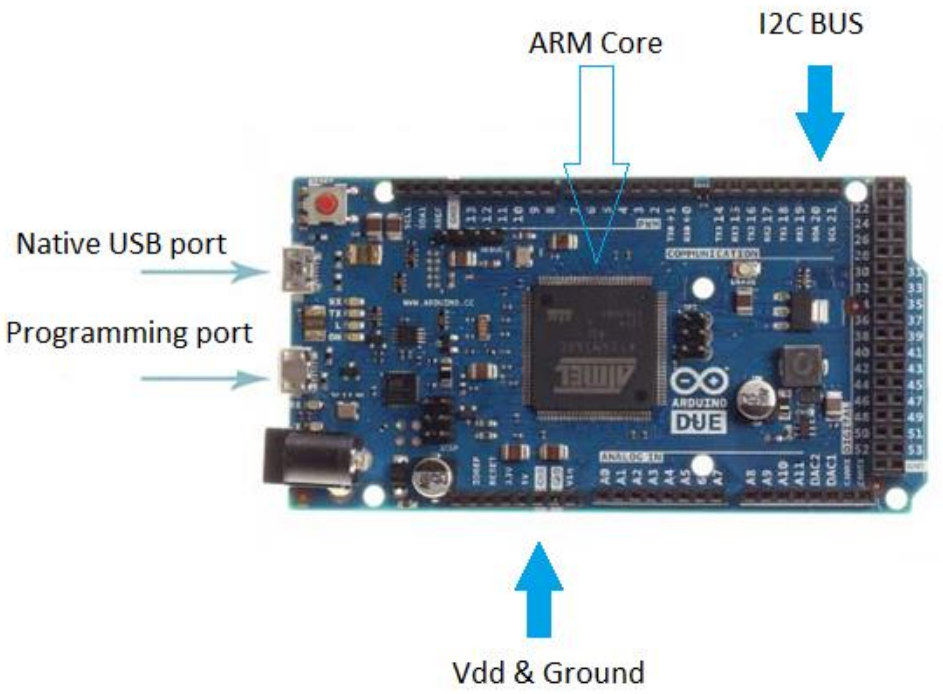

Figure 12. Arduino Due [J]

3.4 The I²C (Inter Integrated Circuit) interface

The I²C mode of communication allows one or multiple master devices to communicate with multiple slave devices as shown in figure 13. For the proposed system, the impedance analyser chip (AD5933) serves as the slave while the Arduino due microcontroller is the master device. The microcontroller can program the slave chip via I²C letting the transmit stage generate an excitation voltage of 200 mV at 5 KHz. The resulting current from the SUT is then picked up by the receive stage and sent to the microcontroller using I²C for further processing on the PC. [11]

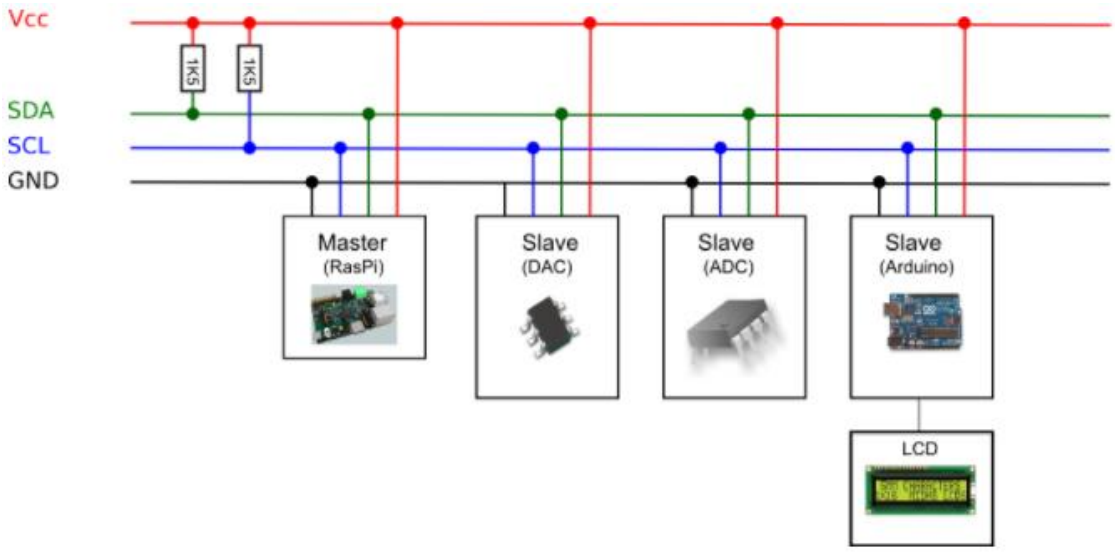

Figure 13. Operation of I²C [11]

The master device uses 8 bits to communicate with the slave device. The 7 MSBs (Most Significant Bit) is the specific address of the slave device (0x0D) and, the LSB (Least Significant Bit) is a write/read bit. The write/read bit is 1 for a read function and 0 for a write function. Next, the slave device sends an ACK (acknowledge) bit to relay that the address has been received as illustrated in the timing diagram in figure 14. If the write/read bit is 0, then a writing process is started by sending one or several bytes to a specified register location in the slave device, or if it is 1 a reading process is started by reading one or more bytes from a specified register location in the slave device. After the transmission is completed, the master generates a stop condition on the bus which then ends the transmission between the master and the current slave.

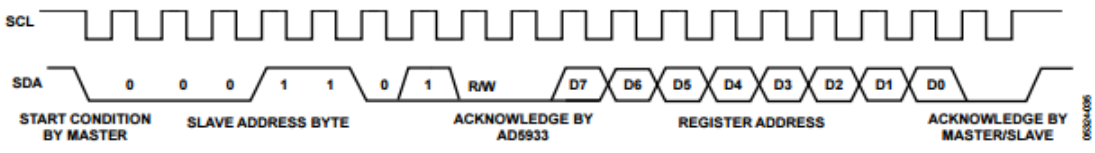

Figure 14. Timing diagram of I²C for AD5933 [9]

3.5 Software for operation

The microcontroller used for the proposed system functions via an Arduino integrated development system (IDE).The IDE comes with built-in libraries. The developed software allows the impedance analyser chip, AD5933 to excite the sample under test with a 200 mV at 5 kHz via a pair of electrodes. After enough time has passed to allow the electrode electrolysis system to stabilize the impedance analyser chip will measure the impedance (Z_T) of the solution under test and send the data to the microcontroller through an I^2C bus. The Arduino language is merely a set of C/C++ functions.

It is necessary to include the <Wire.h> header file in the written program. The wire library allows the microcontroller to communicate with the slave device (AD5933) via I2C. The data sheet of the slave device provides multiple register map as shown in table 3. The transmit and receive stage are configured by programming the relevant registers to get the required outcome.

Table 3. Register map of AD5933 [9]

Register	Name	Register Data	Function
0x80	Control	D15 to D8	Read/write
0x81		D7 to D0	Read/write
0x82	Start frequency	D23 to D16	Read/write
0x83		D15 to D8	Read/write
0x84		D7 to D0	Read/write
0x85	Frequency increment	D23 to D16	Read/write
0x86		D15 to D8	Read/write
0x87		D7 to D0	Read/write
0x88	Number of increments	D15 to D8	Read/write
0x89		D7 to D0	Read/write
0x8A	Number of settling time cycles	D15 to D8	Read/write
0x8B		D7 to D0	Read/write
0x8F	Status	D7 to D0	Read only
0x92	Temperature data	D15 to D8	Read only
0x93		D7 to D0	Read only
0x94	Real data	D15 to D8	Read only
0x95		D7 to D0	Read only
0x96	Imaginary data	D15 to D8	Read only
0x97		D7 to D0	Read only

Registers 0x92 and 0x93 are not used because the internal temperature of the chip doesn't provide relevant information for the proposed system. The flow chart in figure 15 clearly illustrates how the developed program functions.

