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Impedance sensor for rapid enumeration of *E. coli* in milk samples

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ABSTRACT

Detection of pathogenic bacteria in dairy farms and processing plants is important for milk quality control. In this study, a simple *in situ* method for determining the bacterial concentration in milk was developed with an interdigitated microelectrode sensor. This method was based on a punctual measurement of the electrode–milk interface impedance change due to bacterial metabolism in the milk. The interface impedance varied predominately at low frequencies, attributed to the change on the double-layer capacitance. The detection time T_D, defined as the time required to attain a −10% impedance change at 10 Hz, became a practicable means for the estimation of initial *E. coli* concentration in milk samples. Regression analysis resulted in a third-order polynomial relationship between the detection time and the logarithm of the initial *E. coli* concentration (N_0, cells/ml) in a milk sample, i.e., $\log N_0 = 11.48 - 2.33\,T_D + 0.288(T_D)^2 - 0.0154(T_D)^3$ with $R^2 = 0.9988$. The sensor was able to detect *E. coli* in milk of initial concentrations as low as 7 cells/ml. The proposed impedance sensor is suitable for determining bacterial contamination at dairy farms and processing plants.

1. Introduction

Bacterial infection remains a leading cause of death in developing countries, accounting for an estimated 40% of deaths [1]. For instance, the strain O157:H7 of *Escherichia coli* (*E. coli*) is considered to be one of the most dangerous food-borne pathogens [2]. *E. coli* infections are generally caused by eating contaminated food, drinking contaminated water, or coming into direct contact with someone who is sick or with an animal that carries the bacteria. In an attempt to preserve high-quality manufacturing and food production levels and at the same time safeguard the public, the Hazard Analysis and Critical Control Point (HACCP) system has been implemented worldwide [3].

Known as the worst type of *E. coli*, O157:H7 can cause bloody diarrhea, kidney failure and even death. In developed countries, bacterial contamination is also of critical concern, particularly in the dairy industry. Milk is a highly nutritious medium; therefore, many bacteria including pathogenic ones can grow and propagate in it. Many pathogenic bacteria can get access to milk and its byproducts; among these is *E. coli*. The role of milk analysis through quantitative detection of *E. coli* is of utmost importance in the food product assessment and for the promotion of public health. The presence of pathogenic organisms in milk implies a contaminated condition during the milk production and/or processing. So, it is necessary to detect and monitor the bacterial growth during the time when the milk is transported from farms to processing units and from processing units to distributors. Milk spoilage can be avoided by employing a real-time bacterial detection method in every dairy farm and processing unit.

Various techniques have been explored and utilized over the years for the detection and enumeration of microbiological spoilage in food products. Diary plants often use traditional methods such as the standard plate count to determine the bacterial concentration in raw milk. These methods are time-consuming and cumbersome. Recent researches have illuminated a variety of other methods that can simplify the bacterial detection process in milk. These methods include the pH measurement, magnetoelastic sensing, gas sensing and infrared spectroscopy [4–8]. The pH measurement can serve as an indicator of milk spoilage due to the presence of bacterial cells. The increase in strain growth (bacterial cell numbers) will lead to an increase in the amount of acid [4]. Though pH is a user-friendly method, its inaccuracy would likely be a concern of milk plants and manufacturers. The remote-query magnetoelastic sensing is an effective method for use in the remote detection of spoiled milk samples. Huang et al. [5] tested a

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remote-query magnetoelastic sensing platform to quantify the bacterial count of *Staphylococcus aureus* (*S. aureus*) in milk. The magnetoelastic sensor showed a higher sensitivity in milk than in a culture medium because of the higher viscosity of milk. However, it required a longer detection time. Lee et al. [6] proposed a rapid method for monitoring the growth of coliforms in milk samples using an amperometric sensor. The advantages of this method included a shorter detection time, 0.5–2 hours with the detection range of 10^2–10^4 CFU/mL. A major disadvantage of the amperometric sensor was that it cannot continuously monitor the bacterial growth of the same sample over time. Haugen et al. [7] investigated the possibility of developing a gas-sensor array system that detected and monitored the growth of bacteria in milk samples. This gas-sensor array system detected the major volatile metabolites produced by the bacteria during growth and the sensor signals from the pure cultures showed a significant correlation with the cell count. The drawbacks of the gas-sensor array system were its lack of sensitivity and its incapability of detecting very low concentrations of bacteria. Al-Qadiri et al. [8] evaluated the feasibility of applying the visible and short wavelength near-infrared (SW-NIR) diffuse reflectance spectroscopy as a technique to monitor the spoilage of pasteurized skim milk in an industrial setting. In their experimental samples, the visible and SW-NIR diffuse spectroscopy detected the formation of metabolic byproducts from proteolysis and lipolysis caused by bacterial cell growth. This method exhibited accurate results, but required very expensive instruments.

