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Detection Of Escherichia coli In Water Using Capacitance Biosensor

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ABSTRACT

Pathogenic bacteria that are the main causative agent for biological contamination of water have always posed one of the most serious threats to public health, and continue to be specifically dangerous with the rise in antibiotic resistance. The incidence of these infectious agents needs rapid, point-of-care and real-time sensors for their detection and monitoring. Therefore, continuous real-time monitoring provides better microbiological control of the water and helps prevent contaminated water from reaching the households. In this research we have developed a method to detect *E.coli* (*Escherichia coli*) bacteria in water depending upon a large scale capacitance biosensor in the presence and absence of *E.coli* cells. First we show that the sensor is able to detect *E. coli* (Gram-negative) bacteria in water samples. Next, we demonstrate the sensor's ability to measure the bacteria concentration suspended in water sample by comparing the results to those obtained by the traditional measurement using spectrophotometer. The results determine a correlation between the capacitance measurement and the real bacterial concentration which indicate an inversely relationship between the bacterial concentration and the capacitance measurement. Our investigations show that the capacitance biosensor has the potential to be extremely effective at detecting sudden bacterial contaminations found in drinking water in real-time and it is promising for the low-cost monitoring of bacterial growth and the detection of specific *E.coli* concentration due to the consistency and ease-of-use of capacitance measurements.

1. Introduction

With the increase in antibiotic resistance, pathogenic bacteria remain a significant and ongoing threat to public health. Consequently, there is a growing need for the development of rapid, real-time, point-of-care sensors to detect and monitor these infectious agents, given their widespread presence.

One of the pathogens requiring monitoring is the *Escherichia coli* (*E. coli*) bacteria. The presence of these bacteria in water is associated with fatalities and serves as an indicator of the proliferation of other pathogens (Quiroz et al.,2018). Many conventional techniques and methods are used to detect E.coli. Such techniques are sample culture (Lazcka et al.,2007), Polymerase Chain Reaction Methods (Toze., 1999), Immunology-Based Methods (Haglund et al.,1964).

Most of the conventional methods of bacterial detection and identification have enabled the monitoring of real-world samples. These techniques are all limited by the time required and the necessary instrumentation, which can be costly and highly specialized, and the facilities and personnel required to complete these assays.

Impedance-based techniques are some of the most reliable and reproducible characterization methods for electrical systems,

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and information regarding double layer capacitance, diffusive impedance, and charge transfer resistance characteristics can be easily concluded (Ates.,2011).

In general, impedance is known as the effective resistance of an electrical circuit to a component in response to an alternating current (AC). Non-impedance electrical biosensors have emerged as an enhanced alternative to conventional methods for point-of-care or environmental biomolecule detection. This is attributed to their inherent advantages of simplicity and cost-effectiveness.

Electro-Impedance sensors are promising for the real-time, low-cost monitoring of bacterial growth and the detection of specific microbial species due to the consistency and ease-of-use of impedance measurements.

Bacterial cells are prokaryotic cell and very small in size but they have a complex nature and their electrochemical properties are also highly complicated. Cell membranes are comprised of a lipid bilayer, with the lipids oriented such that the polar head groups face the aqueous environment, on both the interior and exterior of the cell, and the hydrophobic hydrocarbons of the lipid tail form the interior of the membrane. This yields a membrane that is highly insulating, (Yang and Bashir., 2008) estimated to be in the range of 10−7 S/ m. (Pethig et al., 1997). The bilayer also has many embedded proteins, some of which are ion channels that can be treated as resistors, such that the resistance across the full membrane can be modeled as “parallel ion channel resistors”. Estimates of the total resistance across cell membranes vary from 105 to 1 M Ω·μm2 . (Pethig.,1979), (Borkholder.,1998). The interior of bacterial cells also contains a variety of charged biomolecules and small molecules, rendering it highly conductive (as high as 1 S/m) (Pethig et al., 1997). Because of the insulating properties of cell membranes, if cells bind to an electrode surface, they reduce the electrode area that is accessible to redox-active molecules in solution, leading to an increase in the interface impedance.

Impedance microbiology (IM), or the monitoring of bacterial cells density by monitoring the electrical parameters of the growth medium, is one of the earliest methods of bacterial detection developed with impedance measurement. It has been used as such for over a century, (Firstenberg-Eden et al.,1984) with early IM monitoring systems composed of two planar electrodes submerged in solution. Such platforms have found extensive use in the development of point-of-care systems (Wawerla et al.,1999). Bacteria can be detected using IM through either a direct or indirect measurement, which is chosen based on the ionic strength of the solution to be measured. The direct detection method involves monitoring the AC impedance at a single frequency at a pair of electrodes immersed in bacterial culture medium (Ur and Brown., 1975), (Silley and Forsythe., 1996). Impedance changes result from ionic metabolites secreted by the bacteria into the solution. If impedance changes are detected beyond a threshold level, the sample is positive for bacteria. Indirect detection may be required if the ionic strength of the solution is too high to detect clear differences due to bacterial ion secretions. (Owens et al.,1989) In this case, CO_2 is instead monitored in a chamber that is isolated from the growth chamber. The rapid speed and ease of use of this technique have led to its broad application in bodily fluid sampling and foodborne pathogen detection (Yang and Bashir., 2008).