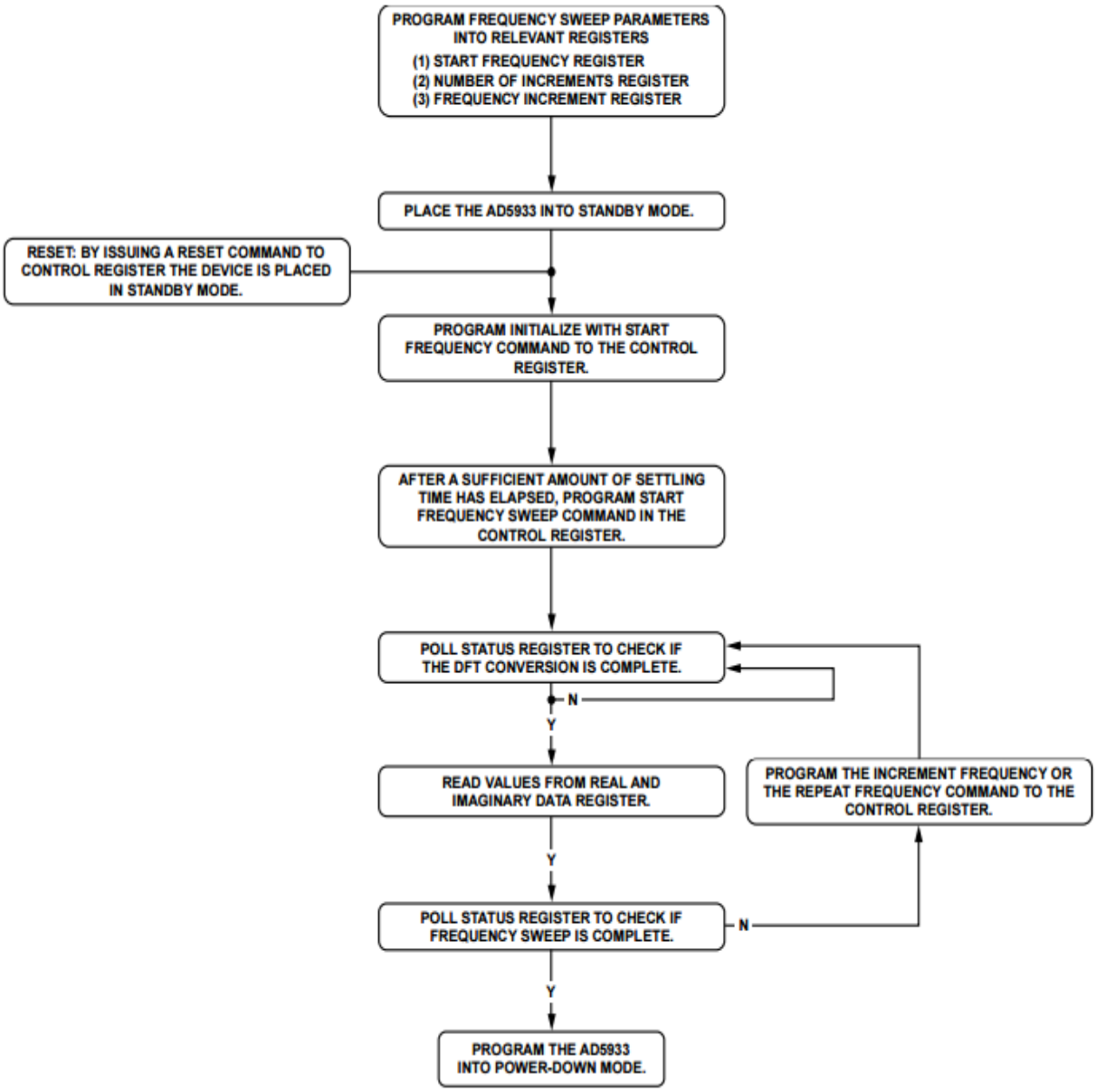

Figure 15. Frequency sweep flowchart [9]

The control register (0x80 and 0x81) have different coding configurations that allow variety of modes as shown in table 4 and 5.

Table 4. Control register (4 MSBs of 0x80) map [9]

D15	D14	D13	D12	Function
0	0	0	0	No operation
0	0	0	1	Initialize with start frequency
0	0	1	0	Start frequency sweep
0	0	1	1	Increment frequency
0	1	0	0	Repeat frequency
1	0	0	0	No operation
1	0	0	1	Measure temperature
1	0	1	0	Power-down mode
1	0	1	1	Standby mode
1	1	0	0	No operation
1	1	0	1	No operation

Table 5. Control register (0x80) map [9]

D10	D9	Range No.	Output Voltage Range
0	0	1	2.0 V p-p typical
0	1	4	200 mV p-p typical
1	0	3	400 mV p-p typical
1	1	2	1.0 V p-p typical

When transmission from the master device to the chip via I^2C begins, the 4 MSB of register 0x80 are programmed as shown in figure 16 and as presented in appendix I. CTRL_REG1 is declared in the code as register 0x80 and writeData is made to be a function that writes on the specified register of the slave device. When the 4 MSBs of the register are 0, the slave device is in a no operation mode. CTRL_REG2 is declared in the code as register 0x81. As shown in table 6, bit D4 must be set in order to put the chip in a reset mode. The last line of the set up code (shown in figure 16) calls programReg function , which sets the PGA gain(set bit D8 for a gain of 1) , programs the settling cycle , programs a start frequency of 5 KHz, writes the increment frequency of 0 (the measurement is done for only 5 KHz) and programs the numbers of frequency sweep.

Equation 10 is used for calculating the start frequency code as recommended by the data sheet of the slave device. Since the start frequency is 5 kHz , using the 16.0 MHz internal clock of the slave it follows that software should program that the value of 0x02 to Register Address 0x82, the value of 0x8F to Register Address 0x83, and the value of 0x5C to Register Address 0x84. This ensures the output frequency starts at 5 kHz. And for a 0 Hz increment step using a 16.0 MHz clock, the value of 0x00 to Register Address 0x85 , the value of 0x00 to Register Address 0x86, and the value of 0x00 to Register Address 0x87 is programmed as shown in equation 11.

$$Start\ frequency\ code = \left(5\frac{KHz}{\left(16\frac{MHz}{4}\right)}\right) * 2^{27} = 028F5C \quad (10)$$

$$Frequency\ increament\ code = \left(0\frac{Hz}{\left(16\frac{MHz}{4}\right)}\right) * 2^{27} = 000000 \quad (11)$$

```
void setup() {
  Wire.begin();
  Serial.begin(9600);

  writeData(CTRL_REG1,0x0); //NO OPERATION

  writeData(CTRL_REG2,0x10);// RESET MODE(D4 IS SET)

  programReg();
}
```

Figure. 16 Configuration of the control register map in the code

Table 6. Control register map of register 0x81 and bit D8 and D11 of register 0x80 [9]

Bits	Description
D11	No operation
D8	PGA gain; 0 = ×5, 1 = ×1
D7	Reserved; set to 0
D6	Reserved; set to 0
D5	Reserved; set to 0
D4	Reset
D3	External system clock; set to 1 Internal system clock; set to 0
D2	Reserved; set to 0
D1	Reserved; set to 0
D0	Reserved; set to 0

The runSweep function is responsible for initializing start frequency and starting frequency sweep. As recommended in table 4, programming the 4 MSBs of 0x80 makes runSweep possible. The function is executed in a loop and is programmed to make measurements every 30 minutes.

