2020 IEEE International Conference on Semiconductor Electronics (ICSE)

Design and Characterization of Impedance based E.Coli Sensor

Siow Yen Tey
School of Electrical Engineering
Universiti Teknologi Malaysia
Johor, Malaysia
siowyen_1014@hotmail.com

Afiqah Mohammad Muzafar Shah
School of Electrical Engineering
Universiti Teknologi Malaysia
Johor, Malaysia
afiqahmuzafar@gmail.com

Mastura Shafinaz Zainal Abidin
School of Electrical Engineering
Universiti Teknologi Malaysia
Johor, Malaysia
m-shafinaz@utm.my

Syafiqah Saidin
School of Biomedical Engineering and Health Science
Universiti Teknologi Malaysia
Johor, Malaysia
syafiqahs@utm.my

***Abstract*—Foodborne illness has become a concern due to high disease rate and huge losses caused. It is critical to detect and identify the pathogen in food by using sensor. However the conventional methods for pathogen detection are quite time-consuming, costly and label-dependent. This paper presents the design, fabrication and characterization of the impedance based biosensor for the detection of *E. coli* in water with features of low cost, rapid detection, easy to use and label-free. The interdigital electrodes and microfluidic system based devices were tested in different concentrations of *E. coli* samples with different structure electrode parameters for sensor characterization. Impedance analyzer was used for monitoring the impedance change to determine the operating frequency. The fabricated interdigital electrodes are also able to discriminate between dry and wet conditions by presenting different impedance outcomes at low frequency. The results depicted in this paper provide a guideline for detection of *E. coli* contamination level at different concentration with corresponding impedance range.**

***Keywords*—E. coli, impedance, interdigital, microfluidic, PDMS**

I. Introduction

According to the World Health Organization (WHO), the foodborne illness or widely known as food poisoning is defined as a disease caused by pathogens that go into a human body through ingestion of food [1]. From a WHO report in 2015, 0.1% of people worldwide are estimated to have foodborne illness symptom after consuming contaminated food. 420,000 people have also died from foodborne illness, including 125,000 children under the age of 5 [2]. In 2000, United States Department of Agriculture (USDA) Economic Research Services reported that medical costs, productivity losses, and costs of premature deaths for diseases caused by major foodborne pathogens total to $6.9 billion per year [3].

Among all pathogens, *Escherichia coli* O157:H7 is one of the major causes of foodborne illness. Based on a 1999 estimate, there were 73,000 cases of infections and 61 deaths occur in the United States each year due to *E. coli* O157:H7 only [4]. Since the *E. coli* O157:H7 has caused a great loss in terms of medical cost and product recall in food industry, it is critical to find a better solution for *E. coli* O157:H7 detection in food products.

Before the biosensors are widely used, the conventional methods of pathogen detection and identification are based on specific biochemical and microbiological identification [5]. These conventional methods are sensitive and they give accurate results. However, these conventional methods have significant limitations in terms of cost, requirement of special facilities, and a long procedural time [6]. The specific instruments for pathogen detection are quite expensive and good laboratory procedures may not be easily accessible in everywhere. Most of the conventional pathogen detection methods are time-consuming that they might takes up to 4 days for initial results and requires waiting for 3 more days for the result confirmation [7]. For some fresh food, the food might lose its best consuming time while waiting for the microbial detection results; otherwise if the food manufacturers do not check for the food contamination possibility due to long procedural time, they might need to undertake the responsibilities for risking on the food quality. There are some label-dependent sensors such as optical biosensors [8, 9]. The sensors might need labeled secondary antibodies to bind with primary antibodies and the antigens in sample and convert them into detectable signals.

The traditional impedance measurement is taken by a pair of electrode rods immersed in a test medium [7]. In these few years, the technology in impedance based sensor has been advanced and the macro-electrodes were replaced by microelectrodes due to its enhancive features of high signal-to-noise ratio, ability for achieving steady state rapidly, low ohmic resistance, and use small amount of sample solution. It is reported that the structure of double interdigitated array microelectrodes (IAM) based flow cell was more sensitive in impedance measurement and able to detect *E. coli* in range of 8.0 and 8.2 x 10^8 CFU/ml after an enrichment growth of 14.7 and 0.8 hours respectively [10]. A gold interdigitated microelectrode (IME) impedance biosensor was able to detect *E. coli* as low as 2.5 x 10^4 CFU/ml in 3 hours with frequency range between 100 Hz and 10 MHz [11]. Another biosensor was developed based on microelectromechanical systems, heterobifunctional cross linkers and immobilized antibodies to detect the presence of *E. coli* in food matrix for concentrations greater than 10^3 CFU/ml [12].