Additionally, an integrated sensor system based complementary metal-oxide semiconductor (CMOS) utilized for monitoring the growth of bacteria by employing an on chip capacitive sensor. The growth of bacteria causes a capacitance change. The platform introduces a unique architecture with two interdigitized electrodes. One serving as a reference and the other as a sensing electrode. These electrodes are exposed to pure Luria-Bertani (LB) medium and *E.coli* suspended in the LB medium, respectively. To facilitate the flow of solutions towards the electrodes, two microfluidic channels are incorporated using a

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direct-write assembly technique (Ghafar-Zadeh et al.,2010).

On the other hand, detection of *E. coli* can be accomplished using bacteriophage or phage organisms as recognition elements. This study presents an integrated sensor system that incorporates these recognition elements for detection. The system monitors changes in capacitance signals when the target bacteria attach to the sensing interface. Signal detection and processing are carried out by a Charge Based Capacitance Measurement circuit. The phage organisms are immobilized on the surface of the capacitor, creating the sensing interface (Yao et al.,2008).

The objectives of this study were to explore the correlation between the sensor's capacitance and the concentration of *E.coli*. In addition to design a detection and monitoring system capable of continuously assessing the concentration of *E.coli* in water.

2. Methodology

2.1 System Design

The system block diagram is depicted in Figure 1, illustrating the components of the system, including the capacitance sensor, capacitance measuring circuit, and display module. To enable continuous monitoring of the presence of *E.coli*, a specific sequence of miniature tasks needs to be performed. Figure 2 presents an algorithm that outlines these tasks. Initially, the algorithm is employed to measure the capacitance of the sensor while immersed in distilled water containing a known concentration of *E.coli*. This process is repeated for multiple trials using various known sample concentrations. Subsequently, the capacitance-concentration (C-[C]) relation will be obtained. Following that, the established correlation will be incorporated into the system to enable direct measurement of the concentration of E.coli bacteria. The final setup will facilitate continuous real-time monitoring of *E.coli* concentration and trigger an alarm when the concentration surpasses a predetermined threshold value.

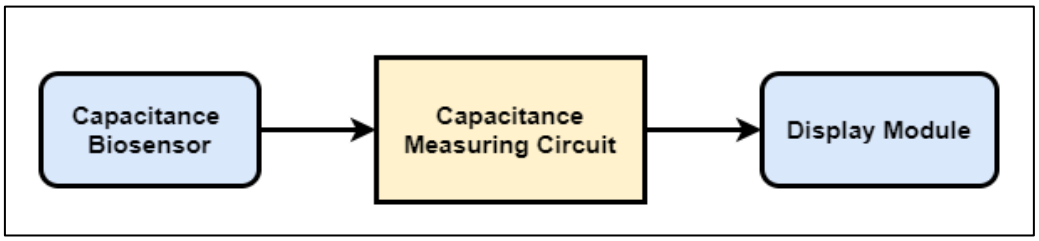

Figure 1. Capacitance-Concentration system block diagram.

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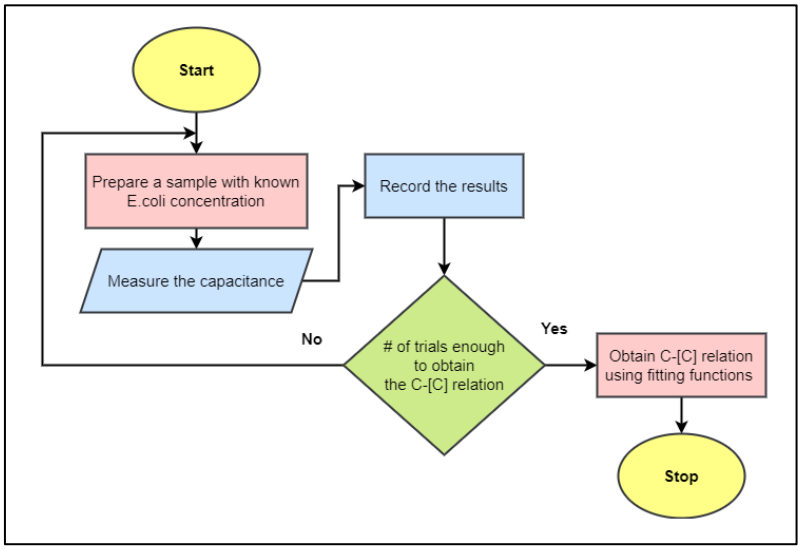

Figure 2. Algorithm for obtaining the C-[C].

2.2 Sensor Design

For the detection of E.coli bacteria, a capacitive sensor was selected. This choice ensures a large-scale, reliable, and non-invasive sensor design. Importantly, this approach eliminates the need for specialized sensors with coated nanomaterials. Figure 3 illustrates the design of the capacitive sensor, which consists of two copper plates encircling a small water tube. This configuration forms a capacitor with curved plates, and the capacitance (C) can be calculated using equation (1) (Pethig.,1979). The experimental works showed a value of 14pF. The dielectric materials encompass the materials of the tube and the sample, which include distilled water and bacteria.

$$C = \frac{\varepsilon * A}{2\rho} * \left[\left(ln\left(tan\frac{\theta 1}{2} \right) - \left(ln\left(tan\frac{\theta 2}{2} \right) \right] \right. \quad (1)$$

Where:
A: is the plate area.
ε: is the dielectric constant.
ρ: is the tube radiance.
$\theta 1$ & $\theta 2$: represent the initial and final angles, respectively, that define the arch-shaped trajectory formed by a plate around the cylindrical tube.