Registers 0x94, 0x95, 0x96 and 0x97 contain the binary representation of the real and imaginary components of the unknown impedance measured at a frequency of 5 KHz and at 200 mV. The binary data is in a 16-bit, twos complement format. As shown in appendix I, to convert this number to an actual impedance value, the magnitude is multiplied by an admittance/code number (gain factor) to give the admittance, and the result inverted to give impedance. The gain factor varies for each ac excitation voltage/gain combination.

3.6 Measurement setup

This sub chapter discusses the measurement setup of the proposed system. The impedance analyser chip (AD5933) is connected as shown in figure 17. As recommended in the data sheet, the DGND, AGND1 and AGND2 pins of the chip are all tied together and connected to the GND pin of the microcontroller. Then the DVDD, AVDD1 and AVDD2 are tied together and connected to the 5V pin of the microcontroller. The SCL and SDA pin of the chip are connected to pin 21 and 20 of the microcontroller respectively. Two pull up resistors (connected to 5V supply) of value 10kΩ are connected to the SCL and SDA of chip as shown in figure 18. The microcontroller (Arduino due) is powered by the PC through a USB cable.

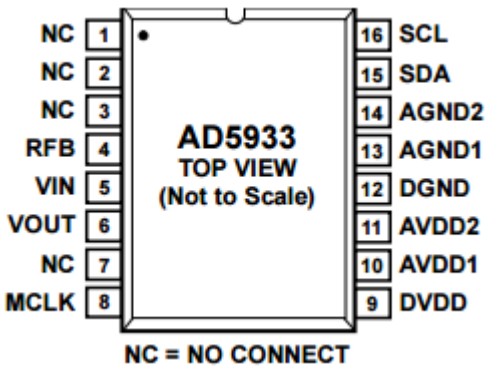

Figure 17. Pin configuration of the chip [9]

A pair of gold plated electrodes are connected to the VIN and VOUT pins of the chip for measuring the unknown impedance (Z_T) of the SUT. R_{FB} (pin 4) is the current to voltage gain setting resistor of the receive stage and is used for the calibration of the measurement. A value of 100 kΩ was selected with consideration for the saturation of the ADC and because the impedance level of the SUT used for the proposed system is at 200 kΩ (as measured using an RLC meter). The data sheet recommends to use an R_{FB} of 100 KΩ for an unknown impedance (Z_T) range between 100 KΩ - 1000 KΩ. For proper calibration the resistor connected between VIN and VOUT which is R_{cal} (where the unknown impedance Z_T is t be connected for measurements) is of the same value as R_{FB}. The gain factor that will be used in the developed software is a result of the calibration formula as shown in equation 12 and 13. The magnitude is the result of the real and imaginary data acquired from the allocated registers of AD5933. [9]

A one point calibration is used even though the data sheet recommends a two point calibration. This is because the proposed system does not conduct a frequency sweep but instead measures the impedance of the SUT at one frequency point (zero frequency band).

$$\text{Gain Factor } = \text{Admitacne}\frac{1}{\text{code}} = \frac{\frac{1}{\text{Impedane}}}{\text{Magnitude}} = \frac{1}{\frac{\text{Rcal}}{\text{Magnitude}}} \qquad (12)$$

$$Impedance = 1/(Gain\ Factor * Magnitude) \qquad (13)$$

The impedance chip (AD5933) is capable of measuring impedance in the range of 1 kΩ to 10 MΩ. With additional circuitry it can measure low impedance levels ranging from 100 Ω to 1 KΩ. The proposed system is interested in measuring the microbial concentration level of drinking water. It is know that drinking water can have an impedance ranging from 500 Ω to 5 KΩ. In order to measure this level of low impedance the impedance chip will need additional circuitry. On the other hand, distilled water, depending on its level of purity can have high levels of impedance and is well within in the measurement range of the chip. Since what is sough is a proof of concept regarding the impedance method, the use of distilled water at an impedance level of 200 KΩ is practical because doesn't require additional circuity.

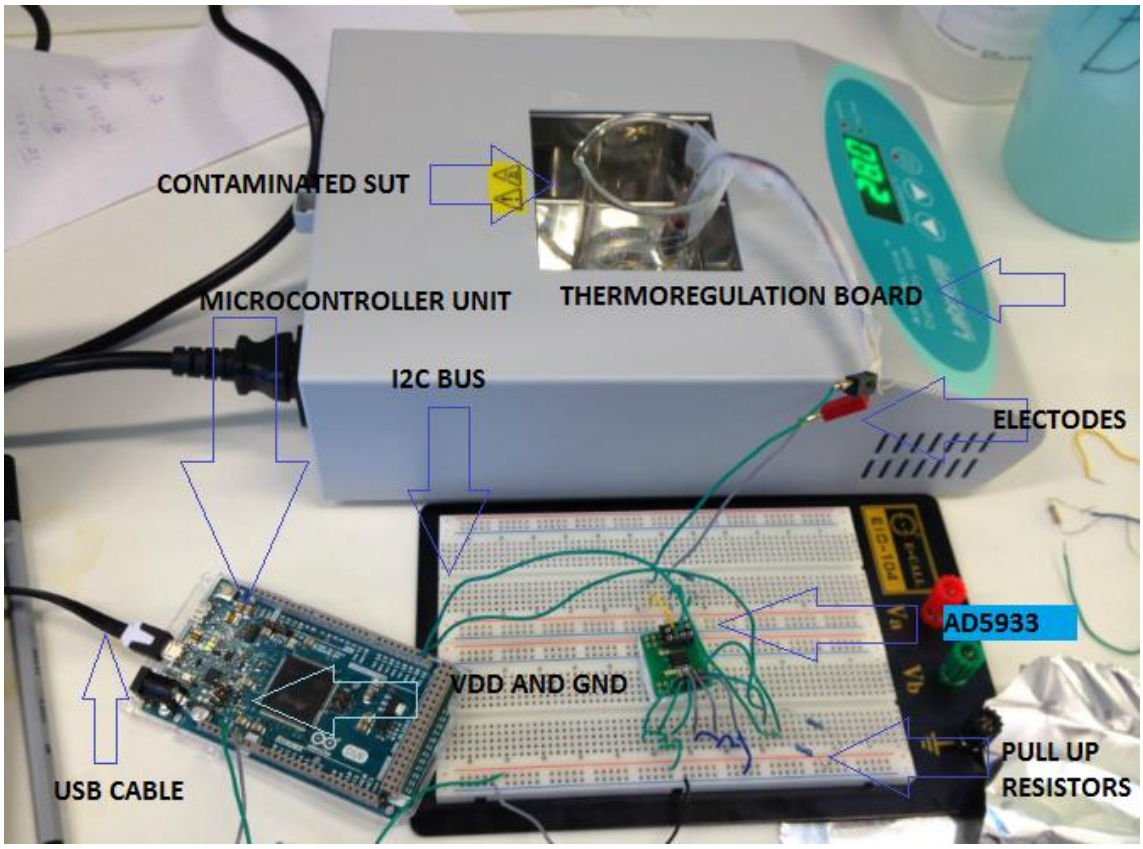

Figure 18. Measurement setup

As thoroughly discussed in chapter two, an environment of regulated temperature is crucial for bacterial growth and the proper execution of the impedance method itself. Without a controlled environment it is difficult to attribute the change in the impedance of the SUT to the increase in the concentration level of bacteria. The measurement set up used for the proposed system was conducted without a confined thermoregulation board which has a critical impact on the measured impedance values.