The impedance microbiology is one of the earliest physicochemical methods for the detection and monitoring of microorganisms, analysis of food preservatives and food hygiene [9–13]. This approach quantifies microorganisms by measuring the change in the medium conductivity during microorganism growth [10]. The change in the solution conductivity is recorded by impedance and conductance techniques. The impedance measurement is commonly preferred to the conductance measurement as it accounts for double-layer capacitance of the system in addition to the resistance of the solution. Conventionally, two-electrode systems consisting of thin metal rods or wires immersed in the medium were used as electrodes in the impedance measurement techniques [14–19]. Impedance spectroscopy analysis has been previously performed for analyzing bacterial cultures. Ur et al. [14] reported an impedance method for quantifying the bacterial concentration related to the change in medium conductivity due to bacterial growth. This idea has been applied in several reports such as Firstenberg-Eden and Zindulis [15] and Felice et al. [16] for analyzing bacterial cultures through impedance measurement by immersing two identical stainless steel electrodes. Firstenberg-Eden and Zindulis [15] evaluated the relationship between the bacterial growth and the resistive and capacitive effects of the impedance. Their data suggested that the microbial growth can be monitored through conductance, capacitance, or impedance variations. In lowly conductive media, the change in the conductance of the media was clearly correlated to bacterial growth. In highly conductive media, the relative change in the capacitive component of the measured impedance was useful for monitoring the bacterial growth. Though the method is suitable for detecting bacteria in lowly and highly conductive media, the bubble/gas formation near the electrodes during bacterial metabolism produced erratic behaviors in the impedance measurements. Felice et al. [16] reported an impedance method based on the measurement of the change in the electrode interface capacitance during the bacterial growth for the quantification of bacterial content in milk. However, in their report, the culture medium prepared through a laboratory protocol was used along with milk samples. Thus, the drawbacks observed in the reports discussed above limited the sensitivity of the conventional two electrode systems. In an attempt to improve sensitivity, several shapes of electrodes have been developed in last few decades. Of these, interdigitated microelectrodes showed higher sensitivities and have been successfully employed for the impedance measurement of bacterial cells during enrichment growth [13,20–22].

Yang et al. employed interdigitated microelectrodes and impedance microbiology for the detection of bacteria in milk samples [20]. In their study, a nutrient culture medium was used for the enrichment growth of bacteria along with spiked milk samples, which have limited applications for *in situ* testing of milk quality. However, studies have shown that the abundance of protein, moisture, and dissolved oxygen in milk *per se* can promote bacteria growth [23].

Our aim was to develop an ultra-sensitive *in situ* method of detecting *E. coli* in milk samples without the use of a nutrient culture medium. In this work, we demonstrated a gold interdigitated microelectrode-based impedance sensor as a simple and direct approach for quantitative detection of *E. coli* in 100% cow milk. In order to fully understand the impedance measured with the interdigitated microelectrode sensor, an equivalent electrical circuit model was used to analyze the impedance spectrum. The technique presented herein is capable of punctual detection of *E. coli* in milk samples. Our results verified the highly sensitive and user-friendly *in situ* procedure for detecting *E. coli* directly in milk samples with a broad *E. coli* concentration range.

2. Materials and Methods

2.1. Chemicals and reagents

Luria Bertani Miller's medium was purchased from AthenaES (Baltimore, MD, United States). Culture medium broth was prepared with deionized water (18.2 MΩ/cm) and sterilized in an autoclave at 121 °C for 1 hour. Acetone and isopropanol were purchased from Mallinckrodt (Cambridge, MA, United States).

2.2. Fabrication of gold interdigitated microelectrode on glass substrate

The gold interdigitated microelectrode sensor was fabricated in-house on a glass chip (1737, Corning) by standard microelectronic fabrication methods. Each chip contained four interdigitated microelectrode sensors and measured 36 mm × 15 mm as depicted in supplemental information Fig. S1(left). Each gold interdigitated microelectrode sensor consisted of a pair of 50-finger electrodes with 8 μm of finger width and spacing. The fabrication process of the gold interdigitated microelectrode sensor is illustrated in Fig. S1(right). Firstly, a standard positive photoresist S1818 was coated on the glass chip (Fig. S1(a)). Then a mask pattern of the interdigitated microelectrode was transferred to the photoresist by exposing the photoresist to UV light (Fig. S1 (b)) and subsequently developing the exposed areas in MF-319 developer (Shipley Far East Ltd., Tokyo, Japan) (Fig. S1(c)). The chip was then rinsed in deionized water and dried by a stream of nitrogen. Once the pattern was defined, 2 nm of chromium was deposited by Q150T ES sputter-coater (Quorum Technologies Ltd., Laughton, UK) in order to improve the adherence of gold to the glass surface (Fig. S1(d)). After the chromium deposition, 50 nm of gold layer was sputtered to form the complete electrodes (Fig. S1 (e)). The excess metal on top of the unexposed photoresist was lifted off by ultra-sonication in acetone (Fig. S1(f)). Fig. S2(a) shows an optical microscopic image of a photoresist pattern created using the photolithographic process and Fig. S2(b) shows an optical microscopic image of a gold interdigitated microelectrode sensor.

2.3. Bacterial culture

E. coli cells were grown in the Luria Bertani Miller's broth overnight at 37 °C till reaching the exponential phase. The cell concentrations were determined by cell counting with a hemocytometer under an optical microscope. *E. coli* cells were then harvested by centrifugation at 5000 rpm for 10 min. The supernatant was discarded after centrifugation and the *E. coli* sediment was used for inoculation of milk samples.

2.4. Spiked milk sample preparation

Fresh milk was purchased from a local supermarket with nutritional facts (per 100 ml) of protein 3.3 g, fat 3.7 g, sodium 41.6 mg, calcium 100 mg, and carbohydrate 4.7 g. Spiked milk samples were prepared by inoculating fresh milk samples with 7.2×10^0, 7.2×10^2, 7.2×10^4, 7.2×10^6, 7.2×10^7, and 7.2×10^8 cells/ml, respectively, of *E. coli.*

2.5. Impedance measurement

All interdigitated microelectrode sensors were cleaned with acetone, isopropanol and deionized water and then dried by a stream of nitrogen prior to measurements. Polystyrene chambers of 1-ml capacity were bound to the sensor substrate with adhesive silicon rubber. The impedance response of the biosensor was measured using an IM-6eX impedance analyzer (Zahner-elektrik CMBH & Co. KG, Germany). Sine waves of 50-mV amplitude with 0-V DC offset were used as the excitation signals to measure the impedance in the frequency range from 1 Hz to 1 MHz. One contact pad of the interdigitated microelectrode sensor was connected to the test and sense probes, and the other was connected to the reference and counter probes on the IM-6 impedance analyzer. Each *E. coli*-inoculated milk sample was introduced to an impedance chamber and the impedance spectrum was recorded during *E. coli* growth, at 37 °C.