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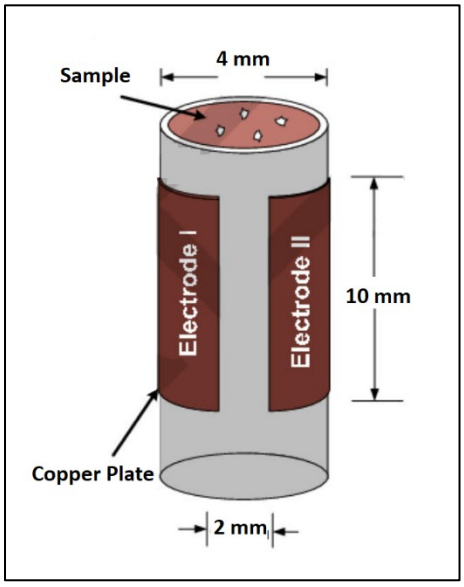

Figure 3. Capacitive sensor design.

2.3 Capacitance Measuring Circuit (CMC)

The capacitance measuring circuit (CMC) will be utilized to measure the sensor capacitance (C). Employing a Resistive-Capacitive (RC) circuit configuration, the CMC is designed. Figure 4 illustrates the RC circuit, with its input-output relationship expressed through a first-order transfer function denoted by equation (2). The product RC parameter is the time constant of the circuit (τ).

$$\frac{V_O}{V_I} = \frac{1/RC}{s+(1/RC)} \qquad (2)$$

$$\tau = RC \qquad (3)$$

Where:
V_O: Represent the RC circuit output voltage (capacitor voltage).
V_I: Represents the input voltage.

Applying the inverse Laplace transform to equation (2) yields the capacitor's output voltage ($V_C(t)$) in the time domain. Within

the presented RC circuit, the E.coli concentration sensor is represented by capacitor $C1$. The time constant of the RC circuit serving as the only parameter for this first-order system. To measure the C, a step input voltage is applied to the RC circuit. Consequently, the capacitor will charges in an exponential manner as dictated by equation (2). At time (t) equal to one time constant ($t = \tau$), the output registers at 63.2% of the final value.

The primary function of the CMC is to gauge the sensor capacitance by determining the time constant of the RC circuit and subsequently calculating the capacitance based on this measured value. To measure the time constant, a timer within a microcontroller will be initiated when the step input voltage is applied. The application of the step voltage is controlled manually by the user through a push button. As the capacitor voltage reaches 63.2% of the final value, a voltage comparator will activate a stop signal and halt the timer. Then the microcontroller will calculate and display C. Figure 5 illustrates the algorithm employed for measuring the sensor capacitance. Figure 6 depicted the complete CMC.

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The depicted CMC in Figure 6 was employed to acquire the capacitance of the sensor for each E.coli sample concentration. An upgrade has been implemented in the algorithm to automate the measurement process. This enhancement relies on periodic initiation of the step input and the timer. As a result, the system is now capable of continuously measuring the sensor capacitance. This capability enables real-time monitoring of the *E.coli* concentration, supported by the presence of the C-[C] relation in mathematical form. Figure 7 illustrates the upgraded algorithm.

