Detection of Escherichia coli and Enterococcus faecalis growth in Ice from Commercial Beverages using Impedance Microbiology and Image Processing Technique

by

Kzandra H. Katigbak
Bradly L. Lomotan
Areej Gabrielle R. Rollon
Hazel Wynne D. Tangonan

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ABSTRACT

Various drinks served in different establishment always come with ice. Ice is the main component in preparing cold drinks- it also bring benefits to human health. However, due to poor sanitation in ice manufacturing, it can also put human health at risk. Escherichia coli and Enterococcus faecalis are the most common bacteria that surrounds the ice. Too much intake of contaminated ice caused by these two bacteria can lead to serious illness. On this study, the researchers presented a prototype that detects the growth of the bacteria, Escherichia coli and Enterococcus faecalis, using impedance microbiology and image processing. In addition, Graphic User Interface (GUI) was also be implemented in this study. GUI displays the captured images of the different phases of the sample and the concentration of bacteria present.

Keywords: ice, impedance microbiology, Escherichia coli, Enterococcus faecalis, image processing, GUI

AUTHORS' BACKGROUND

Your Name	Title*	Research Field	Personal website
Jessie R. Balbin	Full Professor	Signal Processing and Control Systems	jayebalbin@gmail.com
Ernesto Vergara	Full Professor	Signal Processing and Control Systems	emvergara@mapua.edu.ph
Kzandra Katigbak	Student	Signal Processing	khkatigbak@gmail.com
Bradly Lomotan	Student	Signal Processing	bradlyleelomotanv2@gmail.com
Areej Gabrielle Rollon	Student	Signal Processing	areej.gabrielle9@gmail.com
Hazel Wynne Tangonan	Student	Signal Processing	hwdtangonan@gmail.com

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*Title can be chosen from: master student, PhD candidate, assistant professor, lecturer, senior lecturer, associate professor, full professor

Chapter 1

INTRODUCTION

The Philippines is known for its tropical weather. During summer, people tend to seek different ways to cool down and one of the best way is having a cold drink. Filipinos prefer to put ice in their drinks to quench their thirst during sweltering summer days because it is more economical, rather than buying cold drinks served on different establishments that would cost them an amount of money. Due to variation in temperature, the perception of flavor in food and drinks changes. Moreover, ice can also bring danger to human health, in June of 2017, a diarrhea outbreak at the New Bilibid Prison affected 1,212 inmates due to drinking contaminated water. It was also recorded that two of the inmates died because of dehydration and hypovolemic shock. The problem that the researchers would like to address is the unavailability of having a device that can detect the presence of Escherichia coli and Enterococcus faecalis to test the purity of ice. Different food establishments, including carinderias, serve their beverages with ice, however these ice used does not have the assurance of being free from contamination. Any bacteria present in the ice of the beverage can pose a dangerous health risk to human, such as intestinal infection.

A study by Babaan, Poblete, et al entitled "Detection and Classification of Bacteria in Common Street Foods using Electronic Nose and Support Vector Machine" states that Staphylococcus aureus, Enterococcus faecalis and Escherichia coli bacteria are present in street foods sold in the Philippines. Street food cart also offers a wide range of juices. These juices are always served or mixed with ice. Moreover, a study entitled "Enteric bacteria of food ice and

their survival in alcoholic beverages and soft drinks", by Gaglio, Francesca, et al, published in 2017 made at University College Cork, Ireland tested the levels of enteric bacteria that are present in ice cubes that are subjected to alcoholic and carbonated beverages. According to the study, almost all of the ice cubes are contaminated with Enterobacteriaceae while most of the industrial and home made ice cubes are not contaminated with coliforms. The presence of pathogenic microorganisms is attributed to the contamination of the water used in making the ice cubes. Poor sanitation conditions during production and improper handling or packaging also contributes to the contamination of ice cubes. The other types of bacteria can be killed in boiling temperatures. This is why it is safer to consume pre boiled water.

This study will mainly concentrate on coliform bacteria found in ice, specifically Escherichia coli and Enterococcus faecalis. In the Philippines, there is no available device that can test the purity of ice. In this study, the ice will be incubated from a self-developed incubator and will cultivate the bacteria present in the ice. Captured images from a low cost camera will be go through image processing in order to measure the bacterial growth of the sample. The study will also be integrating a Graphic User Interface (GUI) embedded with Raspberry Pi.

The main objective of the study is to detect Escherichia coli and Enterococcus faecalis growth in ice from commercial beverages using impedance microbiology and image processing technique. In addition, the following are the specific objectives of the study, (1) to develop a hardware system that will incubate, measure the impedance, and capture images of a cultured sample taken from commercial beverages' ice, (2) to develop a software, that will process the raw data in detecting the presence of Escherichia coli and Enterococcus faecalis and measure the bacterial concentration in the cultured samples using impedance microbiology and image

processing, (3) to test different ice packages of commercial beverages and in different areas specifically in street food, food courts, fast food chains and etc. and lastly, and (4) to assess the result by comparing it to the actual laboratory result.

The study will raise awareness to consumers of the levels of unknown bacteria present in ice that is used in different beverages sold commercially. The device indicates the level of harmful bacteria present in the melted ice cubes. Consuming food that is contaminated with Escherichia coli and Enterococcus faecalis can arise to immediate health risk. It can cause bloody diarrhea, anemia and even kidney failure. This will also be of good benefit to individuals, especially Filipinos, who monitors their health regularly. The Department of Health and the Food and Drug Administration of the Philippines would benefit from the study as they can use the system for identifying contamination in ice so that they will have the ability to regulate and ensure the safety in ice packages sold in the Philippines.

The study is limited only in testing Escherichia coli and Enterococcus faecalis. These types of bacteria are the common type of bacteria found in untreated water that may cause different harmful effect to humans. All the ice that will be used in the study will be coming from any food establishment including the streets. The effectiveness of the detection of bacteria using image processing will significantly depend on the algorithm used. The GUI will show the microscopic image that was took from the cultured bacteria sample from the melted ice and will indicate the bacteria present.

Chapter 2

REVIEW OF RELATED LITERATURE

2.1 Bacteria

Bacteria are a single-cell organism and prokaryotic cell. It is among the first life forms to emerge on the planet. Bacteria have small loops of DNA also known as plasmids, that can be transferred from one cell to another, either by viruses or intercourse with another bacteria [1]. Having the ability to transmit and receive genes, this makes bacteria adaptable. Bacteria are favorites of molecular biology and genetics engineers given that new genes can easily be inserted into bacteria. It reproduces through binary fission, where a single cell will split into two identical cell. A single bacterial cell could produce more than sixteen million progeny in eight hours [2].