Another important factor for the impedance method is the type of bacteria that is contaminating the SUT. Because of the lack of resources and time the proposed system did not experiment with pathogenic bacterial strains. But it instead measured the change in the impedance of the SUT contaminated when an innocuous plant nitrogen fixing bacteria called Rhizobium acquired from the microbiology lab at Helsinki University. Rhizobium is a slow growing bacteria which might take up to 10 to 12 days to reach C_{TH} (concentration threshold) of about 10^7 cfu/ml as shown in the growth graph in figure 19.

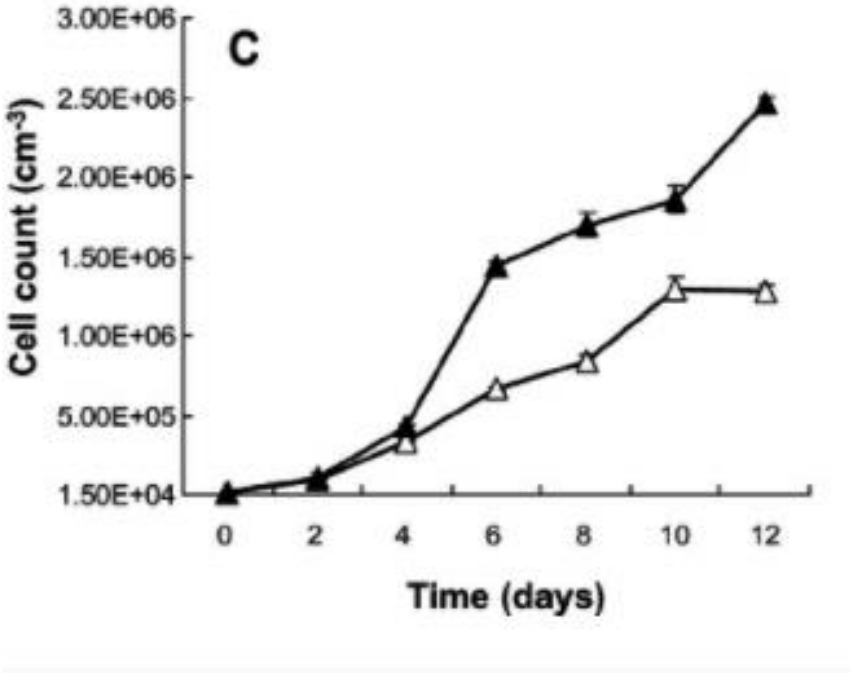

Figure 19. Growth curve of Rhizobium bacteria [12]

There were two types of SUTs used for the measurement set up. The first was a 9 ml distilled water contaminated with 1 ml of Rhizobium and the second was a 9 ml YMB (yeast-mannitol broth) contaminated with 1 ml of Rhizobium. The beaker containing the SUT was put in a thermoregulation board available at the Helsinki University lab as shown in figure 18. The temperature was maintained at 25 ºC, which is suitable for Rhizobium bacteria.

4 Measurements and evaluation

4.1 Impedance measurement for 9 ml distilled water contaminated with 1 ml of Rhizobium

The strain of bacteria contaminating the SUTs is slow growing. It might take up to 6 days (144 hours) until the logarithmic phase of the growth cycle is reached [L]. It is during the logarithmic phase that there is a significant change (5% deviation from the baseline value) in the impedance level of the SUT. It is eveident from figures 20, 21, & 22 that there is 37.3% deviation for the measurements conducted when the SUT is distilled water. This huge change in impedance cannot be attributed to an increase in the microbial concentration of the SUT because of the duration (8 hours) of the measurement. Since

Rhizobium is slow growing, after such a short duration it should still be in the lag phase (no growth phase). Therefore the huge change in impedance might be the result of the instability of the electrode electrolyte system. The change might also be attributed to the lack of proper incubation chamber or thermoregulation board.

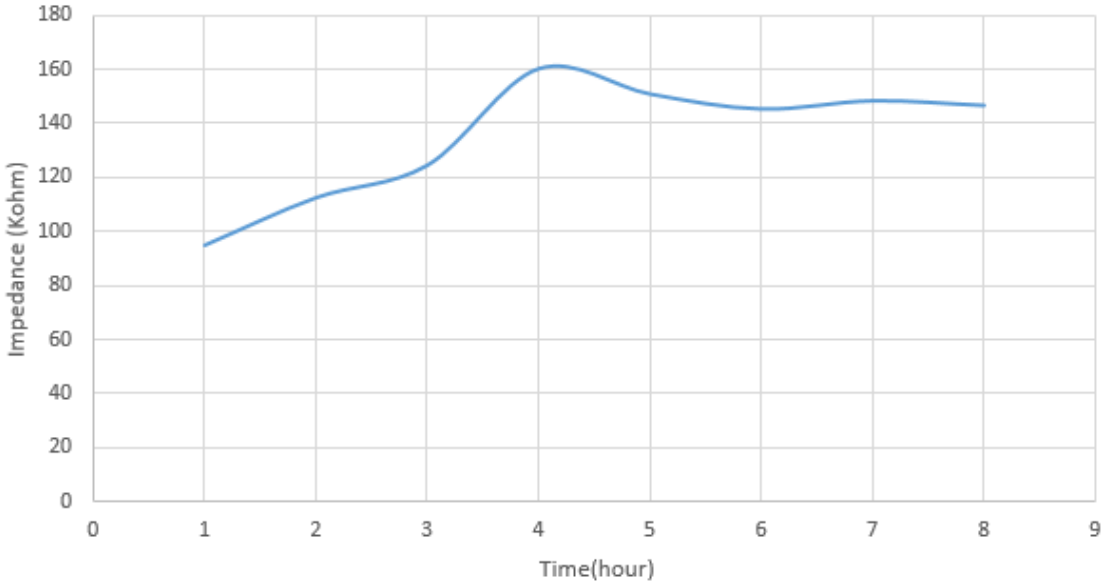

Figure 20. Impedance versus time for 9 ml distilled water contaminated with 1 ml of Rhizobium

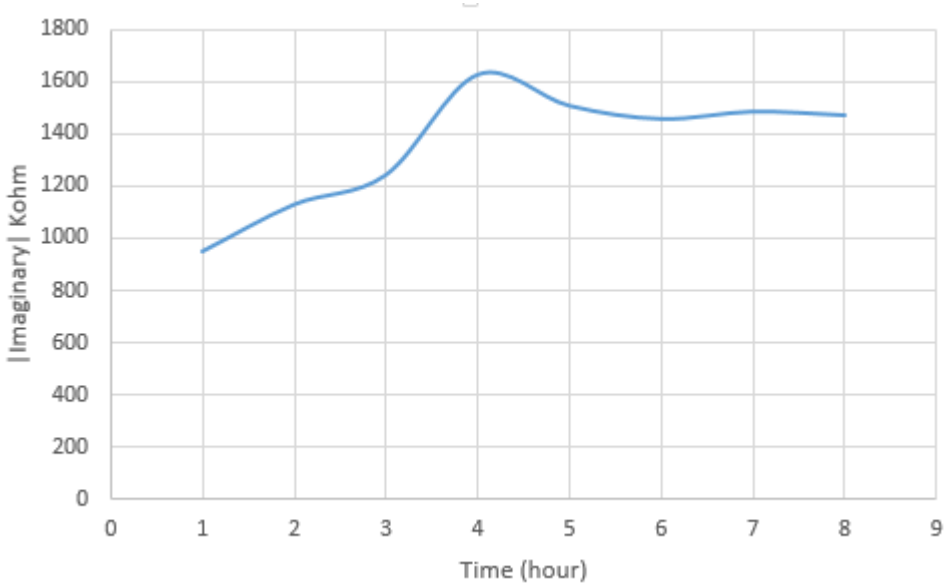

Figure 21. Imaginary data magnitude versus time for 9 ml distilled water contaminated with 1 ml of Rhizobium