3. Results and Discussion

3.1. Characterization of interdigitated microelectrode sensor

Capacitance measurement was used to characterize the fabricated interdigitated microelectrode sensors. The sensor capacitance was measured in air using a HIOKI 3532-50 LCR HiTESTER (HIOKI E.E. Corporation, Nagano, Japan) in the frequency range from 500 Hz to 1 MHz with 100-mV input DC bias. The capacitance measured at 20 kHz was found to be 2.2 ± 0.08 pF. The theoretical value of the capacitance was calculated to be 1.33 pF using the formula proposed by Van Gerwan et al. [24] by assuming the width of and the gap between the electrodes to be 8 μm. The measured capacitance is close to the theoretical value, and the coefficient of variation (CV) was less than 5%. This result evidenced the quality and reproducibility of the developed sensor.

The developed interdigitated microelectrode sensor was electrically characterized over a frequency range from 1 Hz to 1 MHz in milk samples without being inoculated with *E. coli*. The magnitude and phase of the measured electrochemical impedance are shown with solid lines in Fig. 1(a). According to the measured impedance spectrum, the interdigitated microelectrode sensor in milk can be represented by an equivalent electrical circuit shown in Fig. 1(b) [25]. The equivalent circuit consists of two double-layer capacitors (C_{dl}) in series with a solution resistor (R_s). Cdl accounts for the capacitive effect of the ionic species near the electrode surface. R_s represent the bulk electric resistance of the solution (milk). The fitted equivalent circuit's impedance magnitude and phase are shown with cross marks in Fig. 1(a). The mean error in impedance magnitude of the model is less than 0.5% with respect to the measured data. With such a small mean error, the equivalent circuit model C_{dl}–R_s–C_{dl} is suitable for analyzing the impedance behavior in this study.

The measured impedance spectrum (Fig. 1(a)) can be divided into three regions: a capacitive region (with the phase angle close to −90°) in the frequency range from 1 Hz to 10 kHz, a resistive region (with the phase angle close to 0°) beyond 1 MHz and a transitional region that is partly capacitive and partly resistive from 10 kHz to 1 MHz. The milk used in this study contained 0.416 g/l of sodium and 1 g/l of calcium. The abundant sodium (Na^+) and calcium (Ca^{2+}) ions in the milk sample lowered the solution resistance R_s. Because of this, the impedance spectrum shown in Fig. 1(a) had a capacitive region wider than that of the impedance spectrum measured from water.

3.2. Impedance analysis of E. coli growth in milk samples

We inoculated milk samples with different *E. coli* concentrations and allowed the bacteria to grow for 12 hours. Impedance measurements were taken at 0 h, 1 h, 3 h, 5 h, 7 h, 9 h and 12 h during *E. coli* growth in milk. Fig. 2 shows the impedance magnitude and phase spectra measured using the interdigitated microelectrode sensor over the frequency range from 1 Hz to

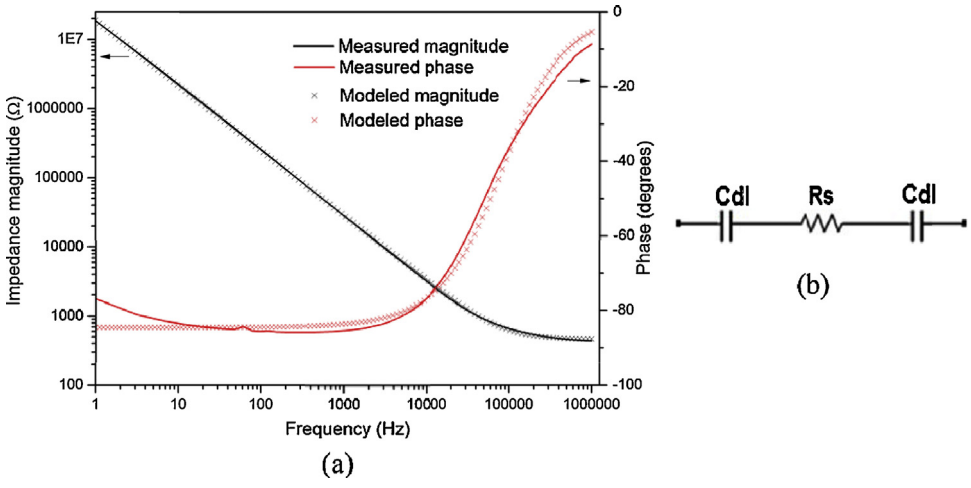

Fig. 1. (a) Bode plot of the impedance spectra measured using an interdigitated microelectrode sensor in milk (without *E. coli*). (b) An equivalent circuit model for the measured impedance.