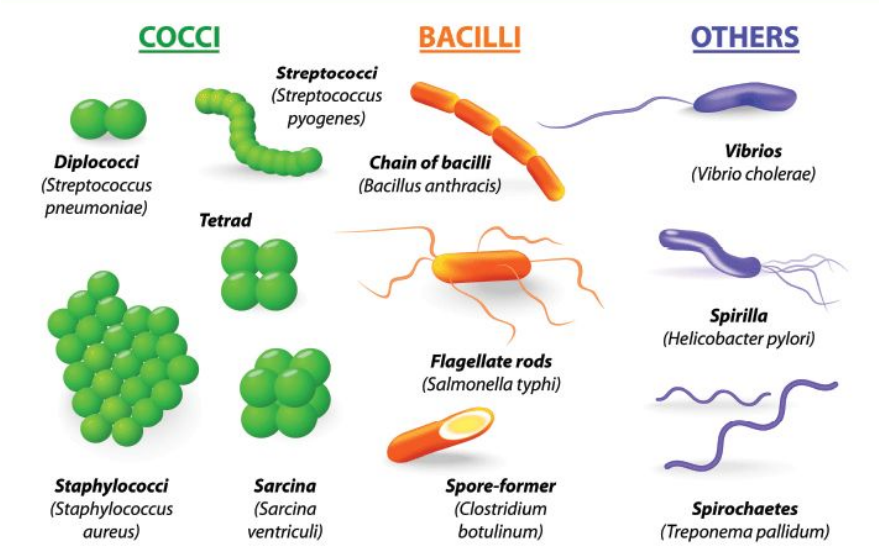


Figure 2.1 Types of Bacteria

2.1.1 Bacterial Growth

Bacterial growth refers to the increase in terms of amount of bacteria present in the controlled incubated environment. In other terms, it is based on proliferation or multiplication. A bacteria sample continues to increase exponentially until a bacterial colony is produced. Generation time is the amount of time needed by one cell to become two cells by using binary fission. It varies from different types of bacteria. Each microorganism requires an optimum and minimum growth temperature. They also require a certain pH level and within this range of temperature and pH level, bacteria can grow and multiply at peak rate [3]. By using an appropriate medium in growing the bacteria, Escherichia coli and Enterococcus faecalis, its bacterial concentration can be estimated within or as low as 3 hours, within this estimated time the sample is considered to be highly contaminated or unsafe [4].

In growing the bacteria, it goes through four different phases namely; lag phase, log phase, maximal stationary phase and death phase. During the lag phase, the bacteria are adjusting itself to the environment, thus growing its size but does not multiply. The cells are replicating their proteins and DNA in preparation for the next phase. The next phase is log phase wherein, the bacterial cells are doubling at a constant exponential rate. The process during the second stage will last until the nutrients present in the medium are already depleted. The third phase is also known as the stationary phase. In this phase, the population growth of the bacteria levels to the growth in dead cells. The finals phase of the bacterial growth is the death phase. Various factor contributed to the death of bacteria, moreover in this phase, the dead bacteria surpass the number of living cells.

2.1.1.1 Growth Rate

The growth rate of bacteria depends on the standard nutritional conditions that also affects the bacterium's generation time. The generation time for bacteria varies depending on the culture medium used. For most common bacteria, the generation time ranges only from 15 minutes to 1 hour [5].

2.1.1.2 Plate Count Agar

Standard Methods Agar is a modified formulation of Plate Count Agar. It is a growth medium used to assess the growth of bacteria in a sample. Yale University suggests to use this standardized medium in culturing bacteria because it is more efficient when performing a plate count on the sample [6].

2.1.1.3 Total Plate Count

Total plate count, also referred to as Heterotrophic Plate Count (HPC) is not only used to assess the quality of water, but also to determine the changes that occurred during the storage process and the distribution of the bacterial growth, in addition, this is also used to examine the efficiency of water treatment process [7]. Hence, this process is commonly used as an indirect indication of pathogen removal, it also indicates whether the number of regrowth organisms may have or may not have sanitary significance. However, it also has its downside, according to the World Health Organization, HPC should not be use to indicate the safety of water because it does not advertise heterotrophic bacteria found in water, instead it highlights the bacteria that can be cultivated [8].

2.1.2 Coliform Bacteria

Coliforms are types of bacteria that resides in the large intestine of warm-blooded animals. It is commonly present in the environment in the form of excretion from animals and humans. Their presence in consumable products can pose a threat to humans. The common type of bacteria in coliforms group are the Escherichia coli and Enterococcus faecalis that can be found mostly in feces. In accordance in testing drinking water, Environmental Protection Agency (EPA) designates a number of coliform bacteria as a standard to ensure the safety of water because of this, the agency established standards for drinking water which are categorized in two: Primary Standards and Secondary Standards [9]. During wet season, these two types of bacteria might be washed away in bodies of water. When the waters are used as source of drinking water and it is not treated properly, the bacteria may end up in the drinking water [10].

2.1.2.1 Escherichia coli (E.coli)

Escherichia coli is a member of the coliforms group and it is often found in the intestine of humans and animals. E.coli is also used for protein expression, for it is more economical, easy to alter and yields high protein. It also enhances the reproductive, digestive and immune health of the human body. However, strains of this kind of bacteria can cause health problems such as, diarrheal disease including Cholera, Typhoid and Dysentery, urinary infection, abdominal pains and fever [11]. Based from the Primary Standard of the Environmental Protection Agency, E.coli should not be detected in a 100mL of drinking water [10]. Sterilized agar is the standard growth medium used in culturing E.coli. And studies suggests that the sample should be place in an

incubator with constant temperature of 37.4 degrees Celsius, the ideal temperature in culturing E.coli [12].

Figure 2.3 Microscopic image of E. coli after Gram staining.

2.1.2.2 Enterococcus faecalis (E.faecalis)

Enterococcus bacteria are known for not living or growing whenever oxygen is present, this characteristic is called anaerobic. This type of bacteria is cultured in high concentration of salt (6.5% NaCl) and has the capability of living in 60 degrees Celsius for short period of time [13]. The illness caused by this bacteria is difficult to administer due to its resistance to antibiotics. Due to Enterococcus' drug-resistance characteristic, healthcare professionals develop various drug options such as ampicillin, tigecycline, daptomycin, linezolid, nitrofurantoin and vancomycin [14].

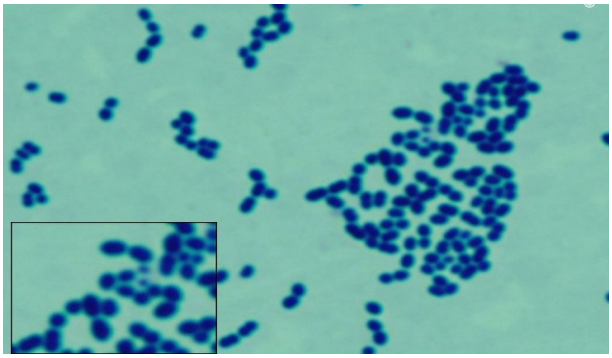


Figure 2.4 Microscopic image of E. faecalis after Gram staining

2.2 Ice

In the Philippines, homemade ice are most commonly made from tap water. Former Philippine Health Secretary, Enrique Oña, said that clear water does not indicate that it is free from impurities, he added that it is not advisable to drink tap water unless it is boiled for safety [14]. The pipes that carries the water supply could also be contaminated or damaged. Other sources of contamination that is related to ice production can come from unsanitary equipments, improper handling, and exposure to unhygienic conditions. This could cause different pathogens to contaminate ice. [15]. In fast food chains and restaurants, many news have emerged that most of the ice used in commercial beverages are contaminated with E. Coli and coliforms. In the study conducted in California State Science Fair, they compared the ice created from the self-serve machine and the drive-thru ice and incubated it for 96 hours. Based on the data recorded, the drive thru ice has a higher bacterial growth than the ice created in a self-serve machine. Mishandling of the ice by the employers is one of the major contributors why the bacterial growth in drive-thru ice is much higher than the ice created in self serve machine.

2.2.1 Benefits of Drinking Cold Beverages

As we all know, drinking a hot and cold beverage have a lot of benefits. One of the benefits in drinking cold beverages is that it can lower the body temperature. It can also increase the metabolism which can help in food digestion [16]. In the study written in Germany that was published in the Journal of Clinical Endocrinology and Metabolism, drinking one-liter of ice-cold water a day can burn 50 calories [17]. Drinking cold water also stimulates adrenaline release. This adrenaline release is essential in triggering the brain to send a "fight-or-flight reaction". Drinking cold refreshments can also switch parasympathetic nervous system to sympathetic nervous system making an individual more alert[18].

