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SIGHTING ELECTRICAL PROPERTIES OF ESCHERICHIA COLI UTILIZING ELECTROLYSIS METHOD BY USING COPPER ELECTRODES

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ABSTRACT

An independent electrochemical method for studying electrical signals from bacteria *Escherichia coli* (*E coli*) was developed by using copper (Cu) electrodes and without any biorecognition element. Current and voltage responses from different colony forming units per ml (CFU/ml) of *E coli* concentrations were observed based on their interaction with the electrolytic solution. An experimental platform was developed to record electrical signal responses in presence of *E coli* Gram negative bacteria. The sensing time to detect presence or absence of *E coli* was around 30 seconds while the total assay time was around 20-25 minutes. The electrical signal responses of *E coli* and aqueous NaCl mixed solution showed different nature which establishes the presence of bacteria. This electrochemical technique has the ability to rapidly detect the presence of *E coli* without using biorecognition element.

KEYWORDS: *E coli*, Electrochemical, Biosensors, Electrodes.

INTRODUCTION

In developing countries because of poor quality drinking water people get various water borne diseases. According to World Health Organisation (WHO) in June 2019 around 2 billion people are affected due to contaminated water.[1] The main reason for lethal diseases like cholera, typhoid, and dysentery is the consumption of contaminated water which has

increased the fatality rate in these countries. Around 600 million people almost 1 in 10 in the world fall sick due to the consumption of contaminated food and water resources, increasing the global burden of disease with 40 lac casualties every year.[2] Diarrhoea is a foremost cause of the mortality of more than 2 million people per year globally, primarily children under the age of five. In fact, each day, nearly 1,000 children die due to inevitable water and sanitation-related diarrhoeal diseases. The significance of water cannot be exaggerated. In fact, sustainable socioeconomic advancement and beneficial subsistence can be built on access to clean water. *E coli* is main contributor to waterborne disease.[3-5] Naturally, *Escherichia coli (E coli)* is a facultative anaerobic bacterium that inhabits the large gastrointestinal tracts of warm-blooded animals and is a major normal flora associated with the human colon.[3-6] It has short doubling time of 20 minutes and is usually present if other coliforms are in the water. The existence of *E coli* in food or water normally signals recent faecal contamination or poor hygienic conditions in food processing facilities.[3-5]

The conventional method for detection are microbiological and molecular biological tests that have the capability to count the bacteria levels in water or other substances, such as Multiple Tube Fermentation (MTF), Plate Count Enumeration Method, Membrane Filter (MF) technique, Polymerase Chain Reaction (PCR) and Enzyme-linked Immunosorbent assay (ELISA), even though the methods are still being used for routine detection of *E coli* in drinking water, it became impractical because the result can only be proved in several days as well as skilled manpower is required in the biological lab to perform the cultivation step and PCR reactions.[4],[7-14] The delay to sense the presence of bacteria rises a demand for more rapid and efficient detection techniques.[10,14] The need of the hour is to develop a simple sensing procedure which reduces the time of detection, and also have the specificity towards the causative pathogens to improve the water quality around the world. Although the present methods are accurate, but they have a lengthen detection time which causes fatality. Thus, our aim is to develop a simple detection technique to sense *E coli* having lesser detection time. The presently available electrochemical biosensors are depending on biorecognition elements, here we want to develop a standalone detection method to detect the *E coli.*[7],[9]–[11],[13],[15]–[20] Thus, to develop the POC (point of contact) detection tool we need to understand the properties of *E coli.* The present work is to find out the electrical signal responses of *E coli* from liquid samples having concentration 10^3 CFU/ml, 10^5 CFU/ml and 10^9 CFU/ml without using biorecognition element.[7] By applying various direct current (dc)

voltages in regular steps to the sample, corresponding electrical signal responses from the sample were observed.[7], [14], [20]–[23]

MATERIALS AND METHODS

Materials

Sodium chloride 99% pure was purchased from SRL (SL60091307), Luria Bertani Agar from HiMedia (M1151-500G), Variable DC power supply (EQUIP-TRONICS, model no. EQ-129), multirange microammeter (TESTRONIX SL NO. 040301), Digital multimeter (DMM), Copper (Cu) electrodes (SWG - 23), Ultrapure water (Marcus) etc.