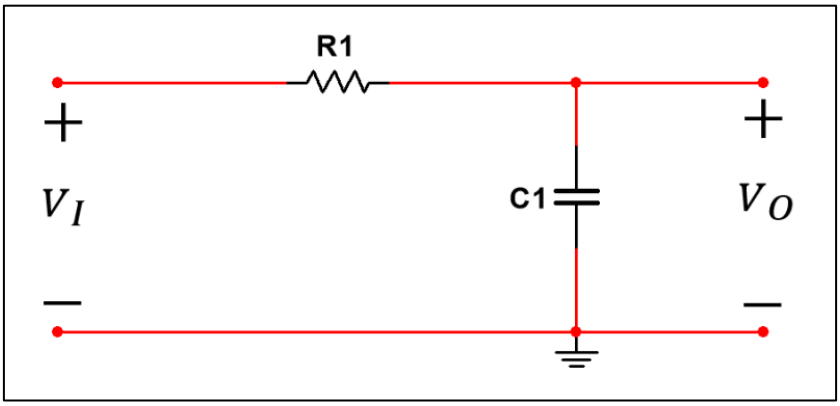

Figure 4. RC circuit

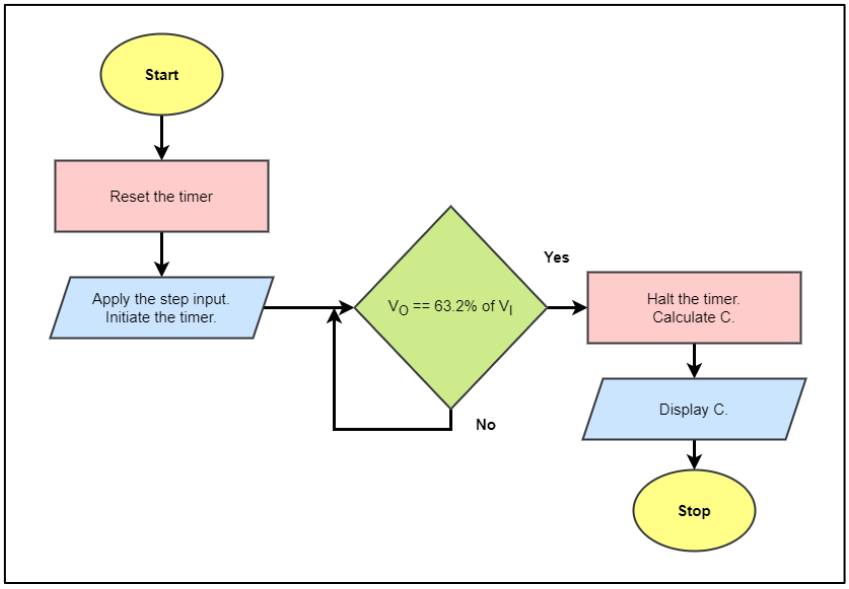

Figure 5. Algorithm for measuring the sensor capacitance

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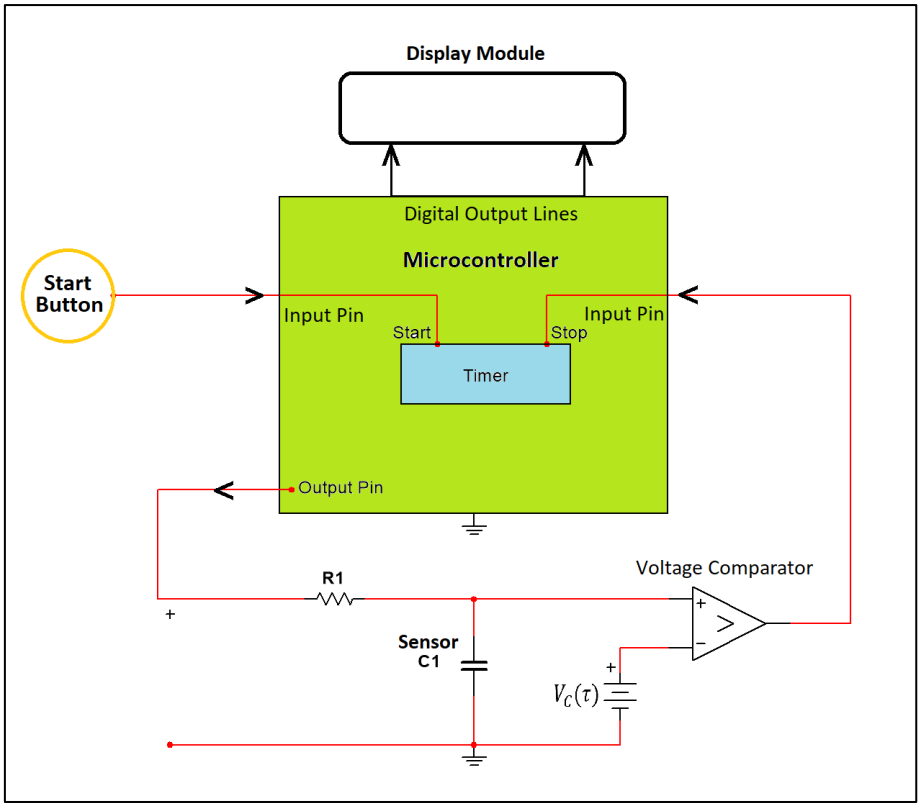

Figure 6. Complete schematic of CMC.