2.3 Impedance Microbiology

In impedance microbiology, the cultivated medium's electrical characteristics is tested. This means that the system relies on the changes of the conductivity of the test medium brought by microbial growth. Pure water has low conductivity level due to the relatively small number of ions. Only adding substances that dissociates into charged ions can change the conductivity of water [19]. Live bacteria releases ionic metabolites that can be measured by an electrode. The electrode is directly connected to the sample medium [20]. The electrode must be isolated from the system to prevent the charging of the sample-electrode that will cause parameter drift, and long time for baseline stabilization meaning it is difficult to calculate the Detect Time. A five percent change of the bacterial content from the initial value will indicate that the sample tested is contaminated with bacteria [21]. The sample is subjected to a constant temperature that ranges from 30◦- 42◦C. The temperature must be held exactly constant. Each 1◦ change in temperature

will result to 1.8% change in the conductance of the solution [19]. A threshold value is set, which is generally in the order of 107 cfu/ml. The time the bacterial growth reaches this value, the initial concentration of bacteria can easily be computed. As the bacterial concentration increase to its threshold value, its impedance, resistance and reactance measured value plotted against time increases. After the threshold value is reached, these values starts to drop [22].

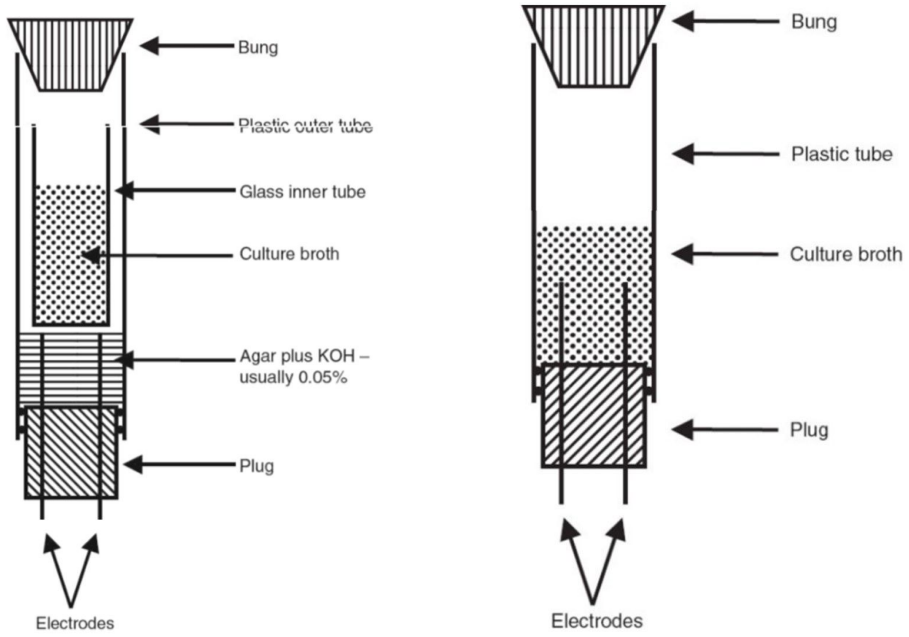


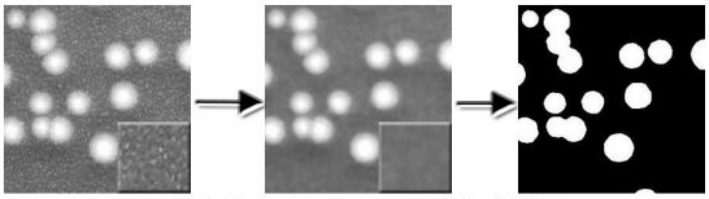
Figure 2.5 Direct impedance technique cell (left) and indirect impedance technique cell (right)

2.4. Standard Plate Count

Standard Plate Count is the process of determining the number of bacterial colonies present with a growth nutrient medium (agar) that is cultured in a controlled environment by the process of incubation. In the traditional laboratory process, the colonies are counted manually and the result is expressed in colony forming units (C.F.U)/g or /mL. The process can also help determine the microbial quality and spoilage level of the test object. A high SPC count may

indicate poor sanitation, inappropriate storage, or problems with the process control. The effectiveness of the SPC data is based on the number of microbes that grows on the specific medium under specific growing conditions. Bacterial colonies can either be too small or it can be overcrowded and may form a cluster of several colonies clumped together. This considerations can be the cause of error in the SPC count [23].

2.5. Image Processing



Pattern detector steps

2.6 Image enhancement

Image processing is an image analysis that examines the image data to solve an imaging problem. Image analysis methods includes major components of a computer vision system, where the system is used to analyze the the images and produce a results. In the development of image processing related to human vision, many images is needed for testing in order to train the system of the application [24].

2.5.1 Noise Filtering

Noise filtering is the process of removing the unwanted noisy component from received signal in order to restore the original data without sacrificing the images' other features [25]. In some cases, noise reduces the quality of the image specially when images are small and has low

contrast level. In this paper, median filtering method is used for removing the unwanted noise in the image produced from the camera installed inside the device.

Median filter is used as the noise filtering process. It is a nonlinear signal processing technology that is based on statistics. The noisy pixel value of the image is replaced by the median value of the neighboring pixel. The values of the pixels are ranked based on their gray levels, the median value is used in order to replace the noisy pixel values.

In the mask, the maximum value of the gray levels of the picture is termed as max, the minimum value of the gray levels of the picture is termed as min, average value is termed as average and median value is med [26]. The adoptive filtering requires two steps:

(1) Initialization: let n=3 (n is the mask size)

(2) Computation: A1 = med-min, A2=med-max

(3) (Judgment: if A1 > 0 and A2 < 0, then turn to the step 2; if not, then enlarge the size of the mask.

2.5.2 Colony Counter

Colony counter is the algorithm that performs splitting and counting operations. The problem in automated colony counting is that initial outputs are not accurate. Some clusters are mistaken for a single colony. Other large clusters of colonies has more than two actual colonies.

Circularity Ratio is used for determining the patterns of thee bacteria inside the petri dish. Circularity ratio is defined as the comparison of the area of the closed shape to the area of a circle. Equation 1 will be used in order to compute for the circularity ratio of the closed shapes

detected in the image. By thresholding the ratio for the shape, the pattern classifier would be able to separate colonies and clusters of colonies.

$$CR = 4\pi \text{ (area)} / \text{(perimeter) } 2 \qquad \textit{Equation 1}$$

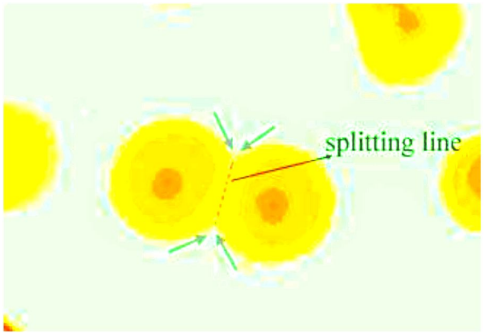


Figure 2.11 Splitting of bacterial clusters

In this paper, Watershed Segmentation algorithm is used for splitting colony clusters. The watershed transform is a region-based segmentation approach. It is based on a landscape that is flooded by water. The watersheds are the divide lines that is similar to the image of rain falling over the region [27]. In using this algorithm, either the basin or the partition can be recognized first. This is done in order to determine the complete partition of the object.Watershed transform is the term used in order to define all the points of a given catchment basin have the same unique label, and a special label, distinct from all the labels of the catchment basins, is assigned to all points of the watersheds [28].