Experimental setup

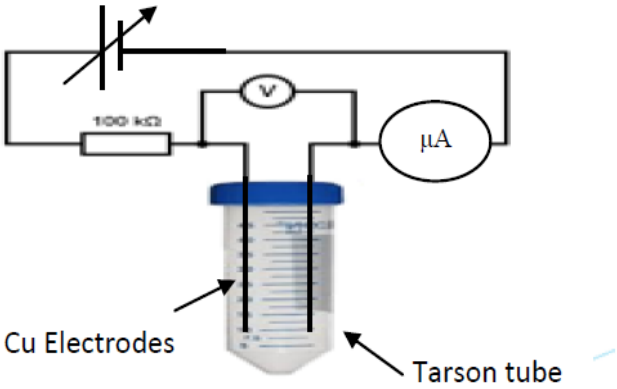


Fig. 1(a): Experimental setup.

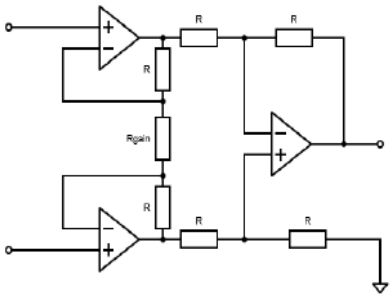


Fig. 1(b): Instrumentation amplifier.

Fig. 1(a) shows the experimental set-up which consists of a 50 cm^3 polypropylene centrifuge tube, a current limiting resistor and copper electrodes. A microammeter and a voltmeter were used to record current and voltage variations respectively. For this research work two different blank electrolyte solutions; ultrapure water and 0.1M aqueous sodium chloride (NaCl) were used. Initially to the blank electrolyte solutions, dc voltages in equal steps were applied and the corresponding voltages across the electrodes as well as current flowing through the circuit were noted. Later to the blank electrolyte solution different concentration of *E coli* was added and similarly voltages were applied and corresponding current flowing through the circuit and the voltage across the electrodes were noted. The following *E coli* concentration 10^3 CFU/ml, 10^5 CFU/ml and 10^9 CFU/ml were used. It was observed that the voltages measured across the electrodes for different applied voltages were having small variations. Hence in order to magnify/amplify this small difference in voltages, an amplifier was used. A variable gain instrumentation amplifier was used for this purpose. The circuit of the instrumentation amplifier is shown in Fig. 1(b).

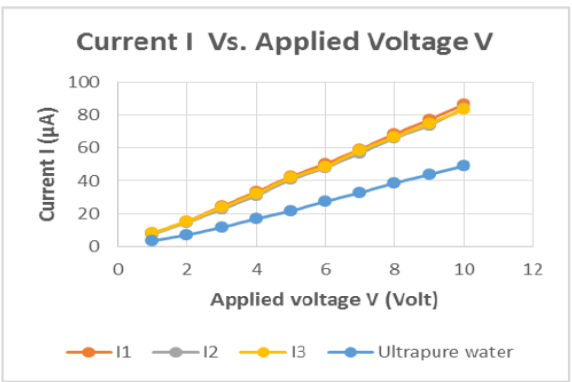
Bacterial growth condition

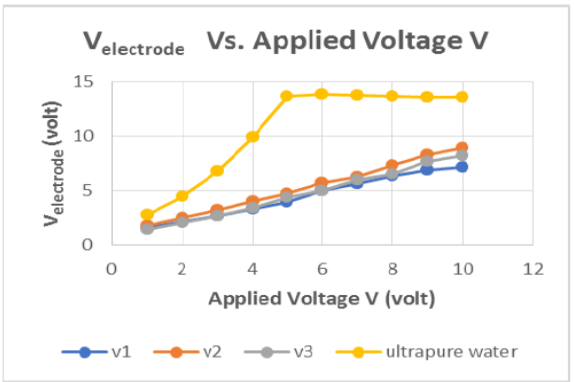
Luria Bertani (LB) Broth was used as a media for our test culture. *E coli* strain MTCC 1697 was grown in 20 ml LB media overnight at 37°C/120 rpm in incubator shaker to obtain concentration of 10^{12} CFU/ml. The *E coli* cells were centrifuged at 2000 rpm to pellet down the cells, the supernatant was discarded, and the cells were reconstituted in 2 different electrolyte solutions i.e. Ultra-pure water and 0.1M aqueous NaCl with the concentration of cells as 10^3, 10^5, 10^9, CFU/ml using 0.5 McFarland standards. To these individual cell concentrations, dc voltages were applied in regular steps and the voltage across the electrodes were observed along with the corresponding current response.