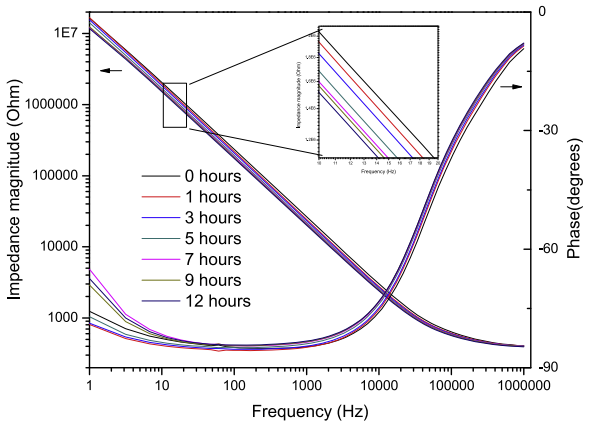

Fig. 2. Bode plot of the impedance spectra of the milk sample inoculated with 7.2×10^6 cells/ml of *E. coli*, measured every hour within 12 hours.

1 MHz at different growth times after inoculating 7.2×10^6 cells/ml of *E. coli* in milk. The impedance spectra measured with the sensor in the frequency range from 1 Hz to 10 kHz consisted predominately of capacitive reactance, as is evidenced by a phase close to −90°. The impedance spectra above 10 kHz gradually approached pure resistance as the capacitive reactance diminished with the increased frequency. Measurable impedance change was observed at frequencies from 1 Hz to 10 kHz and the determination of *E. coli* concentration would be based on the impedance change at 10 Hz. The impedance changes reflected the activity of the growing bacterial cells [25,26]. In this study, the impedance change due to *E. coli* growth in the milk sample was assessed by the percentage change of the impedance measured at a certain instance from the initial impedance measured at 0 h:

$$\text{Percentage change of impedance at a certain instance} = \frac{\text{impedance at the instance} - \text{impedance at 0 h}}{\text{impedance at 0 h}} \times 100 \quad (1)$$

Shown in Fig. 3(a–c) are the time courses of the percentage changes at 10 Hz as *E. coli* growth proceeded in the milk samples with 7.2×10^2, 7.2×10^4 and 7.2×10^6 cells/ml, respectively, of initial *E .coli* concentrations. It can be seen in Fig. 3(a–c) that the impedance Z decreased with time after the time of *E. coli* inoculation (0 h). In order to understand the impedance change caused by *E. coli* growth in milk, the measured impedance spectra were simulated using the equivalent circuit model C_{dl}–R_s–C_{dl} (Fig. 1(b)). This equivalent circuit model was used for curve fitting the measured impedance data along the *E. coli* growth time.

As shown in Fig. 3(a–c), the impedance Z decreased by about 6% at 1 h with all the three initial cell concentrations. It became relatively stable after 1 h with initial *E. coli* concentrations of 7×10^2 cells/ml (Fig. 3(a)) and 7×10^4 cells/ml (Fig. 3(b)).

With initial *E. coli* concentration of 7.2×10^2 cells/ml, the impedance remained almost stable from 1 h to 7 h, but after that it gradually decreased to a −17.6% change at 12 h (Fig. 3a). Similarly,