In order to improve efficiency, the pattern classifier is used twice in the process. The image undergoes pattern classification for the second time after watershed segmentation. If the

blobs still failed to the circularity ratio, it will then be forwarded to resulter and marked as non separable. According to the paper "An Image-Processing Based Automated Bacteria Colony Cluster" this process is similar to the process followed by biologist, therefore this process is recommended for real life applications [29].

Resulter is the final process of the image processing system where the total number of colonies present are presented. The blobs undergoes a process where the small and circular colonies are recorded as one and the larger non-circular colonies (non separable clusters) are tested based on the ratio of the cluster area and the average colony area. The average colony area is automatically computed based on the most populated colony sizes present. Integer division is used in order to determine total colonies present in the cluster in order to minimize error [29].

2.5.3 Pattern Classifier

The definition of circularity ratio is the ratio of the area of the closed shape to the area of a circle (the most compact shape) having the same perimeter, mathematically given as; $CR = 4\pi (area) / (perimeter)^2$. As a shape deviates from circle to rather non convex shapes, the value decreases. By thresholding this ratio for each shape, the pattern classifier module separates patterns into 2 group; colonies, and clusters of colonies. In order to improve efficiency, the pattern classifier test is applied a second time after feeding to the watershed algorithm, which splits the non-circular shaped cluster to finer colony shapes. Following the second pass, a final compactness test is applied, but, this time, its output is no further processed by the watershed algorithm. Instead, if the compactness test still fails, indicating non circular clusters, they are marked as non separable, and they are sent to the module of "resulter", which calculates an estimate number of colonies using division of the area to a "normal" colony area. Due to personal communications with

biologists, this kind of an estimation process was recommended as a common practice for real life applications [29].

Chapter 3

METHODOLOGY

3.1 Introduction

This study will mainly concentrate on detecting coliform bacteria in ice, specifically Escherichia coli (E.coli) and Enterococcus faecalis (E.faecalis). In this study, the ice will be incubated in a self-developed incubator and the bacteria present in the ice will be cultured using medium that provides nutrients for the growth of bacteria. Captured images by the camera in an interval time will go through image processing in order to see the presence of the bacteria. The study will also be implementing a web server with Graphic User Interface (GUI) embedded with the Raspberry Pi Model 3B. This will raise awareness to the consumers regarding the concentration of unknown bacteria present in ice that is used in different beverages sold commercially.

3.2 Process flow

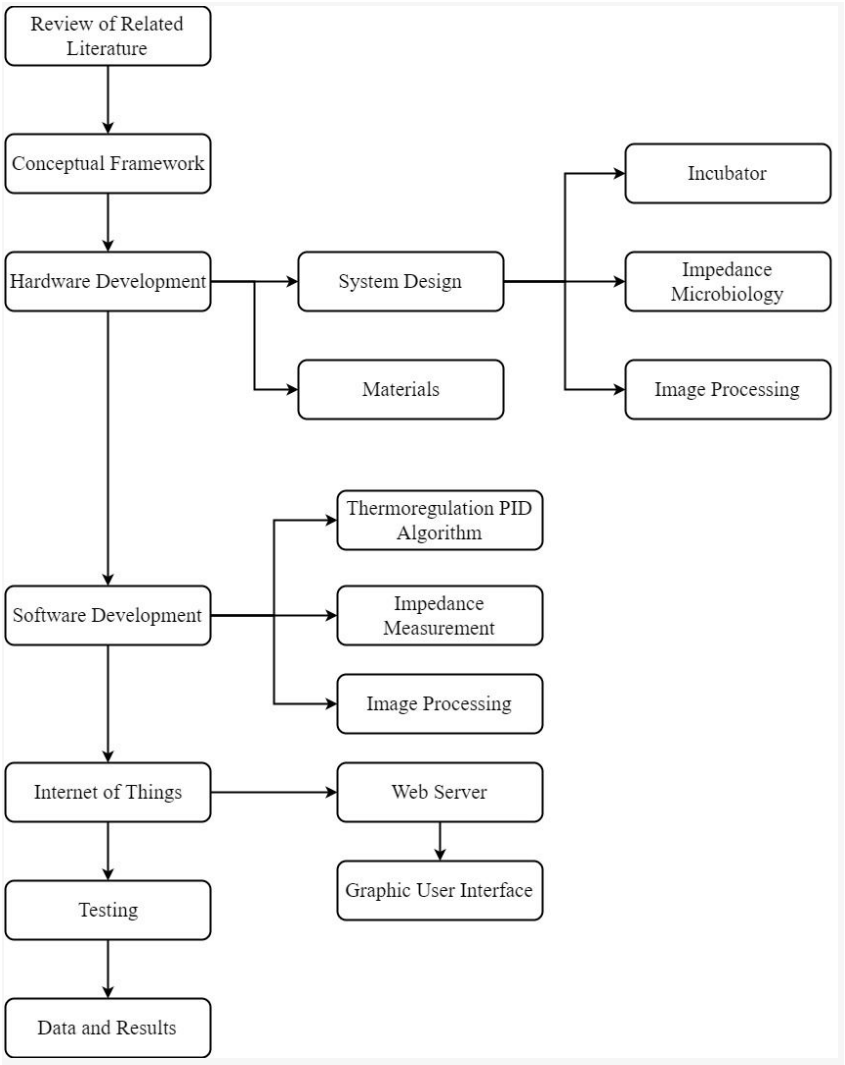


Fig 3.1 Detection of Escherichia coli and Enterococcus faecalis growth in Ice Process flow

The previous figure shows the flow of the development of the study into hardware of the prototype, the software of the prototype, testing and gathering of data.

3.3 Conceptual Framework

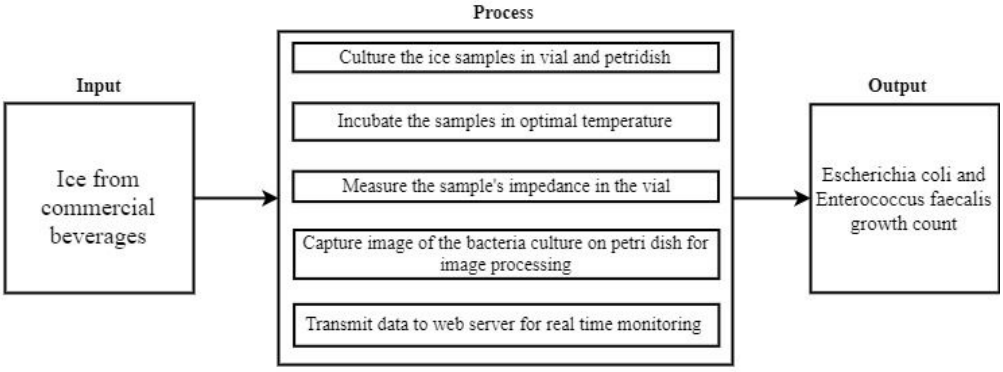


Fig 3.2 Detection of Escherichia coli and Enterococcus faecalis growth in Ice Conceptual Framework

Samples of ice from commercial beverages will be tested in order to determine its bacterial concentration. The process of incubation will be done in 5-24 hours for each sample of data depending on how contaminated is the sample in order to detect and determine the concentration of the bacteria present in ice. The temperature of the incubator will be held constant (37.4◦ C) to culture and preserve the bacteria inside. A petri dish and a vial with built-in electrodes will serve as the container of the sample. The container must be insulated in order to further regulate the temperature inside the incubator since the electrical properties of the bacteria are highly sensitive to temperature change. The impedance of the sample in the vial will be taken in every 5 minutes. Using a low cost camera, an image of the bacterial growth on a petri dish will be taken in a time interval and will be processed and developed through image processing. The gathered data are presented to the graphical user interface which can be accessed through the a web server.