RESULTS AND DISCUSSION

In this study current and voltage responses were recorded for three concentrations of *E coli:* 10^3 CFU/ml, 10^5 CFU/ml, 10^9 CFU/ml with different blank electrolyte solutions, when different dc voltages were applied to them.

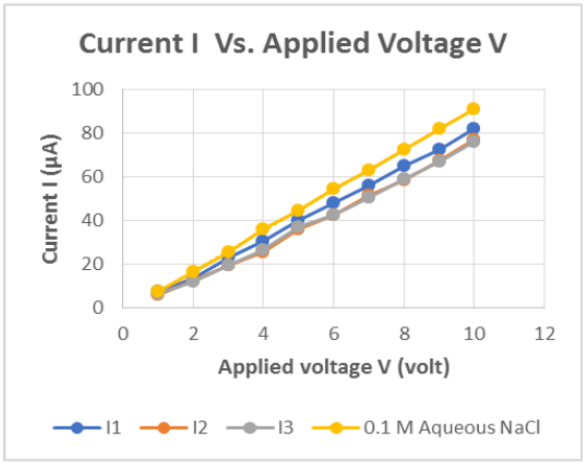
i) When ultrapure water as blank solution with different concentrations of *E coli* was used, the current variations observed for different applied voltages is shown in Fig. 2 (a). From this graph it is observed that the addition of *E coli* cells at different concentration in electrolyte solution (ultrapure water) caused an increase in current response with a difference of 30µA when the applied dc voltage is varied from 6 to 10V. Each *E coli* concentration was tested in triplicates with the consideration of standard deviation. The voltages measured across the electrodes for different applied voltages is shown in Fig. 2(b). The above process was repeated for other electrolyte solution (0.1 M aqueous NaCl), as seen in Fig. 2 (c) the addition of *E coli* cells at different concentration decreased in current response of 10 µA in applied dc voltage range 8 to 10V and Fig. 2 (d) showed variation in the voltage across electrodes with voltage response of 4 volt within the applied dc voltage range of 8 to 10 V indicating the electric properties of bacteria.

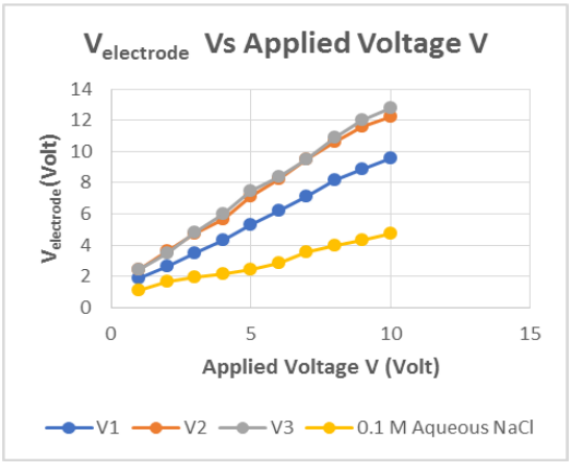




(a) Graph represents Current variations w.r.t Applied voltages of triplicates *E-coli* with ultrapure water. I1= current response for 10^3 CFU/ml, I2= current response for 10^5 CFU/ml, I3= current response for 10^9 CFU/ml	**(b) Graph represents Voltage recorded across electrodes w.r.t Applied voltages of triplicates *E-coli* with ultrapure water. I1= current response for 10^3 CFU/ml, I2= current response for 10^5 CFU/ml, I3= current response for 10^9 CFU/ml**

ii) When ultrapure water and 0.1M aqueous NaCl as blank solution with different concentrations of *E coli* was used, the current variations observed for different applied voltages is shown in Fig. 2 (c). From this graph it is observed that the addition of *E coli* cells at different concentration in electrolyte solution (ultrapure water and 0.1M aqueous NaCl) caused a decrease in current response around 10µA when the applied dc voltage is varied from 8 to 10V. Each *E coli* concentration was tested in triplicates with the consideration of standard deviation. The voltages measured across the electrodes for different applied voltages is shown in Fig. 2(d). In this graph it is observed that voltage across electrodes varies with a variation by 4v when the applied dc voltage is varied from 8 to 10 V indicating the electric properties of the bacteria.