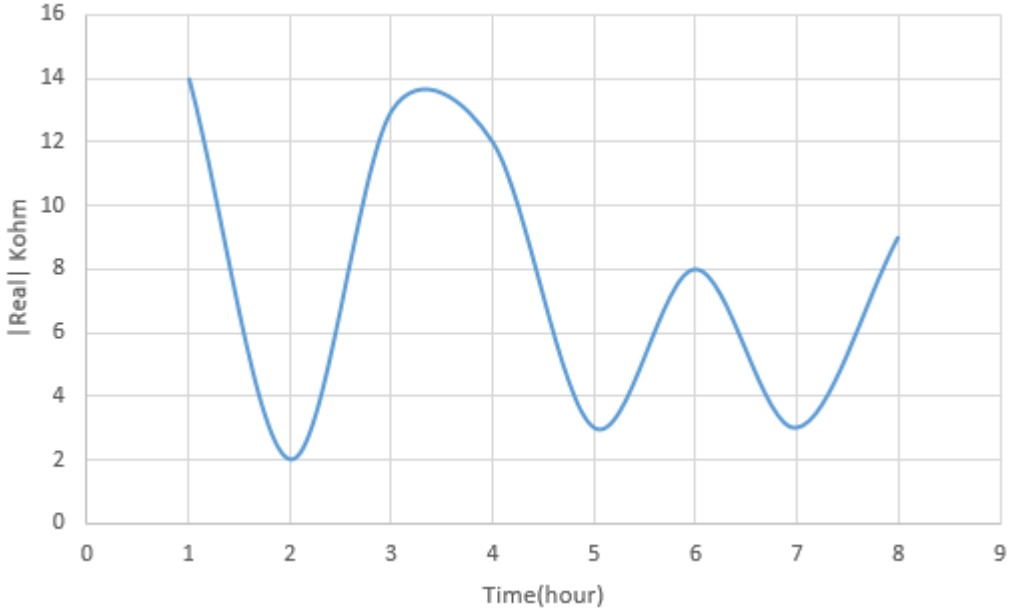

Figure 22. Real data magnitude versus time for 9 ml distilled water contaminated with 1 ml of Rhizobium

4.2 Impedance measurement for 9 ml YMB contaminated with 1 ml of Rhizobium

The SUT containing 9 ml YMB (yeast-mannitol broth) contaminated with 1 ml of Rhizobium was measured for a longer period as opposed to the contaminated distilled water. This was done because the YMB has more nutrients than distilled water. The increase in the amount of nutrients in turn would allow the bacteria to reach the logarithmic phase faster.

When the SUT is YMB it is clear from figures 23, 24 & 25 that a significant change in impedance (5% deviation from the baseline value) doesn't take place for the measurements conducted using the proposed system. Since the measurement was conducted for 120 hours (5days) the lack of significant change in impedance for such a duration is characteristic of the Rhizobium (nitrogen fixing bacteria) bacteria. This is because it might take up to 6 days (144 hours) until the logarithmic phase of the growth cycle is reached.

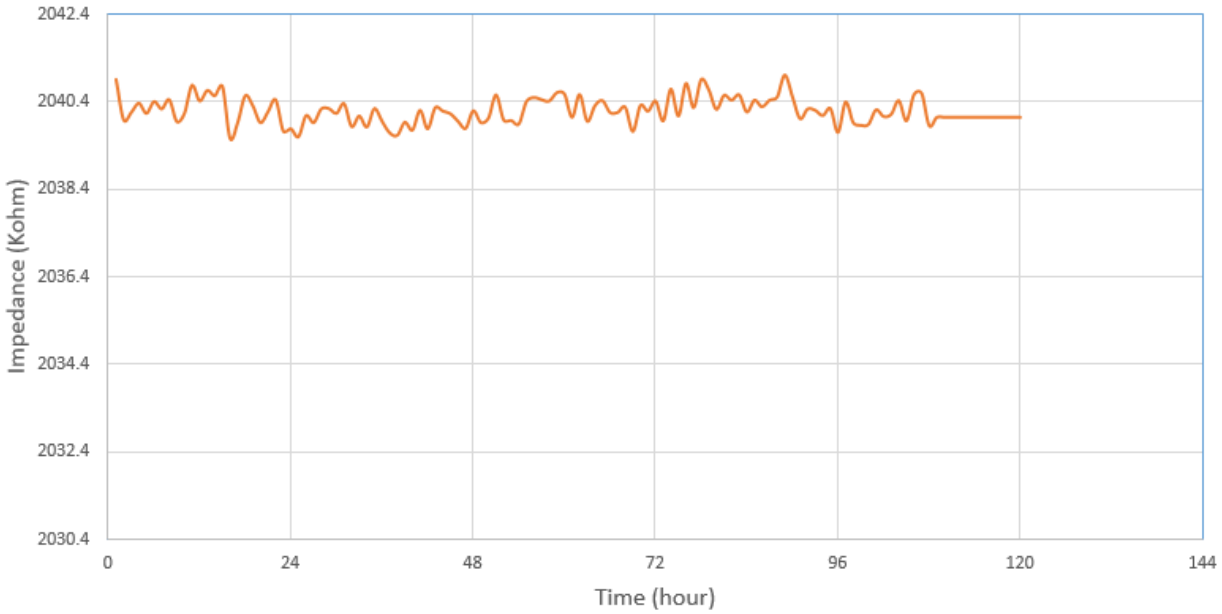

Figure 23. Impedance versus time for 9 ml YMB (yeast-mannitol broth) contaminated with 1 ml of Rhizobium