In this study we present a low cost, rapid detection, easy to use and label-free impedance based biosensor with interdigitated electrodes and polydimethylsiloxane (PDMS)

978-1-7281-5968-3/20/$31.00 ©2020 IEEE

microfluidic system prototype fabricated on printed circuit board (PCB).

II. Materials and Methods

A. Design Structure and Working Principle

In this study, the impedance based sensor was mainly constructed by copper interdigital electrodes, using the PCB. The side ends of the alternate electrodes were connected to the positive and negative terminals respectively. The upper side of copper interdigital electrodes was covered with PDMS which acts as a protection to the electrodes from contamination. PDMS is also a microfluidic system for the sensor which handles small volume and fixed position of the sample in contact with the interdigitated electrodes. The both ends of PDMS microfluidic system was connected to two tubes for inlet and outlet of the samples. Fig.1 illustrates the cross section layer view of the impedance based sensor used in this study. Basically, there were two sets of sensor dimension, namely sensors A and B with different parameters as shown in Fig. 2.

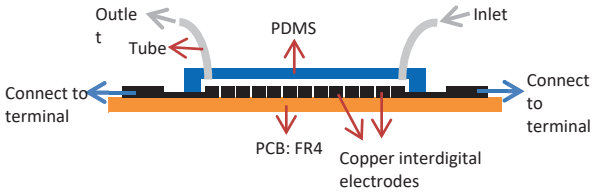

Fig. 1. Impedance based sensor cross section view

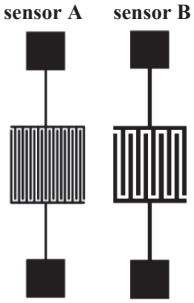

Fig. 2. Interdigital electrodes top view for sensors A and B

The working principle of the interdigital electrodes is based on capacitive principle as same theory has been applied in the electrode fabrication, that is dielectric exists between two conductors with opposite polarities [13]. When the AC voltage is applied, a potential difference as a function of time is formed between positive and negative terminals. The electric field lines would bulge from one electrode to another of opposite polarity as illustrated in Fig. 3.

When the impedance analyzer is connected to the interdigital electrodes, the impedance changes due to the changes in characteristics of electric field lines would be measured. The impedance Z consists of a real part (resistance, R) and an imaginary part (reactance, X) that shown as below:

$$Z = R + jX \quad (1)$$

X can also be explained based on the capacitance (C) and frequency (f) as follows:

$$X = \frac{1}{2\pi fC} \quad (2)$$

where,

$$C = \frac{\varepsilon_o \varepsilon_r A}{d} \quad (3)$$

A is the effective sensing area of the electrodes, d is the effective spacing between adjacent positive and negative electrodes, which is represented by symbol S in Fig. 3, ε_o and ε_r are the dielectric constant of free space and the relative dielectric constant of the material, respectively.

If any material or sample is placing close to the electrodes, the generated electric field lines will penetrate through the material and cause the field lines characteristics to be varied. These changes can be identified in the outcome result; alternately we can study the attribute of the material under test (MUT) based on the outcome changes.

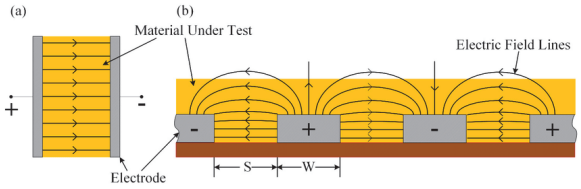

Fig. 3. The operating principle of the interdigital electrodes [13]

B. Fabrication of Interdigital Electrodes and PDMS

In this study, the interdigital electrodes structures were first designed in Proteus software and fabricated on PCB by etching process. There were two sample sets of interdigital electrodes which difference in the structure parameters of number of electrodes, N, electrode trace width, W and space between electrodes, S. Since the area of the sensing area of electrodes was set to be 20 mm x 20 mm for both samples, it indicates that the greater the number of electrodes N, the shorter electrode traces width W and gap between electrodes G. As shown by Fig. 2, the structure parameters are (N=10, W=0.6 mm, S=0.4 mm) for sensor A while (N=5, W=1.0 mm, S=1.0 mm) for sensor B.