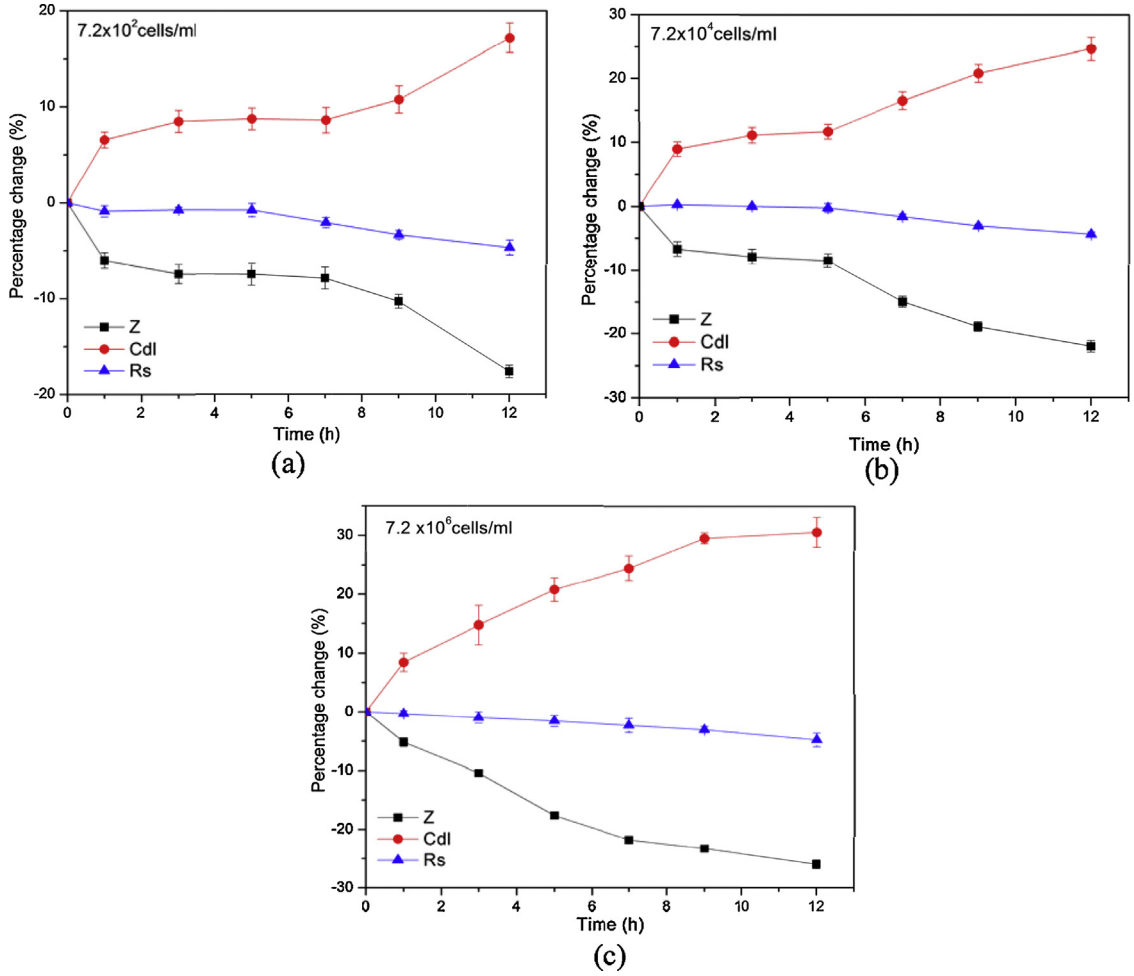

Fig. 3. Percentage changes in impedance *Z*, double-layer capacitance C_{dl}, and solution resistance R_s during *E. coli* growth in milk with different initial cell concentrations (a) 7.2×10^2 cells/ml, (b) 7.2×10^4 cells/ml, and (c) 7.2×10^6 cells/ml. Each of the error bars is the standard deviation of 4 measurements.

with initial *E. coli* concentration of 7.2×10^4 cells/ml, impedance change remained almost stable from 1 h to 5 h, but after that it gradually decreased to a −22% change at 12 h (Fig. 3b). This impedance behavior followed the growth kinetic profile of a bacterial culture. The growth time before 7 h and that before 5 h with the initial 7.2×10^2 and 7.2×10^4 cells/ml, respectively, may include the growth lag phase, where the cells get used to the media, and the time needed by the cells to grow sufficiently to affect the impedance by a measurable amount [26]. After the lag phase, the bacteria entered the logarithmic growth phase, which involved an exponential increase in the number of cells. This effect was reflected by a decreasing trend of impedance after 7 h and 5 h with initial *E. coli* concentrations of 7.2×10^2 and 7.2×10^4 cells/ml, respectively.

As shown in Fig. 3(c), the impedance measured in the milk samples initially inoculated with 7×10^6 cells/ml of *E. coli* gradually decreased in the first 12 hours. This indicates the logarithmic growth phase of *E. coli* from the initial time of inoculation (0 h) due to the high cell concentration. At 1 h, the ionic concentration near the electrode surface could have considerably increased due to the release of ions or other intracellular materials from the cells into the milk sample [27]. When *E. coli* cells were taken from the cell growth medium and inoculated into a milk sample, their extracellular ionic concentration changed. To maintain their cellular activity in the new environment, the cells adjusted their intracellular solute concentrations. During the growth time, *E. coli* cells metabolized non-charged large molecules into charged small molecules. The increase in the ionic concentration in the milk sample resulted in decreasing the solution resistance R_s with the growth time [28]. However, as seen in Fig. 3(a–c), R_s only decreased slightly over the 12 hours; the decrease in Z was mostly due to the increase in C_{dl}. Due to high ionic content of the medium (milk), the increase in ionic content caused by bacterial growth was very small compared with the ionic content already present in the medium. So the change in the resistive impedance was very small and therefore no significant change in R_s was observed during bacterial growth. Similarly, the difference in R_s with different bacterial concentrations was small. However, despite the high ionic content of milk, the change in ionic composition resulted during bacterial growth in the milk was sufficient to cause a significant change in the C_{dl}. This conforms to the fact shown in Fig. 2 that the capacitive impedance prevailed over the resistive impedance at low frequencies.

According to previous studies, the double layer capacitance can be affected by the following two factors during bacterial growth: (i) Metabolic activity by bacterial cell growth could increase the double-layer capacitance [20]. (ii) Attached bacteria and biofilm could decrease the double-layer capacitance [29]. From the result of C_{dl} analysis in Fig. 3(a–c), the increasing trend in C_{dl} values beyond 7 h, 5 h, and 1 h, respectively, with the three different initial *E. coli* concentrations indicates the metabolic activity generated by growth of *E. coli* cells in the milk samples. Metabolic activities during *E. coli* growth in milk possibly converted uncharged or weakly charged substances in the milk, such as proteins, lipids, and carbohydrates, into highly charged substances, such as amino acids, acetates, lactates and other metabolic products [16]. These highly charged ions caused an increase in the dielectric permittivity and decrease in the thickness of the double layer [20,25,30]. These changes resulted in an increase in the double-layer capacitance and consequently a decrease in the impedance. The increasing trend in C_{dl} due to metabolic activity of *E. coli* cells in the milk sample started at different growth times with different initial cell concentrations: 7 h with 7.2×10^2 cells/ml, 5 h with 7.2×10^4 cells/ml, and 1 h with 7.2×10^6 cells/ml (Fig. 3(a–c)); the starting time reduced with increasing initial *E. coli* concentrations. With a high initial cell concentration (such as 7.2×10^6 cells/ml), the ionic substances produced by *E. coli* metabolism will be higher and hence the change in C_{dl} was observed almost from the time of *E. coli* inoculation. Whereas with a low initial cell concentration (such as 7.2×10^2 cells/ml), the number of ionic substances produced by *E. coli* metabolic activity is not enough to produce a measureable change in C_{dl} or Z until 7 h.