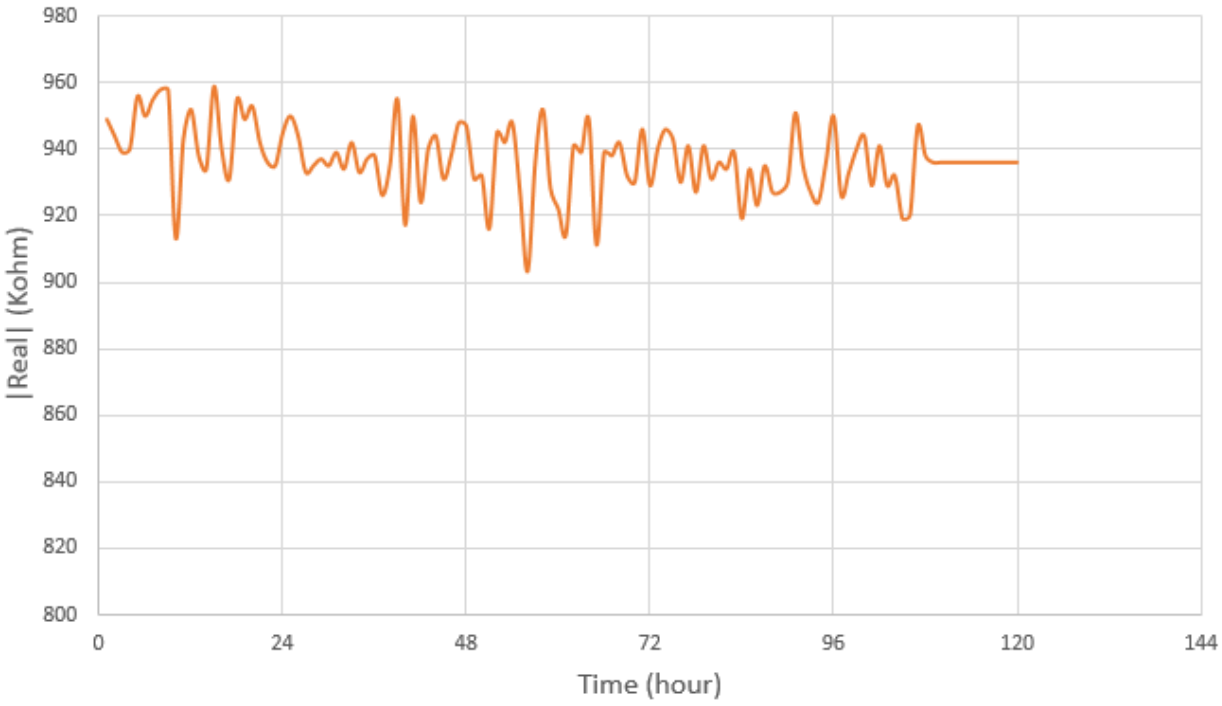

Figure 24. Real data magnitude versus time for 9 ml YMB (yeast-mannitol broth) contaminated with 1 ml of Rhizobium

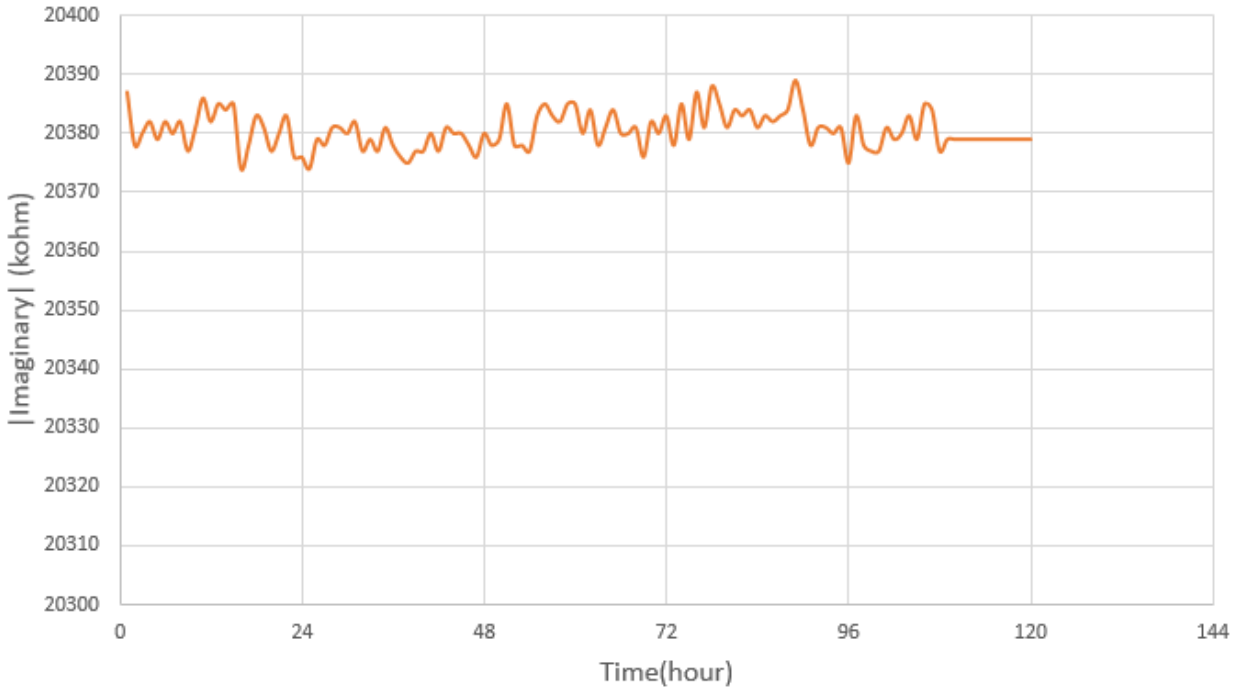

Figure 25. Imaginary data magnitude versus time for 9 ml YMB (yeast-mannitol broth) contaminated with 1 ml of Rhizobium

5 Possible improvements

There are plenty of improvements that can be made to make the functionality of the proposed system better. The proposed system was lacking in many aspects. Nonetheless this doesn't take away from the immense potential that the proposed system has. The system can benefit from improved software and hardware. The following are a list of improvements that can alter the proposed system significantly

1. An external oscillator provided for AD5933 would result in a more stable measurement.
2. Providing additional circuitry would make it possible to measure low impedance levels. Designing an analog board specifically for the impedance range of drinking water would allow the proposed system to go beyond proving the concept of impedance microbiology.
3. Make additional measurements by conducting a frequency sweep. From such a measurement, the bacterial concentration level can be characterized for a range of frequencies.

4. Designing and including an incubation chamber would also make it possible to attribute changes in impedance only to the increase in the concentration level of bacteria in the SUT.
5. Working in collaboration with institutions that are capable of providing faecal coliforms and handling such types of bacteria with an environment equipped with tools of precaution so that the results from the proposed system can serve in preventing immediate threats to drinking water quality.
6. Providing a battery as a power supply for the microcontroller, writing a better software that is capable of assessing the concentration level of the SUT and adding a wireless interface such as a Bluetooth module to make remote measurements possible so that the proposed system becomes independent.

6 Conclusion

Using the principles of impedance microbiology, a system was proposed for detecting the concentration level of bacteria in water. The proposed system employs an impedance analysing chip, AD5933. The chip is interfaced with an Arduino due microcontroller via I^2C. The microcontroller is connected to a PC through a USB cable. A software has been developed using the Arduino IDE that is capable of measuring the impedance level of an SUT.

The proposed system measures the change in the impedance level of an SUT via a pair of electrodes at a frequency of 5 KHz. The calibration process for the impedance chip was carefully evaluated to avoid saturating the ADC of the receive part of the chip. Measurements were conducted for an SUT containing contaminated distilled water and another SUT containing contaminated YMB. An SUT containing YMB was measured for a longer period because it has more nutrients than distilled water. Both SUTs provided results that could be attributed to the growth characteristic of the contaminating bacteria though there were also other possible explanations.

Despite the limitations of time and resources, the proposed system can serve as proof of concept for the impedance method. The proposed system employs a principle that has immense potential and is capable of overtaking traditional microbial detection methods. With improvements in both the hardware and software of the system, it can be very useful in maintaining the quality of drinking water thereby preventing the catastrophic consequences of microbial pollution of drinking water.