In order to obtain the PDMS in the desired shape, the mold with opposite shape was prepared. The PDMS mold was first designed by using AutoCAD software with dimensions shown in Fig. 4. The mold for the PDMS was then fabricated by 3D printing. For the PDMS fabrication, the PDMS resin was prepared by mixing the base elastomer and curing agent (Dow Corning® Sylgard 184 Part A and Part B) at a ratio of 10:1 and stirred for 5 minutes. The mixture was then put in the desiccator about 40 minutes until all the air bubbles disappeared. After that, the mixture was poured into the molds. The mold with PDMS was put inside the fridge to remove any excessive air bubble for few minutes before setting on the table in room temperature. After one day the PDMS should be able to peel off from the mold and the fabricated PDMS layer obtained as in Fig. 5.

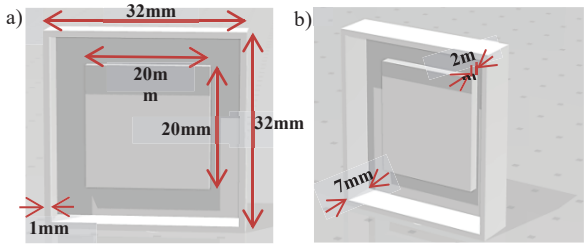

Fig. 4. PDMS mold with dimensions designed in AutoCAD software (a) PDMS mold top view (b) PDMS mold 3D view

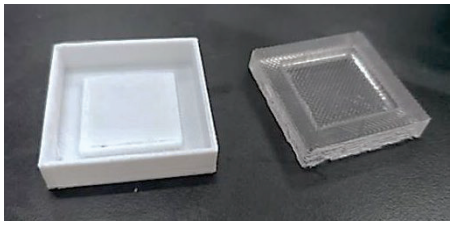

Fig. 5. PDMS mold and the fabricated PDMS microfluidic system

C. Assembly Process

After the interdigital electrodes and PDMS microfluidic system were fabricated, they were assembled together to form a complete sensor. This was performed by using uncured PDMS to combine the PCB surface with the PDMS layer. Same PDMS mixing ratio but uncured one will be used; the uncured PDMS mixture was applied as an adhesion layer onto the PDMS surface with the PCB. Then the PDMS and PCB were combined together and kept in room temperature for one day. Fig. 6 shows the devices after assembly completed.

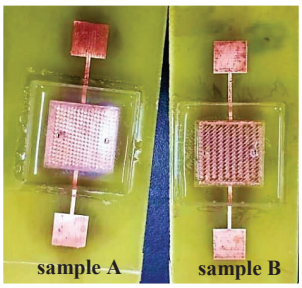

Fig. 6. Assembled devices for sample A and B

D. Measurement Setup for Microbial Sensing Response

The *E. coli* samples with six concentrations level (10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 CFU/ml) had been prepared with dilution method. Based on the impedimetric biosensors working principles, the impedance measurement would give vary results when biological reaction took place. The impedance analyzer will supply small AC voltage through the terminals to the electrodes which produce current flows through the sensors and the excitation voltage can be observed at certain frequency range. The impedance changes due to the interaction of electrodes with sample interface were measured and analyzed using electrochemical impedance spectroscopy (EIS) techniques which has been widely used in pathogen detection. For this study impedance analyzer IM3570 was used for data acquisition and the responses were measured in frequency from 1 kHz to 1 MHz. The measurement setup is as illustrated in Fig. 7.

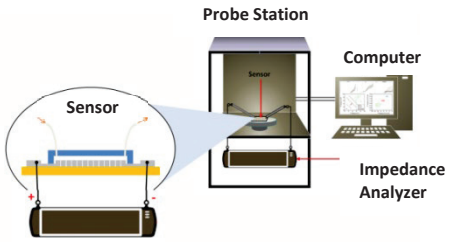

Fig. 7. Measurement setup for the experiment

III. Results and Discussion

A. Sensor Characterization

Sensors A and B with different structure parameters in distance between electrodes d (space between electrodes S) are compared to identify the relationship between spacing of electrodes and the sensitivity in impedance measurement. The *E. coli* samples with concentration range between 10^1 and 10^6 CFU/ml were tested in both sensors A and B and the impedance outcomes were shown in Fig. 8. It is noted that sensor A has higher impedance measurements than sensor B in all range of concentrations. For sensor B with wider spacing between electrodes, it did not show significant changes in impedance outcomes as sensor A in Fig. 8 when different *E. coli* concentrations were tested. Since the surface area contact for the binding of bacteria cells with the electrodes increases, it improves the sensitivity in impedance measurement.