3.4 Hardware Development

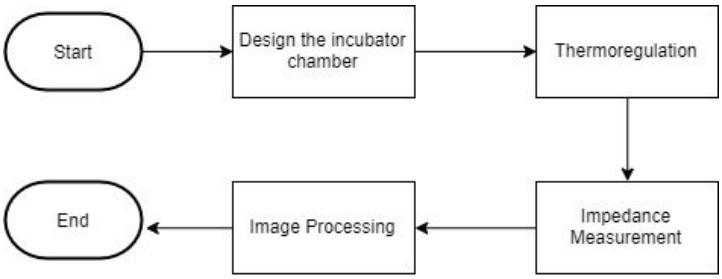


Fig. 3.3 Process Flow of Hardware Development

The hardware development includes the process of producing prototype device. The steps includes designing of the incubator chamber, thermoregulation, impedance measurement and image processing. The design of the incubator is illustrated at Figure 3.4.

3.4.1 Incubator Design

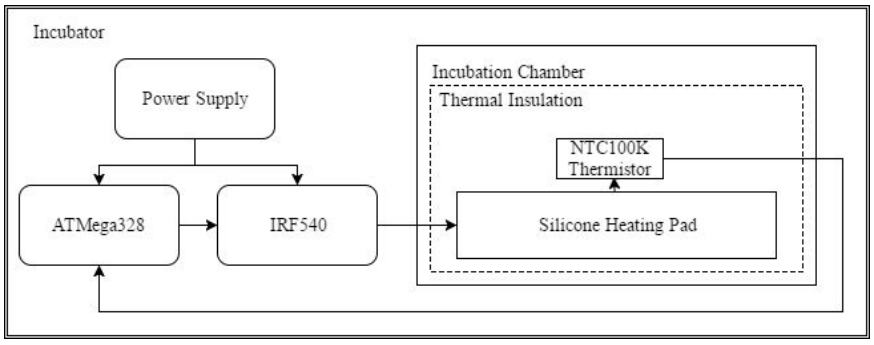


Fig. 3.4 Block Diagram of the Incubator

An incubator is a device that is used to maintain environmental condition suitable to the organism. This specific incubator does only regulate the temperature in the chamber. The inner walls of the chamber will be thermally insulated to improve the regulation of temperature. Its structure is 3D printed using an FDM printer in order to have the exact dimensions that we require. The electronics will be isolated from the incubation chamber.

3.4.2 Thermoregulation

The thermoregulation of the incubator will be done by an open-sourced ATMega328-based Microcontroller. A 12V 50W Silicone Heating Pad was used a means of increasing the temperature in the chamber, it has an integrated NTC100K Thermistor to measure the temperature. An N-Channel IRF540 MOSFET was used for switching the silicone heating pad instead of a mechanical relay because it has a faster switching characteristics suited for the logic control of the ATMega328 and PID. A copper tube was used to hold the vial to have a better thermal spread.

3.4.3 Impedance Measurement System

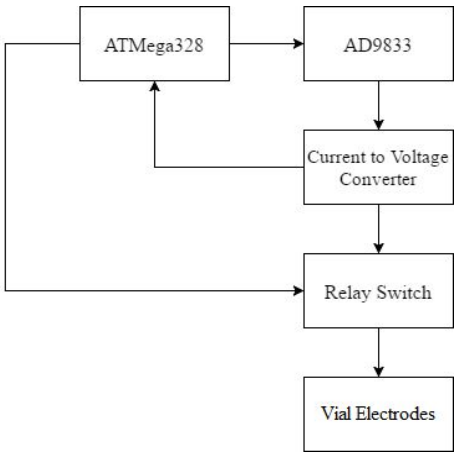


Fig. 3.5 Block Diagram of the Impedance Measurement Hardware

Aside from the thermoregulation, the ATMega328-based Microcontroller will also be responsible in measuring the the impedance of the sample. It will control the AD9833 to generate a 100mV peak-to-peak, 200 Hz sinusoidal wave test voltage (Vin) at a time interval of 5 minutes. Only when the electrical characteristics are being measured when the test

signal is being applied. A current to voltage converter is used to measure the Vout in order for us to calculate for the impedance. A relay was used to isolate the connection.

3.4.3.1 Vial Design

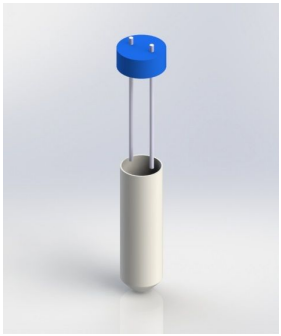


Figure 3.6 A modified 50mL Polypropylene Vial

The polypropylene vial was modified to accommodate a stainless steel electrodes. Stainless steel was used because there is a risked that the metal ions produced by the electrodes can be toxic to the bacteria present in the sample.

3.4.4 Image Processing Hardware

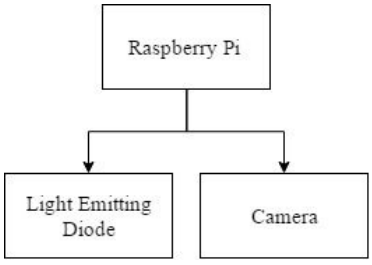


Fig. 3.7 Block Diagram of the Image Processing Hardware

The hardware components of the image processing setup are all controlled by the raspberry pi module. These materials includes the LED diode and the Camera. The purpose of

the led LED is to illuminate the petri dish when an image is need to be taken by the camera. This image will undergo image processing through the use of the Raspberry Pi.

3.4.5 Prototype Assembly

The image processing hardware and the impedance measurement hardware will communicate through the serial interface of the Raspberry Pi and Arduino Nano. As the Raspberry Pi would be responsible transferring the data to the internet.

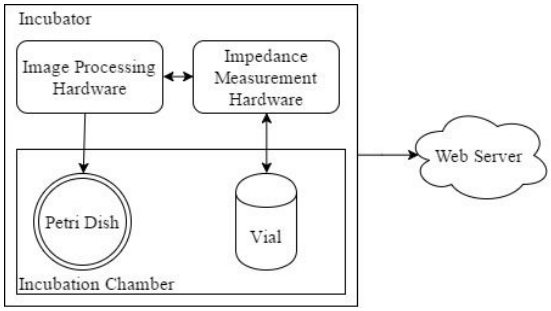
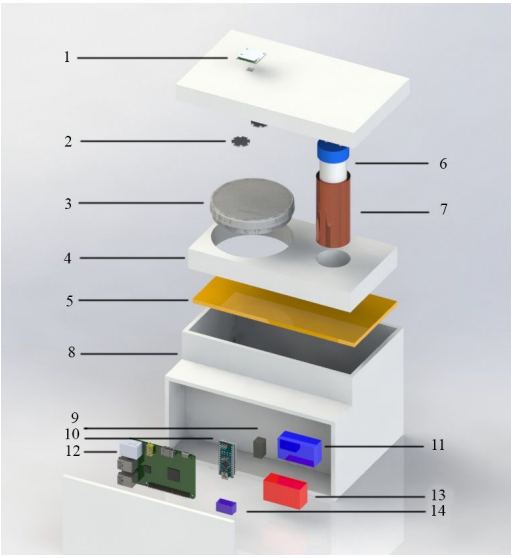