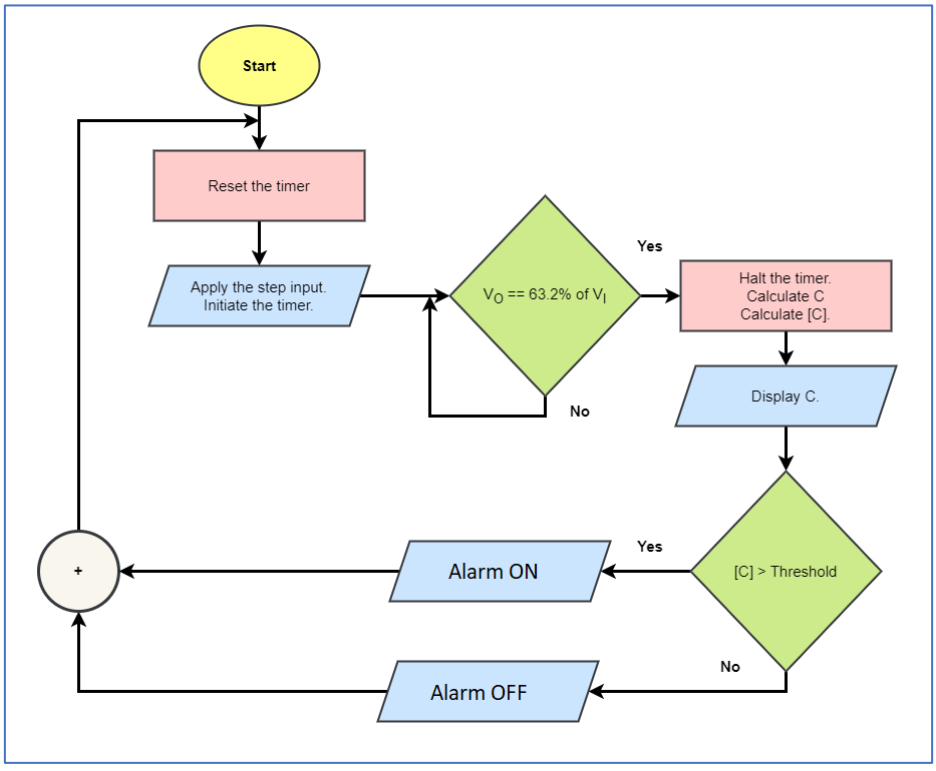

Figure 7: Real-time E.coli concentration measuring algorithm.

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2.4 System Implementation

This section presented the implementation details of the system, featuring the practical circuit for the proposed design shown in **Figure 8**.

Several modifications and new components have been incorporated. To enable the use of operational amplifiers with moderate slew rate, a significantly large resistor, R1, was selected to slow down the capacitor charging process. However, this decision gave rise to two issues.
The first problem is related to the input impedance of the comparator, which acts as a parallel resistor connected to the capacitor, thereby altering the circuit's time constant. Unfortunately, comparators with very high input impedance in the Giga Ohms range are not readily available. The second issue arises from noise interference across the large resistor, essentially causing it to act as an antenna.

To tackle these problems, an instrumentation amplifier was employed. It is connected across the capacitor terminals to measure the voltage difference. This configuration serves as a voltage buffer by providing a very high input impedance (in the Giga Ohms range) between the capacitor output and the voltage comparator (Borkholder.,1998). Additionally, it ensures a high common mode rejection ratio, effectively eliminating common noises that may appear at the two inputs (Firstenberg-Eden et al.,1984). To maintain the noise balance between the two input terminals, another resistor (R2), with the same value as R1 is connected between the inverting terminal and the ground.

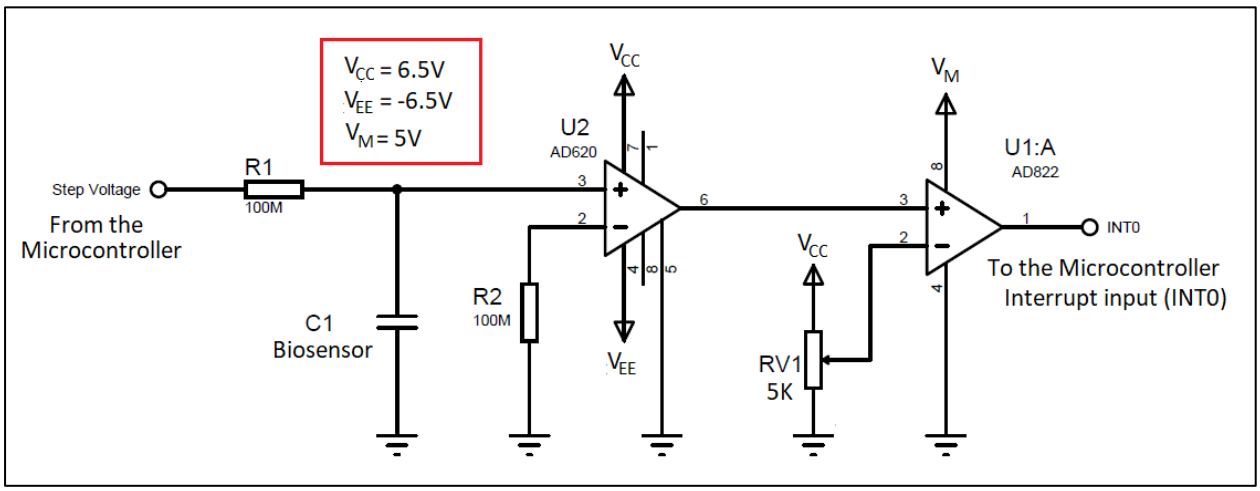

Figure 8. Practical conditioning circuit in the CMC.

Figure 9 displays the schematic diagram of the microcontroller, while Figure 10 illustrates the algorithm implemented within the microcontroller. The microcontroller is responsible for measuring the concentration of *E.coli* every 50ms. As mentioned earlier, it applies a step input voltage of (3.6 V) and initiates a digital timer simultaneously. The microcontroller then waits for an interrupt signal to be received from the voltage comparator, with the comparator output connected to the external interrupt pin (INT0). The comparator negative terminal is adjusted at 2.27 V using potentiometer (RV1).

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At this point, the biosensor capacitor has reached 63.2% of its final value (2.27 V), prompting the microcontroller to stop the timer. The capacitance is then calculated using equation (3). Subsequently, the E.coli bacteria can be determined using C-[C] relation. Finally checking if the concentration exceeds the threshold value or not (Figure 10).