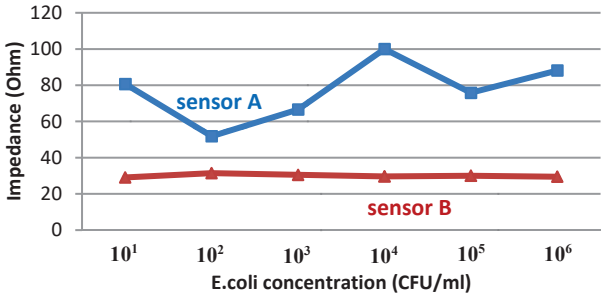

Fig. 8. Impedance response towards different *E. coli* concentration for sensor A and B

B. Comparison with Non-microfluidic Device

To determine the significant of microfluidic device for the impedance based biosensor. The configuration of non-microfluidic sensor is the same as the fabricated sensor but without the microfluidic device. The *E. coli* samples with concentration range between 10^4 and 10^6 CFU/ml were tested for both sensors and the impedance measurements for microfluidic and non-microfluidic sensor A were shown in Fig. 9. As the bacteria sample is confined in small volume for microfluidic device and provides higher signal-to-noise, sensor with microfluidic device detected higher and appreciable change in impedance measurement for different *E. coli* concentration.

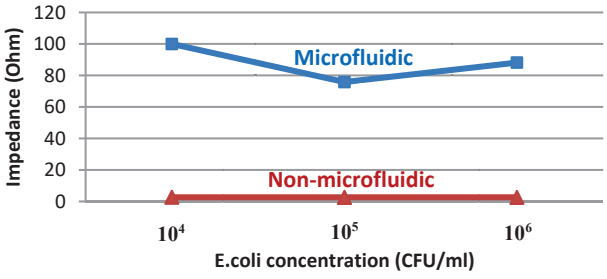

Fig. 9. Impedance response on *E. coli* concentration for sensors with microfluidic and non-microfluidic devices

C. Concentration Response

Different concentration of *E. coli* samples were used (from 10^1 to 10^6 CFU/ml) to test for the functionality of the impedance based sensor. Theoretically, when the concentration of *E. coli* increases, the impedance response increases as well. The impedance responses decreased

gradually and became less reactive as the frequencies increase. This is due to the relaxation of small dipole species (water molecules) and leads to the minimization of the effect of bacteria bound to the sensor [12]. However, the experimental results were randomly different from what was expected as shown in Fig. 10 for sample A.

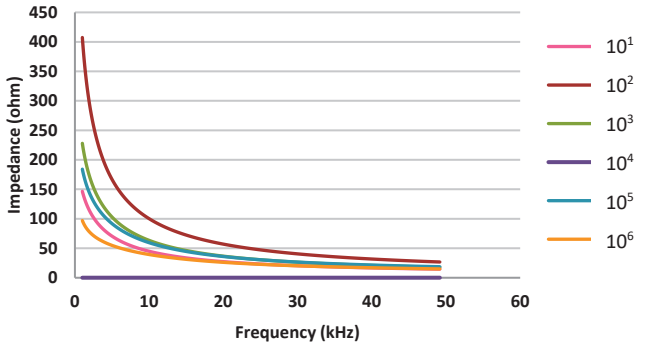

Fig. 10. Graph of impedance against frequency for different *E. coli* concentrations

D. Time Response

By keeping the response of sensor at a constant concentration of *E. coli* sample (10^5 CFU/ml), the impedance responses at different time intervals was recorded. The impedance values at certain frequencies within the intervals of 30 minutes as shown in Fig. 10 for samples A and B.

This time response is used to investigate the minimal time for the sensor to differentiate between impedance responses when *E. coli* sample is applied. From the results shown in Fig. 10, it is suggested that sample B was able to give faster response than sample A as the impedance response for sample B is distinguishable for each frequency from the beginning. Time response showed that the impedance measurement of the *E. coli* sample is not a function of time that it might be reduced after excess time.