Fig. 3.8 Block Diagram of the Prototype Assembly



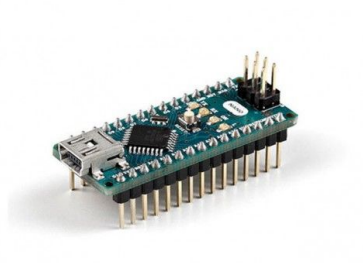
1) Raspberry Pi Camera module V2; 2) LED; 3) Petri dish; 4) Copper Holder; 5) Silicone Heating Pad; 6) Vial; 7) Copper Tube; 8) Incubator Chamber; 9) DPDT Relay 10) Arduino Nano; 11) Current to Voltage converter; 12) Raspberry Pi 13) MOSFET; 14) AD9833

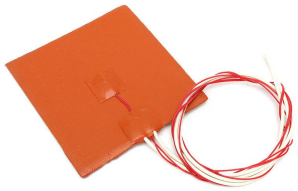
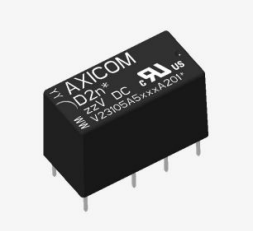
Figure 3.9 Exploded view of the assembly

A vial and a petri dish is inscribed in the incubator. The Image processing hardware are associated with the petri dish for the automated colony count, while the impedance measurement hardware are associated to the vial. The results gathered from the image processing and Impedance measurements are connected to the web server.

3.4.6 Materials

Table 3.1 List of Materials

COMPONENT	DESCRIPTION
Raspberry Pi 3 model B 	● Quad Core 1.2GHz Broadcom BCM2837 64bit CPU ● 1GB RAM ● 4 USB 2 ports ● 4 Pole stereo output and composite video port ● Upgraded switched Micro USB power source up to 2.5A
Raspberry Pi Camera module V2 	● Has a Sony IMX219 8-megapixel sensor ● Can be used to capture and take high definition images and videos. ● It supports 1080p30, 720p60 and VGA90 image and video modes ● Accessible through MMAL and V4L APIs
Arduino Nano 	● Microcontroller: ATmega328 ● Operating Voltage (logic level): 5 V ● Input Voltage (recommended):7-12 V ● Input Voltage (limits): 6-20 V ● Digital I/O Pins: 14 (of which 6 provide PWM output) ● DC Current per I/O Pin: 40 mA ● Flash Memory: 16 KB (ATmega168) or 32 KB (ATmega328) of which 2 KB used by bootloader ● SRAM: 2 KB (ATmega328) ● EEPROM: 512 bytes (ATmega168) or 1 KB (ATmega328) ● Clock Speed: 16 MHz

Silicone heating pad 	• Low thermal mass • Has an excellent electrical insulation. • The heaters can operate in wide range of temperatures (-76 ◦ F to 446 ◦ F). • Ability to profile the watt density • Excellent thermal control • Maximum Watt Density is 60 watts/in^2
AD9833 Waveform Generator 	• 12.65 mW power consumption at 3 V • 0 MHz to 12.5 MHz output frequency range • 28-bit resolution: 0.1 Hz at 25 MHz reference clock • Sinusoidal, triangular, and square wave outputs • 2.3 V to 5.5 V power supply • No external components required • 3-wire SPI interface • Extended temperature range: −40°C to +105°C • Power-down option 10-lead MSOP package
DPDT Relay 	• DPDT contact configuration • Monostable DC coils available based on ordering • Switching capacity of 3A at 220VDC, 250VAC • 1.5KV (10/700µs) between coil and contacts • Coil voltage range from 3VDC to 48VDC • 20mm x 10mm x 11mm size
1W Light Emitting Diode 	• Forward Current : 0.35 A • Peak Forward Current: 0.8 A • Reverse Voltage : 5 V • Power Dissipation: 1W • Electrostatic discharge: ±2000 V • Operation Temperature: -40~+80 ℃ • Storage Temperature: -40~+100 ℃
IRF540 MOSFET Module 	• 1-Controlling side: VCC(Signal+): 3~6V, no more than 6V • Maxium Frequency: 200KHz 2-Controlled side (Left side on the picture) : VCC: 10~100V DC • Maxium Current: 10A

Current to Voltage Converter Module 	• Size:45*25*18mm • Work voltage:DC 5-30V • Operating temperature:-20 ~ 60 Centigrade • Maximum error: 10mv • Accuracy: +/-0.2% • Response time: <10ms

3.5 Software Development

This shows how the process flow for the software design of the device.

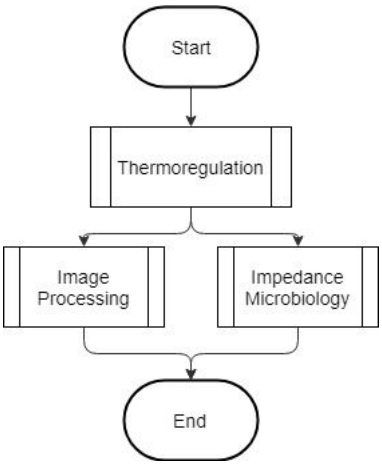


Figure 3.10 Flowchart of software development

Thermoregulation will keep the temperature inside the incubator at a constant temperature. Each 1。change in temperature will result to 1.8% change in the conductance of the solution. Image processing will be responsible for the automated colony count. Impedance Microbiology will measure the solutions bacterial concentration.

3.5.1 Thermoregulation

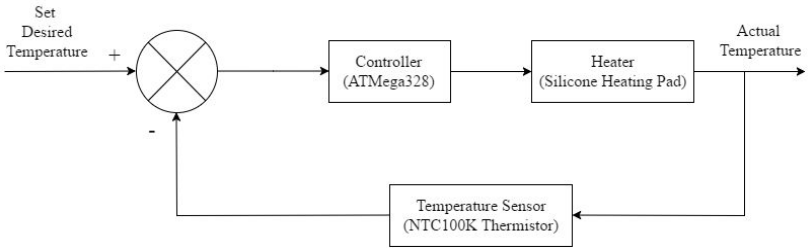


Fig. 3.11 Block Diagram of the Thermoregulation

The control of the temperature inside the chamber will require a closed-looped proportional - integral - derivative control to have the stability needed. A simple on and off closed-looped system would not be ideal for an incubator as there would be a large deviation in temperature. As the electrical characteristics of the bacteria are highly sensitive to temperature change.

The design project will be using the PID control system in regulating the temperature inside the incubator chamber. In PID, three terms are summed to produce a control output. These are the tuning parameters for the PID control: Kp, Ki, and Kd. Applying the PID control law consists of applying properly the sum of three types of control actions: the proportional action, an integral and derivative action.

3.5.2 Impedance Microbiology

This section discusses how impedance microbiology is utilized. The Flowchart below show the concept of impedance microbiology.

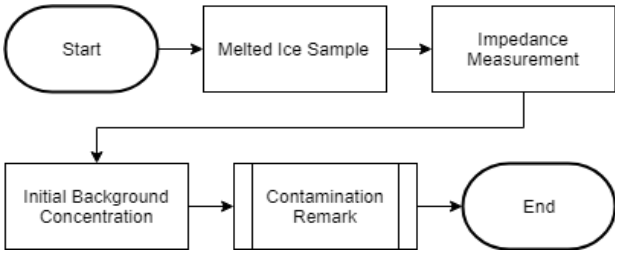


Figure 3.12 Flowchart of Impedance Microbiology

To start the process of impedance microbiology, a melted ice sample is instilled in a vial. This vial is designed to accommodate the electrodes for the impedance measurement. The bacteria multiplies exponentially therefore, its impedance measurement also changes exponentially. Knowing this, the initial bacteria concentration can be known in order to indicate whether the sample is contaminated or not.