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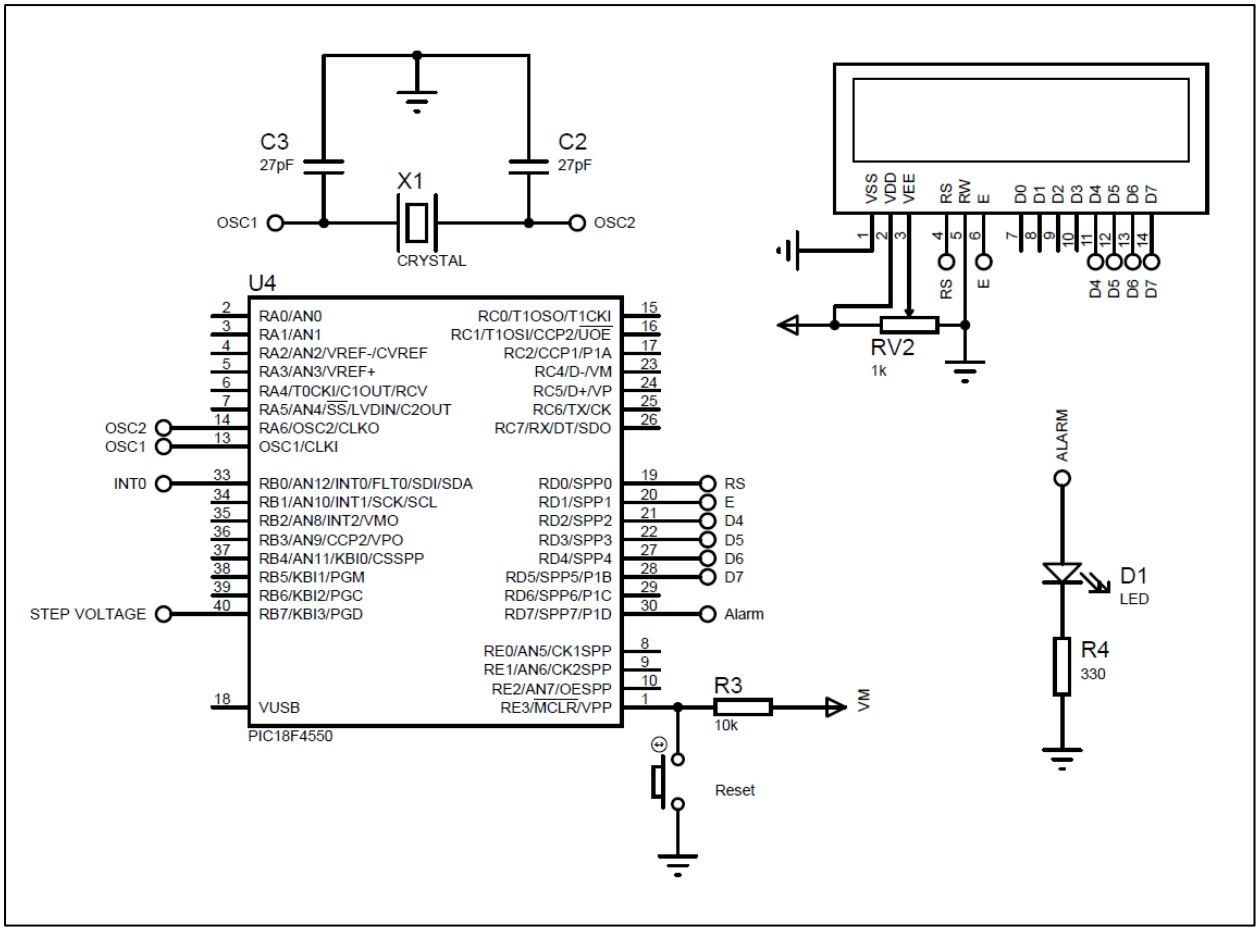

Figure 9. Microcontroller schematic diagram with display module.