It is difficult to verify that a local increase of charged substances near the electrode surface could produce such a big capacitance change without significantly altering the solution resistance. Since it is not possible to directly measure the double-layer capacitance,

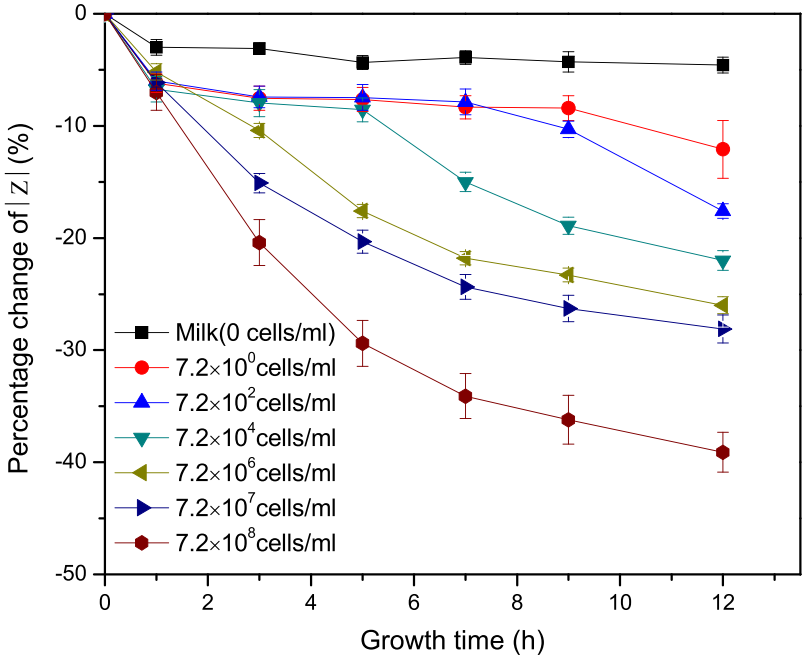

Fig. 4. The percentage change of the measured impedance at 10 Hz in milk with initial *E. coli* concentrations ranging from 7.2×10^0 to 7.2×10^8 cells/ml. Each of the error bars is the standard deviation of 4 measurements.

the hypothesis of the relative change between C_{dl} and R_s is still under discussion. However, as shown in Fig. 3, the simulated results (C_{dl} and R_s) can provide a plausible explanation for the experimental data (Z) [31–33].

To confirm the acidification of milk by *E. coli* metabolism, the pH value was measured. During the 12 hours of measurement, the pH value of milk without *E. coli* was about 6.7, whereas in *E. coli* infected milk samples, the pH value decreased over the 12 hours.

3.3. Quantification of E. coli in milk samples

In this study, the relative impedance change at 10 Hz was used to quantify the initial *E. coli* concentration in a milk sample. Fig. 4 shows the growth curves (percentage impedance change vs. growth time) with various initial *E. coli* concentrations. With initial *E. coli* concentrations of 7.2×10^6, 7.2×10^7 and 7.2×10^8 cells/ml, decreases in impedance were observed from the initial time of cell inoculation. This represented the "log phase" of bacterial growth, during which they grew exponentially and produced a huge amount of by-products and hence the decrease in impedance was observed from the time of *E. coli* inoculation. With low initial *E. coli* concentrations of 7.2×10^0, 7.2×10^2 and 7.2×10^4 cells/ml, the "log phase" was observed after a "lag phase". This was because the ionic concentration during bacterial metabolism was not high enough to change the impedance by a measurable amount in the early growth times. The duration of "lag phase" was shortened with increasing initial *E. coli* concentrations.

In this study, the time corresponding to a 10% decrease in impedance, i.e., a −10% change in impedance, was defined as the detection time T_D. Fig. 5 shows a plot of the initial *E. coli* concentration as a function of the detection time obtained from the measured results shown in Fig. 4. Using this assay protocol, a standard curve was obtained that was fitted with a third-order polynomial (Fig. 5). The relationship between the detection times (T_D, h) and the initial cell concentration (N_0, cells/ml) in the milk samples was found to be $\log N_0 = 11.48 - 2.33\ T_D + 0.288(T_D)^2 - 0.0154(T_D)^3$ with $R^2 = 0.9988$. The detection time for an initial cell concentration between 7.2 and 7.2×10^8 cells/ml was between 10.6 and 1.4 h.

Impedimetric analysis with interdigitated electrodes has been previously performed for a number of different microbiological assays. Yang et al. [20] used interdigitated microelectrode-based impedance biosensor for rapid detection of viable *Salmonella Typhimurium* in a selective growth medium (5 ml) and milk samples (0.5 ml milk + 4.5 ml growth medium). Selenite cystein broth supplemented with trimethylamine oxide and mannitol (SC/T/M) was used for the selective growth of *S. Typhimurium*. They detected a ~30% relative variation of the double-layer capacitance and a 0.5% relative variation of the solution resistance at 10 Hz after 16 h of incubating a bacterial culture of 5 ml with an initial inoculum of 2.06×10^2 CFU/ml. The main differences between their work and our present task are the growth medium and sample volume. In the present study, we used milk as the growth medium for *E. coli* and the impedance measurements were carried out in a sample volume of 1 ml. Varshney and Li [25] presented a double interdigitated microelectrode-based impedance biosensors to detect *E. coli* cultures in a lowly conductive yeast–peptone–lactose–trimethylamine oxide (YPLT) growth medium. They specially prepared the YPLT medium without adding salts such as sodium chloride and disodium phosphate in order to minimize the conductivity. Their results showed a ~46% relative variation of the C_{dl} and a 33% variation of the R_s at 1 MHz with an initial inoculum of 5.4×10^4 CFU/ml. Thus, Yang et al. [20] and Varshney and Li [25] analyzed bacterial cultures with a growth medium prepared through a lab protocol. However, monitoring *E. coli* growth in milk without using a laboratory-prepared enrichment growth medium has not been carried out before. In the present study, interdigitated microelectrode sensors were successfully used for analyzing *E. coli* growth in milk samples without using any laboratory-prepared enrichment growth medium. Based on the experimental results, the sensor's response to uncontaminated milk is significantly different from that of the *E. coli* contaminated milk samples. This finding leads to a new option for testing bacterial contamination in milk samples *in-situ*.