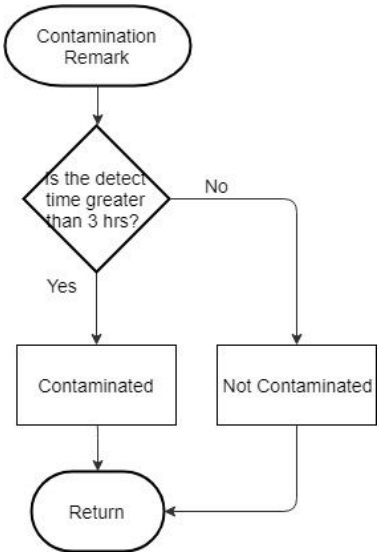


Figure 3.13 Flowchart of contamination remark

In order to classify whether the sample is contaminated or not, we need to compare the change of bacterial content to the initial bacterial concentration value. The moment of 5%

change on the solutions impedance reading indicates the detect time and whenever the detect time is greater than 3 hours, the solution is contaminated..

3.5.3 Image Processing

This section discusses about the Image Processing technique used in the paper.

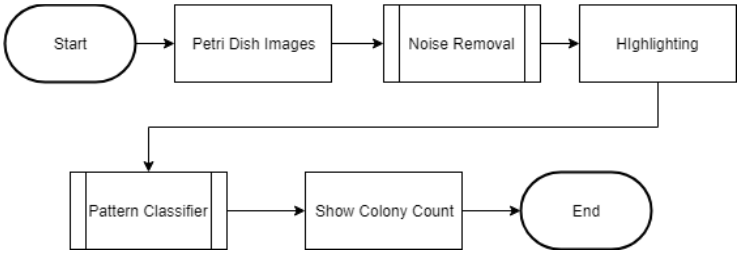


Figure 3.14 Process Flow of Image Processing

As the system starts, the petri dish images will be gathered from the incubator. It will then be processed for noise removal.

3.5.3.1 Algorithm Development

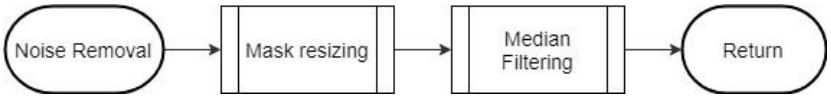


Figure 3.15 Flowchart of the Noise Removal

Under noise removal, the image will go through mask resizing and Median Filtering. This processes will enhance the quality of the image by removing unnecessary parts.

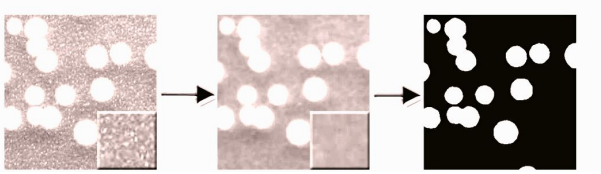


Figure 3.16 example of noise removal

From left to right, the first image is the original image from the petri dish culture. After the process of noise removal, it will result to the image in the middle. After highlighting the suspected bacterial colonies are highlighted, it will be result the the rightmost image.

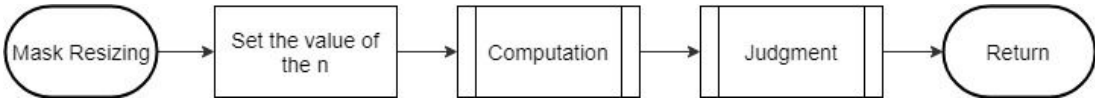


Figure 3.17 flowchart of mask resizing

Under mask resizing, the size of filtering mask (n x n) is modified. Usually, filtering mask is an odd number. After setting the value of n, the grayscale values will undergo computation. The overall image will have a minimum, medium, and maximum values for its grayscale. These three will be represented by min, med, and max respectively.

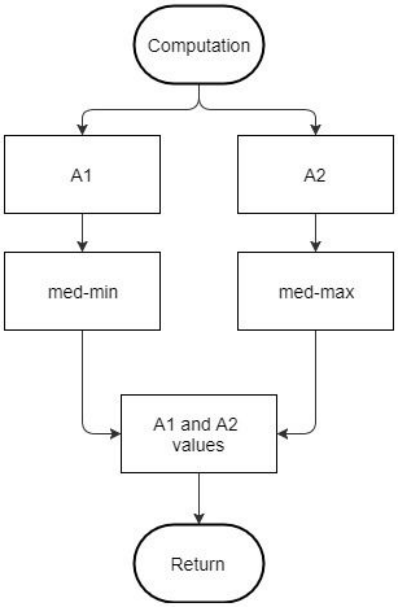


Figure 3.18 flowchart of computation in noise filtering

A1 is equated to med - min gray level, while A2 is equated to med - max value. The values of this computation will be transferred to the judgment process where the values are interpreted.

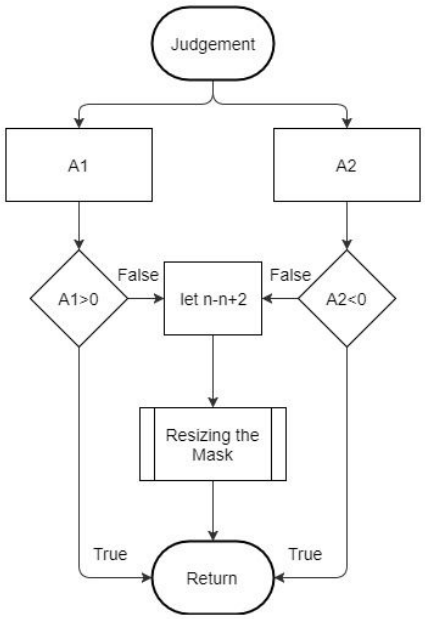


Figure 3.19 flowchart of the judgement process

If A1 is greater than 0 and A2 is less than 0, the data will be passed to median filtering. If not, changing the value of n by incrementing it by 2, should be done and returned to the first process of noise reduction.

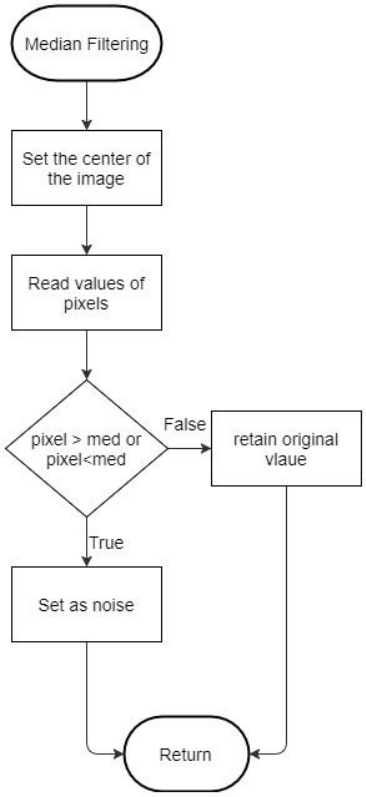


Figure 3.20 flowchart of the Median Filtering

In median filtering, the center of the image is identified first. The grayscale value of the pixels will be compared to the medium color value of the whole image. If the pixel value is not equal to the medium level, that pixel will be considered as a noise, if it is equal to medium, it will retain its value and be set as an object to be tested. All objects that were not filtered are considered bacterial colonies. Under highlighting, the image will be further enhanced and the bacterial colonies will be highlighted.