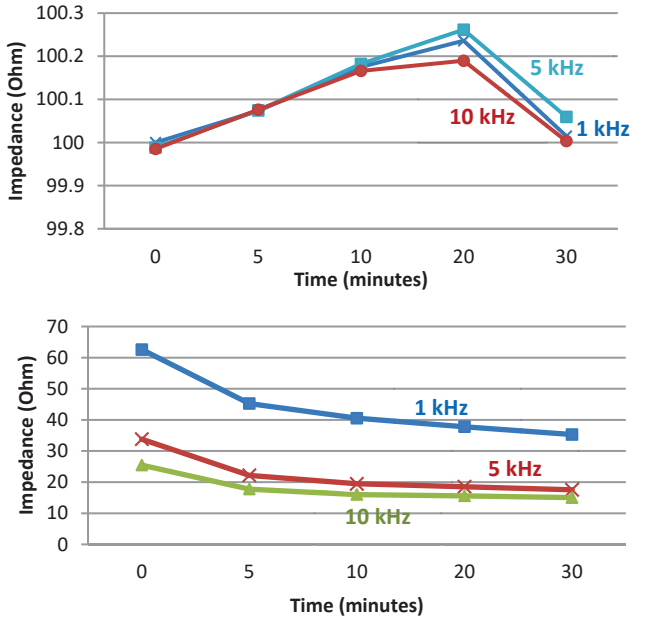

Fig. 11. Time response of impedance with 10^5 CFU/ml *E. coli* for (a) sample A (b) sample B

IV. Conclusion

The device with interdigital electrodes and PDMS layer structures have been fabricated in this study for the use as impedance based sensor. Several comparisons were made to find out the conditions for optimum impedance results. Based on the experimental results, sensor A with more number of electrodes and shorter width between electrodes is more sensitive that sensor B. Microfluidic device which concentrated the bacterial cells over the active region of sensor showed significant changes in impedance measurement than sensor without microfluidic device. The proposed device also able to give faster response as evidenced in time response analysis although there are some fluctuating values of impedance responses at different *E. coli* concentrations obtained during the experiment.

Acknowledgement

Authors would like to express gratitude to the financial support from Universiti Teknologi Malaysia. This work was conducted in Micro-Nano Mechatronic Laboratory in School of Electrical Engineering, UTM and the *E. coli* samples were prepared by Biomedical Science Laboratory in School of Biomedical Engineering, UTM.

References

[1] N. D. WHO, "Food safety and foodborne illness," *biochimica clinica,* vol. 26, no. 4, p. 39, 2002.

[2] S. Ali *et al.*, "Disposable all-printed electronic biosensor for instantaneous detection and classification of pathogens," *Scientific reports,* vol. 8, no. 1, p. 5920, 2018.

[3] S. Hoffmann and T. D. Anekwe, "Making sense of recent cost-of-foodborne-illness estimates," 2013.

[4] CDC (Centers for Disease Control and Prevention). (2019). *Disease Information-E.coli O157:H7.* Available: https://www.cdc.gov/ecoli/

[5] V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa, and C. Adley, "An overview of foodborne pathogen detection: In the perspective of biosensors," *Biotechnology advances,* vol. 28, no. 2, pp. 232-254, 2010.

[6] P. Leonard *et al.*, "Advances in biosensors for detection of pathogens in food and water," *Enzyme and Microbial Technology,* vol. 32, no. 1, pp. 3-13, 2003.

[7] S. Ghosh Dastider, S. Barizuddin, N. S. Yuksek, M. Dweik, and M. F. Almasri, "Efficient and Rapid Detection of Salmonella Using Microfluidic Impedance Based Sensing," *Journal of Sensors,* vol. 2015, pp. 1-8, 2015.

[8] S. Z. Hossain *et al.*, "Multiplexed paper test strip for quantitative bacterial detection," *Analytical and bioanalytical chemistry,* vol. 403, no. 6, pp. 1567-1576, 2012.

[9] C. Pöhlmann, I. Dieser, and M. Sprinzl, "A lateral flow assay for identification of Escherichia coli by ribosomal RNA hybridisation," *Analyst,* vol. 139, no. 5, pp. 1063-1071, 2014.

[10] M. Varshney and Y. Li, "Double interdigitated array microelectrode-based impedance biosensor for detection of viable Escherichia coli O157:H7 in growth medium," *Talanta,* vol. 74, no. 4, pp. 518-25, Jan 15 2008.

[11] M. Dweik, R. C. Stringer, S. G. Dastider, Y. Wu, M. Almasri, and S. Barizuddin, "Specific and targeted detection of viable Escherichia coli O157:H7 using a sensitive and reusable impedance biosensor with dose and time response studies," *Talanta,* vol. 94, pp. 84-9, May 30 2012.

[12] S. M. Radke, E. C. Alocilja, S. M. Radke, and E. C. Alocilja, "Design and Fabrication of a Microimpedance Biosensor for Bacterial Detection," *IEEE Sensors Journal,* vol. 4, no. 4, pp. 434-440, 2004.

[13] Y. Yuan *et al.*, "Development of an Internet of Things Based Electrochemical Microfluidic System for Free Calcium Detection," *Applied Sciences,* vol. 8, no. 8, p. 1357, 2018.