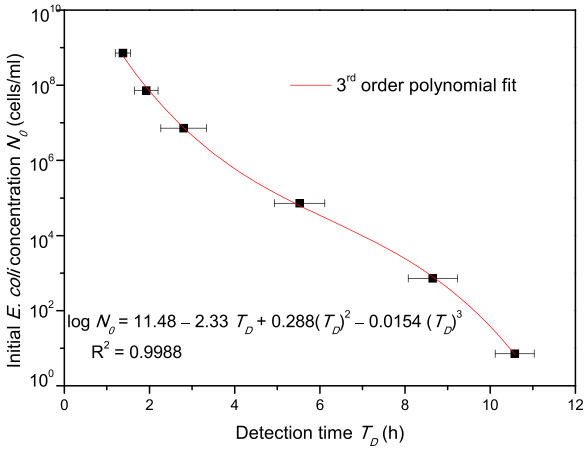

Fig. 5. The relationship between logarithm of the initial *E. coli* concentration and the detection time. Each of the error bars is the standard deviation of 4 measurements.

Our proposed label-free punctual impedance measurement technique can detect very low concentration of *E. coli* (7 cells/ml) without electrode modification. Normalized impedance change with respect to the impedance measured at the initial time (0 h) was used to plot the calibration curve for bacterial quantification in milk. This difference method effectively revealed the impedance change due to *E. coli* growth in milk more clearly and indicates that this method was not predominantly affected by various proteins and ions present in the milk sample. Hence, the normalized impedance change at a fixed frequency in an interdigitated microelectrode sensor is applicable to bacterial concentration determination in milk. Thus, our proposed device demonstrates the possible *E. coli* detection in milk samples *in situ* with a large detection range. This *in situ* bacterial growth monitoring approach for *E. coli* detection is highly promising for 1 cell/ml in a test sample. Therefore, this study proves that the interdigitated microelectrode impedance sensor is an effective tool for performing punctual measurements for bacterial quantification. This approach could be used to determine bacterial contamination for quality control of milk samples in dairy farms and processing plants.

4. Conclusion

In this study, quantitative detection of *E. coli* in cow milk samples was attained using interdigitated microelectrode impedance sensors without using enrichment medium for *E. coli* growth. Our experiment results showed that *E. coli* growth in milk causes a measurable impedance change at low frequencies (1 Hz–10 kHz). An equivalent circuit analysis indicated that the impedance change was mainly due to a change in the double-layer capacitance, which was related to the breakdown of nutrients in milk during *E. coli* growth. The change in impedance showed dependence on both the growth time and the initial concentration of *E. coli*. A third order polynomial relationship between the detection time and the logarithm of the initial *E. coli* concentration was obtained. Our sensor exhibits a high sensitivity for detecting a wide range of

E. coli concentration, 7.2×10^0 to 7.2×10^8 cells/ml, in milk samples. Our sensor could be applicable for *E. coli* detection in dairy processing industry.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.electacta.2015.09.029.