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Appendix I

Main program file

```
#include <Wire.h>

#define AD5933_ADDR 0x0D
#define ADDR_PTR 0xB0

#define CTRL_REG1 0x80
#define CTRL_REG2 0x81
#define STATUS_REG 0x8F

#define START_F_REG1 0x82
#define START_F_REG2 0x83
#define START_F_REG3 0x84

#define INCR_F_REG1 0x85
#define INCR_F_REG2 0x86
#define INCR_F_REG3 0x87

#define INCR_NUM_REG1 0x88
#define INCR_NUM_REG2 0x89

#define NUM_SCYCLES_R1 0x8A
#define NUM_SCYCLES_R2 0x8B

#define REAL_DATA_REG1 0x94
#define REAL_DATA_REG2 0x95

#define IMAG_DATA_REG1 0x96
#define IMAG_DATA_REG2 0x97

#define TEMP_R1 0x92
#define TEMP_R2 0x93
```

```
const float MCLK = 16.776*pow(10,6); // AD5933 Internal Clock
Speed 16.776 MHz
const float start_freq = 5*pow(10,3); // START FREQENCY AT 5KHz
const float incre_freq = 0; // Set freq increment, FREQUENCY
DOESN'T INCREASE THEREFORE IT REPEATS 5KHz
const int incre_num = 384; // Set number of increments; < 511

char state;

void setup() {
  Wire.begin();
  Serial.begin(9600);

  writeData(CTRL_REG1,0x0); //NO OPERATION

  writeData(CTRL_REG2,0x10);// RESET MODE(D4 IS SET)

  programReg();
}


void loop(){

  //Read state and enter FSM
    if(Serial.available()>0) {
      state = Serial.read();

      //FSM
      switch(state) {
        case 'A':  //Program Registers
          programReg();
          break;
```

```
        case 'B':
          runSweep();
          delay(1.8*pow(10,6));//MEASURE EVERY 30 MINUTES
          break;

     }

    }

}




void programReg(){

  // Set Range 1, PGA gain 1
  writeData(CTRL_REG1,0x01);

  // Set settling cycles
  writeData(NUM_SCYCLES_R1, 0x07);
  writeData(NUM_SCYCLES_R2, 0xFF);

  // Start frequency of 1kHz
  writeData(START_F_REG1, Freqcode(start_freq,1));
  writeData(START_F_REG2, Freqcode(start_freq,2));
  writeData(START_F_REG3, Freqcode(start_freq,3));

  // Increment by 0 kHz
  writeData(INCR_F_REG1, Freqcode(incre_freq,1));
  writeData(INCR_F_REG2, Freqcode(incre_freq,2));
  writeData(INCR_F_REG3, Freqcode(incre_freq,3));

  // Points in frequency sweep (100), max 511
  writeData(INCR_NUM_REG1, (incre_num & 0x001F00)>>0x08 );
```

```
  writeData(INCR_NUM_REG2, (incre_num & 0x0000FF));

}


void runSweep() {
  short re;
  short img;
  double freq;
  double mag;
  double phase;
  double gain;
  double Impedance;
        double GF;
        double FFW;
        double wt;
        double BF;
        double tot = 0;
        double magcount = 0;
        double impcount = 0;
        double avgmag;
        double totimp = 0;
        double avgimp;
  int i=0;

  programReg();

  // 1. Standby '10110000' Mask D8-10 of avoid tampering with
gains
  writeData(CTRL_REG1,(readData(CTRL_REG1) & 0x07) | 0xB0);

  // 2. Initialize sweep
  writeData(CTRL_REG1,(readData(CTRL_REG1) & 0x07) | 0x10);

  // 3. Start sweep
  writeData(CTRL_REG1,(readData(CTRL_REG1) & 0x07) | 0x20);
```

```
  while((readData(STATUS_REG) & 0x07) < 4 ) {   // Check that
status reg != 4, sweep not complete
    delay(100); // delay between measurements

    int flag = readData(STATUS_REG)& 2;


    if (flag==2) {

      byte R1 = readData(REAL_DATA_REG1);
      byte R2 = readData(REAL_DATA_REG2);
      re = (R1 << 8) | R2;

      R1  = readData(IMAG_DATA_REG1);
      R2  = readData(IMAG_DATA_REG2);
      img = (R1 << 8) | R2;

      freq = start_freq + i*incre_freq;
      mag = sqrt(pow(double(re),2)+pow(double(img),2));
                        tot = tot+mag;
                        magcount = magcount+1;
                        GF = (1/.22)/19.06;
                        Impedance = 1/(GF*mag);
                        if (Impedance < 100){
                          impcount = impcount+1;
                          totimp = totimp+Impedance;
                        }


      // phase = atan(double(img)/double(re));
      // phase = (180.0/3.1415926)*phase;  //convert phase angle
to degrees

      // Phase Calibration
```

```
      // sys_phase = 118;
      // phase = phase - sys_phase;

      // gain = (1.0/197760)/9786.98;
    //    impedance = 1/(gain*mag);

      Serial.print("Frequency: ");
      Serial.print(freq/1000);
      Serial.print(",kHz;");

      Serial.print(" Magnitude: ");
      Serial.print(mag);
      Serial.print(",kOhm;");

                        Serial.print(" Impedance: ");
      Serial.print(Impedance);
      Serial.println(",");

      Serial.print(" Reactance: ");
      Serial.print(img);
      Serial.println(",");

      // break;  //TODO: for single run, remove after debugging

      //Increment frequency
      if((readData(STATUS_REG) & 0x07) < 4 ){
        writeData(CTRL_REG1,(readData(CTRL_REG1) & 0x07) | 0x30);
        i++;
      }
                        avgmag = tot/magcount;
                        avgimp = totimp/impcount;
                        wt = 118/2.205;
                        FFW                                          =
(0.396*(pow(1.54,2)/(avgimp*1000))+0.143*wt+8.399)*1.37*2;
                        BF = ((wt-FFW)/wt)*100;
```

```
    }

  }
                        Serial.print(" Avg Mag: ");
      Serial.print(avgmag);
      Serial.print(",");

                        Serial.print(" Avg Impedance: ");
      Serial.print(avgimp);
      Serial.print(",");

                        Serial.print(" % Body Fat: ");
      Serial.print(BF);
      Serial.print(",");

  //Power down
//  writeData(CTRL_REG1,0xA0);
  writeData(CTRL_REG1,(readData(CTRL_REG1) & 0x07) | 0xA0);
}



void writeData(int REG_addr, int data) {

 Wire.beginTransmission(AD5933_ADDR);
 Wire.write(REG_addr);
 Wire.write(data);
 Wire.endTransmission();
 delay(1);
}


int readData(int REG_addr){
  int result;

  Wire.beginTransmission(AD5933_ADDR);
```

```
  Wire.write(ADDR_PTR);
  Wire.write(REG_addr);
  Wire.endTransmission();

  delay(1);

  Wire.requestFrom(AD5933_ADDR,1);

  if (Wire.available() >= 1){
    result = Wire.read();
  }
  else {
    result = -1;
  }

  delay(1);
  return result;
}


byte Freqcode(float freq, int n){
  long val = long((freq/(MCLK/4)) * pow(2,27));
  byte code;

    switch (n) {
      case 1:
        code = (val & 0xFF0000) >> 0x10;
        break;

      case 2:
        code = (val & 0x00FF00) >> 0x08;
        break;

      case 3:
        code = (val & 0x0000FF);
        break;
```

```
      default:
        code = 0;
    }

  return code;
}
```

Appendix II

Figure. 1 Test tubes containing Rhizobium bacteria and YMB used for the measurements.