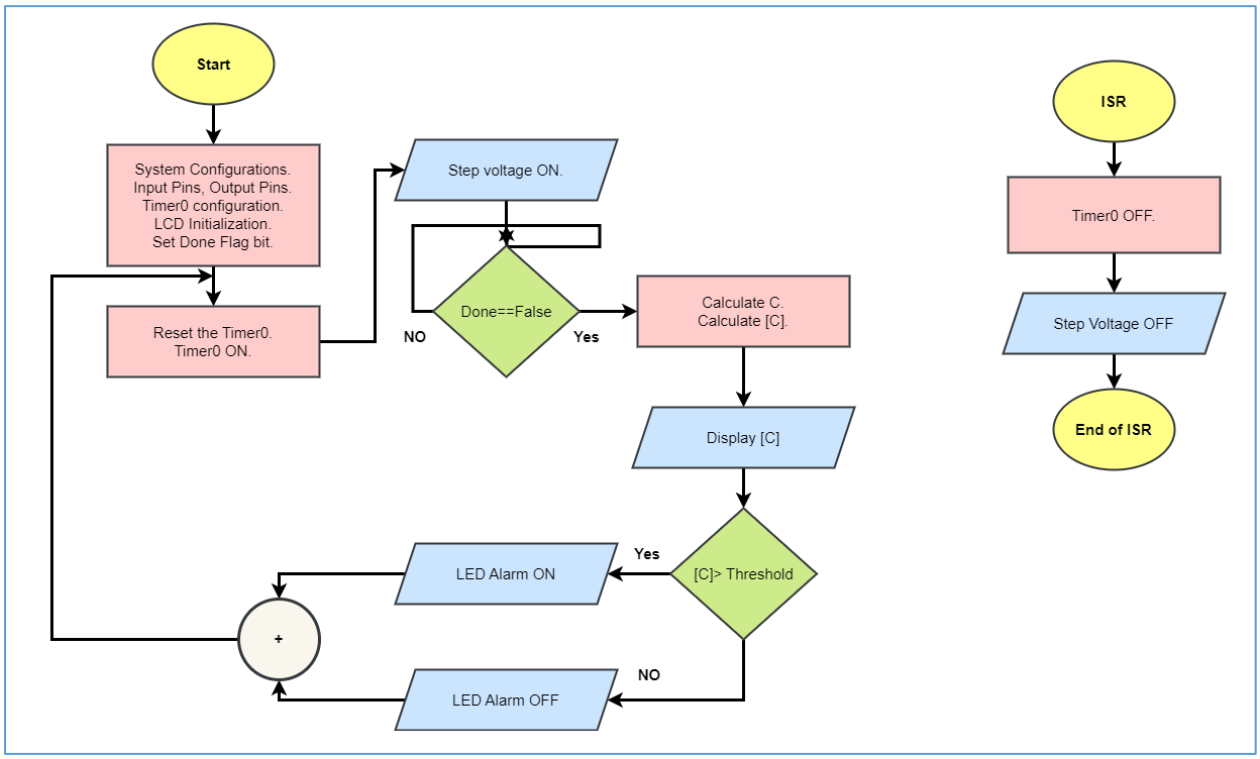

Figure 10. *E.coli* concentration and detection algorithm within the microcontroller.

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3. Results and discussion

Through this study, a design for capacitive biosensor and a bacteria concentration and detection system is discussed in details. In this section the results of system validation and *E.coli* detection are presented.

3.1 Capacitance Measuring Circuit

In the initial phase, the capacitance measuring circuit underwent testing. This involved measuring the capacitance of a known capacitor (22pF) and examining the output of each stage in the process.
Figure 11 depicts the output of the instrumentation amplifier circuit, represented by the blue signal. This output reflects the voltage across the capacitor when a 3.6 V step input voltage, indicated by the yellow signal, is applied. Notably, it is evident that the final value of the output aligns with the step input after approximately 5τ. In Figure 12, the blue signal represents the outputs of the voltage comparator, while the yellow signal represents the input signal. It is evident that the output signal is delayed by approximately 2.5 ms from the input signal. This delay corresponds to the time required to charge the capacitor to one time constant. According to these results, the capacitance obtained to be:

$$C = \frac{\tau}{R} = \frac{2.5ms}{100M} = 25pF$$

It is important to highlight that the input capacitance of the instrumentation amplifier, approximately 2pF, functioned as a parallel capacitor to the biosensor. As a result, the measured capacitance of 23pF closely approximates the actual capacitance. In conclusion, the microcontroller displays a capacitance value of 23.37pF. The results obtained demonstrate that the capacitance measuring circuit is capable of accurately measuring capacitance within the low pF range, while maintaining minimal noise interference and without introducing any signal distortion.

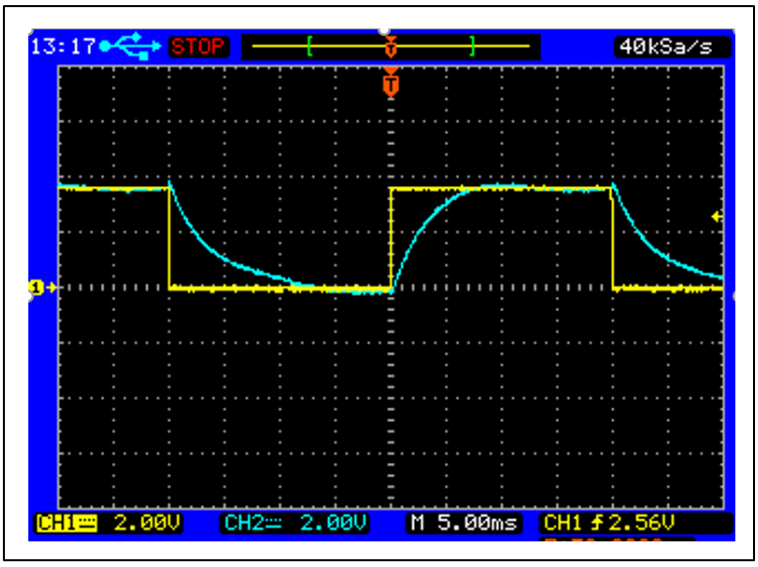

Figure 11. Output voltage of the first stage (Blue trace) in response to a step input (Yellow trace).