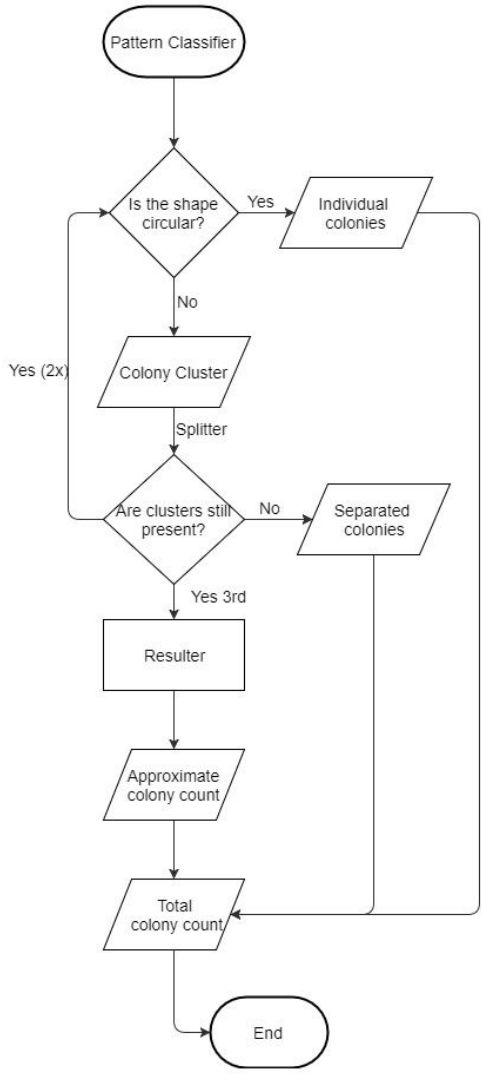


Figure 3.21 Flowchart of the pattern classifier.

E. coli and E. faecalis are bacterias that fall under circular form of colonies. Under pattern classification, it will identify whether the highlighted part, supposedly are bacterial colonies, are in circular in shape. Those that overlap and are not in the shape of circle will be considered as multiple clustered colonies. In order to determine the right value of colony clusters, a splitting process will occur which will be derived from the watershed segmentation

process. If after the 3rd time of splitting and still the colony is clustered, it will then be transferred to the resultant.

$$N = A / Ac \qquad \textbf{(3.1)}$$

The resultant uses equation 3.1 to solve for the approximate colonies present in the cluster. If the colony cluster can't be separated, a process of dividing the area of the cluster (A) to the area of the colony (Ac) will be done in order to result to an estimate values of the colonies present. This is called as "Resulter". The total colony count will be the summation of the individual colonies, separated colonies, and the approximate colony count from the resultant.

3.7 Testing

The testing section of the study discusses the entire process of the system, as well as, the expected outputs.

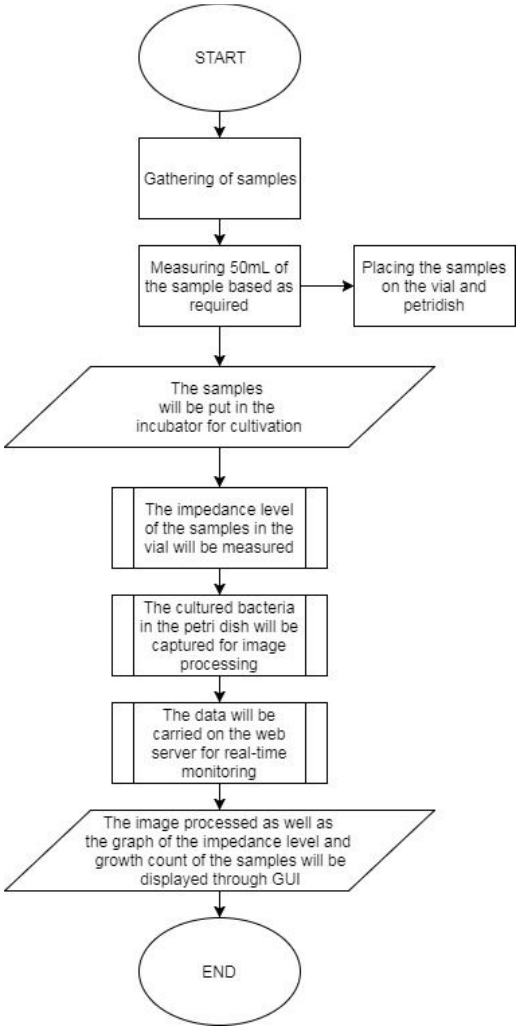


Figure 3.7 The entire flow process of the system

A few ice used in commercial beverages from different establishments will be gathered to use as sample in the study. The ice will be kept in a cool container as soon as it is acquired preventing it from melting and preserving any possible bacteria. During the testing period, a 30 mL of water from the ice will be put into the vial and petri dish.

Figure 3.8 vial (left) and petri dish (right)

The figure shows, 50 mL polypropylene vial and 90 mm petri dish, the materials used in the incubation and cultivation of the samples.

The step-by-step process of culturing bacteria (Escherichia Coli and Enterococcus faecalis)

Step 1: Preparing the Petri Dishes

1) Prepare the medium that will provide nutrients to the bacteria. Agar and Sodium Chloride are the nutrients used to culture the bacteria, Escherichia coli and Enterococcus faecalis respectively. Boil the solutions. After the boiling solutions, the powder should be completely dissolved and the liquid should be clear in color. Allow the solutions to cool for minutes before proceeding.

2) Prepare the petri dishes. Petri dishes should be sterilized before using it for growing bacteria. Pour the warm solutions into the petri dish and quickly cover the dish to prevent contamination of any airborne bacteria. Wait until the solutions cool and harden.

3) Refrigerate the petri dishes. The petri dishes with the solutions should be placed in a refrigerator until prepared to proceed with the testing. If the samples are ready for testing, allow the petri dishes to reach room temperature before introducing the samples.

Step 2: Growing Bacteria

1) Put bacteria to the petri dishes. Collect bacteria from the sample and simply transfer it to the petri dish. Swiping the swab in any surface of the component that contains the sample is the easiest way to transfer bacteria, and smear it to the surface of the hardened solution.

2) Label and seal the petri dishes once the bacterium is introduced.

3) Incubate the petri dishes wherein the bacteria can easily be develop.

Step 3: Capturing Images of the Cultured Bacteria

1) The camera will be capturing an image of the cultured bacteria at time interval.

2) The captured images will undergo image processing to spot the bacteria easily.

3.8 Data and Results

Table 3.1 The Result of the Laboratory Testing and Theoretically Gathered Data

Sample	**Laboratory Testing Result**	**Experimental Theoretical Values**		**Remarks**
		Colony Count (CFU)	**Detection Time (minutes per CFU/mL)**	
1	37	32	446	SAFE
2	225	228	280	SAFE
3	141	149	350	SAFE
4	1825	1375	124	NOT SAFE
5	660	626	156	NOT SAFE

Table 3.1 shows the sample results of the laboratory testing and theoretical gathering of data. The five (5) samples in each trial undergone impedance measurement, and colony-forming unit test. The results between the laboratory test results and the colony count done by the device shows an increasing amount of margin of error as the number of colonies increased. Some of the clustered colonies are counted as one. It can be observed that as the petri dish gets more crowded of the colony productions from the bacteria, the harder it gets for the device to accurately identify the number of bacterial colony present. The detection time of the impedance measurement reflects the concentration of the bacteria. If more bacteria are present in the medium, the threshold limit of the bacterial solution can be reached faster. Therefore, the detection time is inversely proportional to the colony count test.

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