(c) Graph represents Current variations w.r.t Applied voltages of triplicates *E-coli* with ultrapure water and NaCl. I1= current response for 10^3 CFU/ml, I2= current response for 10^5 CFU/ml, I3= current response for 10^9 CFU/ml	**(d) Graph represents Voltage recorded across electrodes w.r.t Applied voltages of triplicates *E-coli* with ultrapure water and NaCl. I1= current response for 10^3 CFU/ml, I2= current response for 10^5 CFU/ml, I3= current response for 10^9 CFU/ml**

CONCLUSION

From this study we arrive to a conclusion that when voltage is applied to the bacterial sample prepared in ultra-pure water and 0.1M aqueous NaCl, the Gram-negative *E coli* bacteria in electrolyte solution affects the value of the current. Because whenever voltage is applied to

this electrolyte solution there are dissociation of NaCl ions as shown in Fig. 3(a)and hence due to this there is decrease in current. Premise is that when concentration is increased of Gram-negative bacteria in ultra-pure and 0.1M aqueous NaCl, this results to the restriction for flow of ions in the electrolytic solution. Hence there is a decrease in current which is due to dissociation of ions in the electrolyte solution partially on Cu electrode and *E coli* cell membrane. Thus, this change in current is due to the presence of bacteria. The *E coli* competes with ions in the electrolysis process and gets involved in electrolysis process which contributes to partial flow of current given low values. But when voltage is applied through the *E coli* and ultrapure water, the Gram-negative *E coli* increases the flow of ions and due to this there is an increase in the current value as shown in Fig. 3(b)

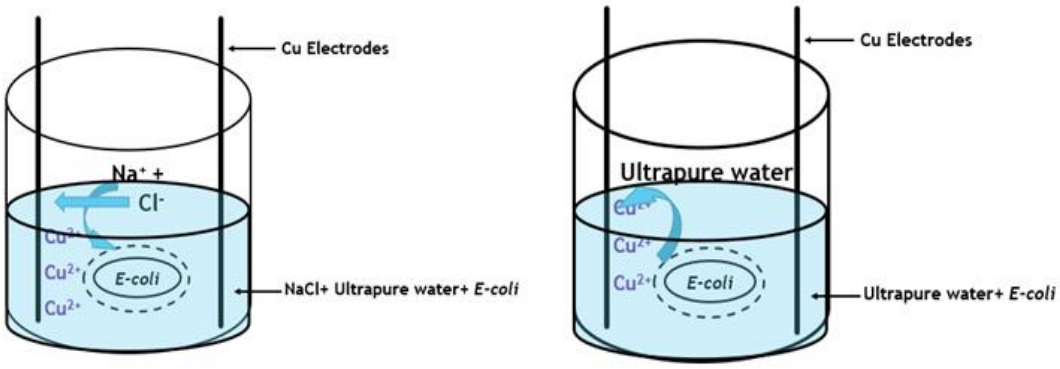


Fig. 3(a) **Fig. 3(b)**

Fig. 3: Schematic representation of electrolysis process.

Consumption of contaminated drinking water being serious issues around globally. *E coli* is the major contributor to the contaminated water resources, thus identification and quantification of bacteria become essential need of human for healthy life. The result reflected by this study about *E coli* is that it has a property to response to electrical signal, this property can be used for designing the simple detection method for *E coli* in various contaminated samples. This research will help for developing biosensor which can overcome the present detection techniques like PCR, ELISA, optical sensing methods which are depended on enzymes or biorecognition elements to detect the *E coli*. The property with slight modification will be able to detect presence or absence of *E coli* within 30 sec and total assay time is within 30 min. But it is specificity of electrode that can be enhanced by use of biorecognition elements which gives rise to detection of specific class of bacteria and its concentration. This experimental data can be used as model to develop a biosensor for

detection of *E coli*. Future study will focus on improving the detection limit and evaluating its use for other bacterial species of *E coli.*

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