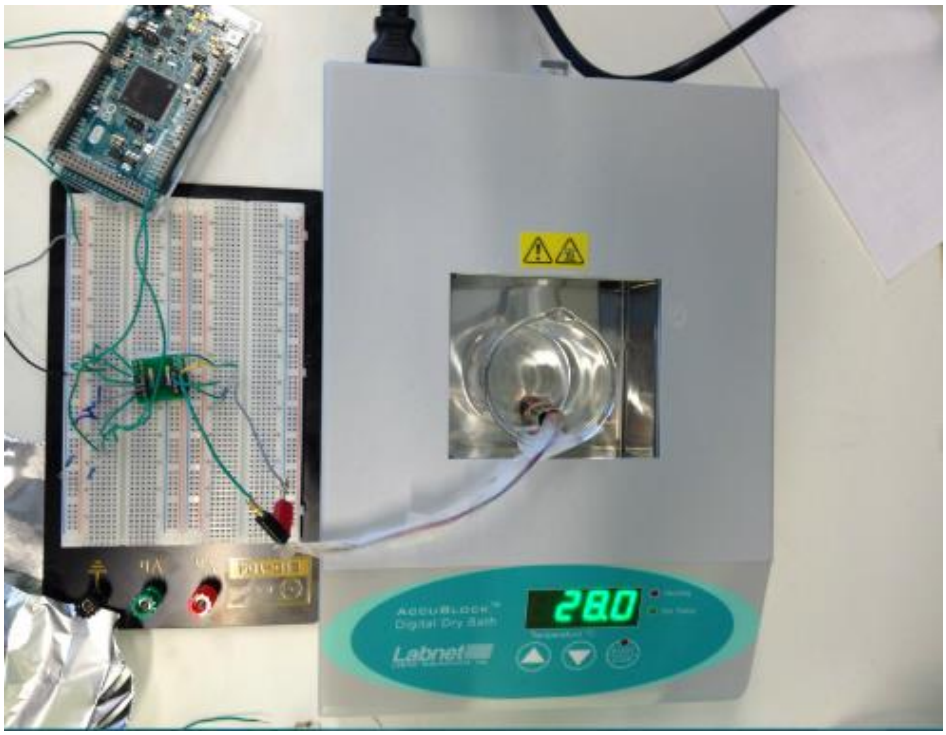

Figure. 2 Measurement setup

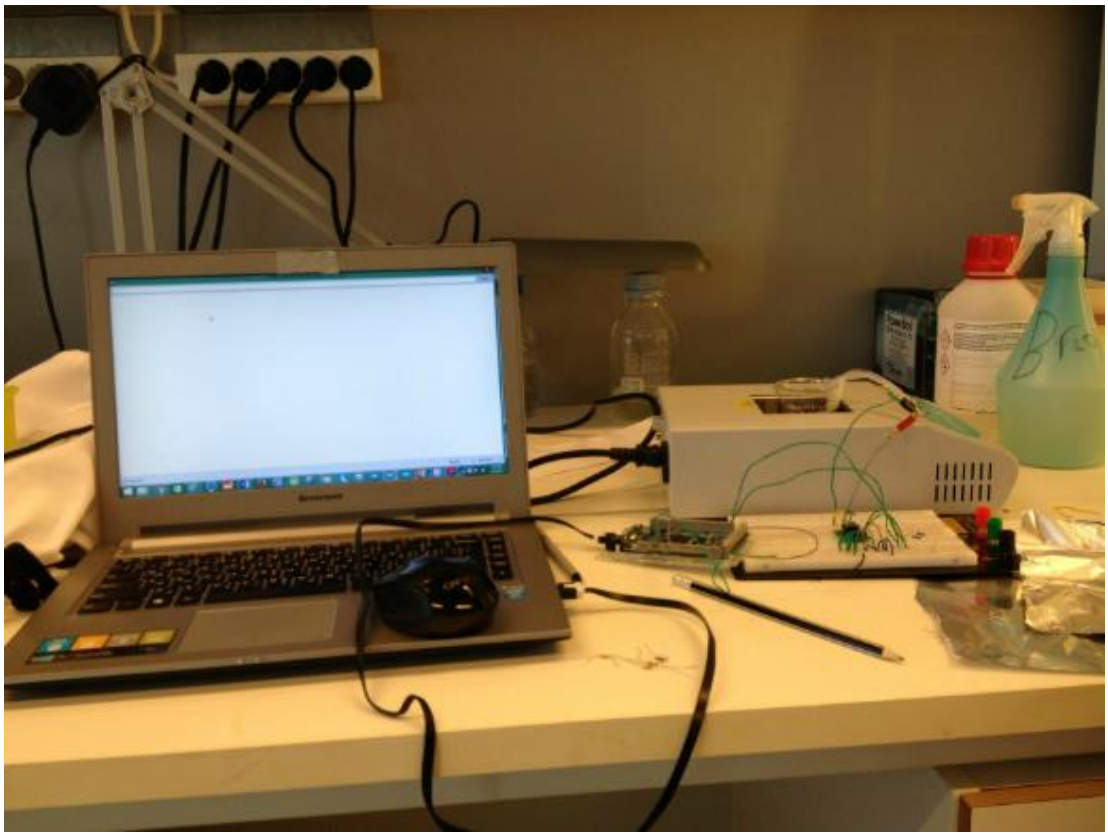

Figure. 3. Measurement setup

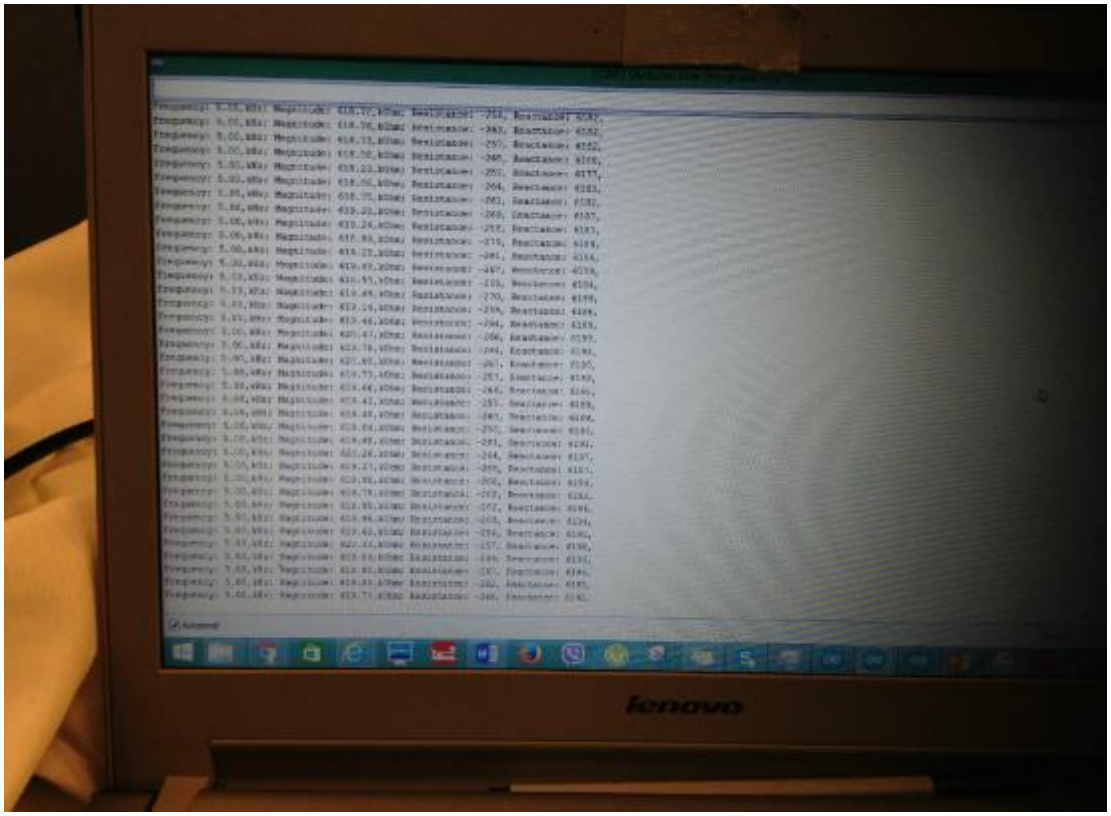

Figure. 4 .Measurement setup (Receiving measured data)