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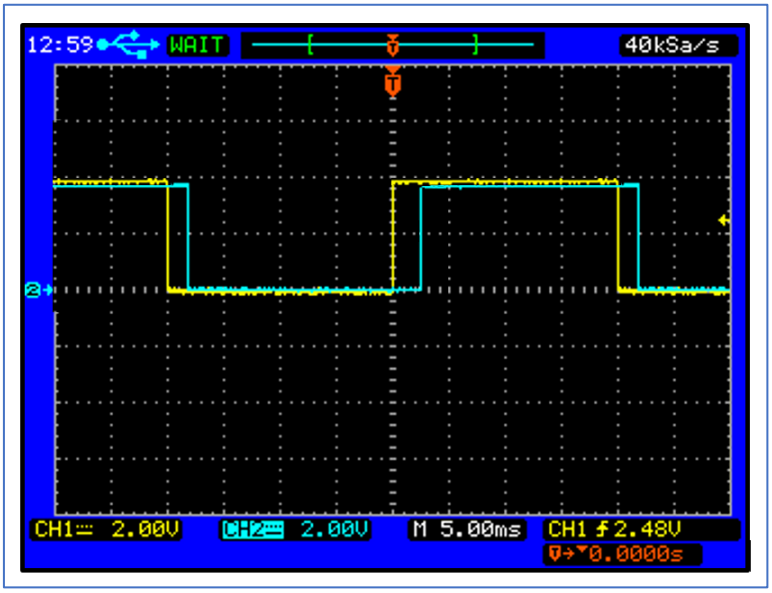

Figure 12. Output of the voltage comparator circuit (Blue trace).

3.2 Capacitance-Concentration Relation

To explore the correlation between biosensor capacitance and *E.coli* concentration, several samples were prepared to analyse the capacitance behaviour. Each sample comprised distilled water with a known concentration of *E.coli*. The concentration of *E.coli* in each sample was initially measured using spectrophotometry. Subsequently, the same sample was applied to the biosensor, and the system designed for this study conducted capacitance measurements. This procedure was repeated for multiple samples, and table 1 presents the corresponding capacitance values for each sample.

Table 1
Biosensor capacitance for each E.coli concentration

	S1	S2	S3	S4	S5
$[C](unit)$	0	0.291	1.27	5.596	7.975
$C\ (pF)$	14	32	25	20.5	20

Key: S1 = sample#1 S2 = sample#2 S3 = sample# S4 = sample#4 S5 = sample#5

The aforementioned results indicate a significant impact of *E.coli* presence on capacitance, with an inverse relationship observed between E.coli concentration and biosensor capacitance. This relationship can be characterized as logarithmic. Notably, the capacitance demonstrates a sharp fluctuation at the initial stages. This phenomenon can be attributed to the abrupt alteration in the dielectric properties of distilled water. Additionally, other factors that were not investigated in this study may also influence the dielectric constant. For instance, the distribution of bacteria within the water sample, temperature variations, or the impact of electric fields on the E.coli membrane could all play a role. Based on the current findings, it has been demonstrated that the presence of

bacteria in distilled water can be successfully detected. This outcome has significant implications for minimizing water contamination and preventing the consumption of water contaminated with *E.coli*.

The authors in (Rydosz et al.,2016) introduce a novel label-free microwave sensor coated with T4 bacteriophage gp37 adhesion. This sensor is highly sensitive and can detect *Escherichia coli* B (*E. coli* B) by recognizing its bacterial host lipopolysaccharide (LPS). The change of the sensors' capacitance and conductance as a subject to LPS presence is an indicator of the detection. The measured changes are well above the limit of the detection. The presented method requires a special fabrication techniques and high-frequency costly microwave capacitance detection devices.
Another study presents an aptamer-functionalized capacitance sensor array capable of real-time monitoring of bacterial growth and antibiotic susceptibility. The sensor array exhibited increasing capacitance over time when culturing *E. coli* and *S. aureus*, even at a low bacterial concentration of 10 CFU/ml.
With the selectivity of aptamers, bacteria could be identified within one hour using the aptamer-functionalized capacitance sensor array. This method requires at least one hour to detection bacteria existence. Furthermore, it requires a special capacitor sensor fabricated with sensitive materials (Jo et al.,2018).

4. Conclusions

In this study, the concentration measurements and real-time detection of *E.coli* using a large scale capacitance biosensor was performed and developed. The practical results showed that's the capacitance biosensor capable of sensing the existence of *E.coli* suspended in water. Furthermore, the results demonstrated the biosensor ability to measure *E.coli* concentration in real-time. The results determined a correlation between the capacitance measurement and the real bacterial concentration which indicate an inversely relationship between the bacterial concentration and the capacitance measurement.
Additionally, a real-time *E.coli* detection system was designed and implemented, primarily incorporating a capacitance measuring circuit. The validation results of the system confirmed its ability to measure the sensor capacitance in real-time.
In conclusion, the effectiveness of the proposed capacitance biosensor, which is large-scale, low-cost, and simple, was revealed. It has the potential to be highly effective in real-time detection of sudden bacterial contaminations in drinking water. Moreover, the biosensor showed promise for low-cost and on-site monitoring of *E.coli* growth.

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