References

[1] D. Ivnitski, I. Abdel-Hamid, P. Atanasov, E. Wilkins, Biosensors for detection of pathogenic bacteria, Biosensors and Bioelectronics 14 (1999) 599–624.
[2] R.L. Buchanan, M.P. Doyle, Foodborne Disease Significance of Escherichia coli O157:H7 and Other Enterohemorrhagic E. coli, FOODTECHNOLOGY 51 (1997) 69–76.
[3] J. Niza-Ribeiro, A.C. Louzã, P. Santos, M. Lima, Monitoring the microbiological quality of raw milk through the use of an ATP bioluminescence method, Food Control 11 (2000) 209–216.
[4] H.I. Fromm, K.J. Boor, Characterization of Pasteurized Fluid Milk Shelf-life Attributes, Journal of Food Science 69 (2004) M207–M214.
[5] S. Huang, S. Ge, L. He, Q. Cai, C.A. Grimes, A remote-query sensor for predictive indication of milk spoilage, Biosensors and Bioelectronics 23 (2008) 1745–1748.
[6] Y.-G. Lee, H.-Y. Wu, C.-L. Hsu, H.-J. Liang, C.-J. Yuan, H.-D. Jang, A rapid and selective method for monitoring the growth of coliforms in milk using the combination of amperometric sensor and reducing of methylene blue, Sensors and Actuators B: Chemical 141 (2009) 575–580.
[7] J.E. Haugen, K. Rudi, S. Langsrud, S. Bredholt, Application of gas-sensor array technology for detection and monitoring of growth of spoilage bacteria in milk: A model study, Analytica Chimica Acta 565 (2006) 10–16.
[8] H.M. Al-Qadiri, M. Lin, M.A. Al-Holy, A.G. Cavinato, B.A. Rasco, Monitoring Quality Loss of Pasteurized Skim Milk Using Visible and Short Wavelength Near-Infrared Spectroscopy and Multivariate Analysis, Journal of Dairy Science 91 (2008) 950–958.
[9] O.N. Okigbo, G.H. Richardson, Detection of Penicillin and Streptomycin in Milk by Impedance Microbiology, Journal of food protection 48 (1985) 979–981.
[10] J. Strassburger, J. Hossbach, R. Seidel, Application of Impediometry to Rapid Assessment of Liquid Culture Media, Zentralblatt für Bakteriologie 274 (1991) 481–489.
[11] P. Silley, S. Forsythe, Impedance microbiology—a rapid change for microbiologists, Journal of Applied Bacteriology 80 (1996) 233–243.
[12] M. Wawerla, A. Stolle, B. Schalch, H. Eisgruber, Impedance Microbiology: Applications in Food Hygiene, Journal of food protection 62 (1999) 1488–1496.
[13] R. Gomez-Sjoberg, D.T. Morisette, R. Bashir, Impedance Microbiology-on-a-Chip: Microfluidic Bioprocessor for Rapid Detection of Bacterial Metabolism, Microelectromechanical Systems, Journal of 14 (2005) 829–838.
[14] A. Ur, D.F. Brown, Impedance monitoring of bacterial activity, Journal of medical microbiology 8 (1975) 19–28.
[15] R. Firstenberg-Eden, J. Zindulis, Electrochemical changes in media due to microbial, Journal of Microbiological Methods 2 (1984) 103–115.
[16] C.J. Felice, R.E. Madrid, J.M. Olivera, V.I. Rotger, M.E. Valentinuzzi, Impedance microbiology: quantification of bacterial content in milk by means of capacitance growth curves, J Microbiol Methods 35 (1999) 37–42.
[17] B.C. Towe, V.B. Pizziconi, A microflow amperometric glucose biosensor, Biosensors and Bioelectronics 12 (1997) 893–899.
[18] C. Berggren, B. Bjarnason, G. Johansson, An immunological Interleukine-6 capacitive biosensor using perturbation with a potentiostatic step, Biosensors and Bioelectronics 13 (1998) 1061–1068.
[19] V.M. Mirsky, M. Mass, C. Krause, O.S. Wolfbeis, Capacitive approach to determine phospholipase A2 activity toward artificial and natural substrates, Analytical chemistry 70 (1998) 3674–3678.
[20] L. Yang, Y. Li, C.L. Griffis, M.G. Johnson, Interdigitated microelectrode (IME) impedance sensor for the detection of viable Salmonella typhimurium, Biosensors & bioelectronics 19 (2004) 1139–1147.
[21] L. Yang, Y. Li, Detection of viable Salmonella using microelectrode-based capacitance measurement coupled with immunomagnetic separation, Journal of Microbiological Methods 64 (2006) 9–16.
[22] R. Gómez, R. Bashir, A.K. Bhunia, Microscale electronic detection of bacterial metabolism, Sensors and Actuators B: Chemical 86 (2002) 198–208.
[23] R.P. Singh, B.A. Anderson, The major types of food spoilage: an overview, in: R. Steele (Ed.), Understanding and measuring the shelf life of food, Woodhead Publishing Ltd., 2004, pp. 3–23.
[24] P.V. Gerwen, W. Laureyn, W. Laureys, G. Huyberechts, M.O.D. Beeck, K. Baert, J. Suls, W. Sansen, P. Jacobs, L. Hermans, R. Mertens, Nanoscaled interdigitated electrode arrays for biochemical sensors, Sensors and Actuators B-chemical 49 (1998) 73–80.
[25] M. Varshney, Y. Li, Double interdigitated array microelectrode-based impedance biosensor for detection of viable Escherichia coli O157:H7 in growth medium, Talanta 74 (2008) 518–525.
[26] J. Paredes, S. Becerro, F. Arizti, A. Aguinaga, J.L. Del Pozo, S. Arana, Interdigitated microelectrode biosensor for bacterial biofilm growth monitoring by impedance spectroscopy technique in 96-well microtiter plates, Sensors and Actuators B: Chemical 178 (2013) 663–670.
[27 L. Yang, Electrical impedance spectroscopy for detection of bacterial cells in suspensions using interdigitated microelectrodes, Talanta 74 (2008) 1621–1629.
[28] H.E. Ayliffe, R. Rabbitt, An electric impedance based microelectromechanical system flow sensor for ionic solutions, Measurement science & technology 14 (2003) 1321–1327.
[29] S. Kim, G. Yu, T. Kim, K. Shin, J. Yoon, Rapid bacterial detection with an interdigitated array electrode by electrochemical impedance spectroscopy, Electrochimica Acta 82 (2012) 126–131.
[30] M.J. Sparnaay, The electric double layer, 1st ed., Pergamon Press, Sydney, 1972.
[31] X. Muñoz-Berbel, F.J. Muñoz, N. Vigués, J. Mas, On-chip impedance measurements to monitor biofilm formation in the drinking water distribution network, Sensors and Actuators B: Chemical 118 (2006) 129–134.
[32] O.K. Okoth, K. Yan, L. Liu, J. Zhang, Simultaneous Electrochemical Determination of Paracetamol and Diclofenac Based on Poly (diallyldimethylammonium chloride) Functionalized Graphene, Electroanalysis (2015) , doi:http://dx.doi.org/10.1002/elan.201500360.
[33] S. Brosel-Oliu, N. Abramova, A. Bratov, N. Vigués, J. Mas, F.-X. Muñoz, Sensitivity and Response Time of Polyethyleneimine Modified Impedimetric Transducer for Bacteria Detection, Electroanalysis 27 (2015) 656–662.