Sensors **2002**, *2*, 219-232

sensors

ISSN 1424-8220

http://www.mdpi.net/sensors

Remote Query Resonant-Circuit Sensors for Monitoring of Bacteria Growth: Application to Food Quality Control

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Received: 15 June 2002 / Accepted: 17 June 2002 / Published: 22 June 2002

Abstract: This paper presents a technique for *in-situ* remote query monitoring of bacteria growth utilizing a printed thin or thick-film sensor comprised of an inductor-capacitor (LC) resonant circuit. The sensor, which is placed within the biological medium of interest and remotely detected using a loop antenna, measures the complex permittivity of the medium. Since bacteria growth increases the complex permittivity of a biological medium the LC sensor can be used to determine bacteria concentration. This paper presents results on monitoring of three different bacteria strains, *Bacillus subtilis*, *Escherichia coli* JM109, and *Pseudomonas putida*, demonstrating application of the sensor for monitoring bacteria growth in milk, meat, and beer. Due to its low unit cost and remote query detection, the sensor is potentially useful for commercial scale monitoring of food quality.

Keywords: Sensor, Resonant circuit, Wireless, Remote query, Passive, Complex permittivity, Bacteria, Food quality

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Introduction

Bacteria are a major source of contamination in water and food supplies resulting in both food poisoning and disease outbreaks [1]. Although constant monitoring of bacteria concentrations can reduce food-related illnesses, counting bacteria in a biological food medium is a time consuming and

difficult task due to the complex nature of bacteria and the biological medium. Today most bacteria counts are still determined by standard plate count (SPC) or psychrotrophic bacteria count (PBC) [2], which are labor and time intensive. Microbiological impedance devices [3-8] provide a less labor intensive way to estimate bacteria count in a biological medium. Microbiological impedance devices measure the permittivity and/or impedance spectra of the biological medium by immersing two or three electrodes into the medium and measuring the voltage at a constant current. The bacteria concentration or even the strain of the bacteria can then be calculated from the measured permittivity and/or impedance spectra [9-11].

One of the major applications of microbiological impedance devices is to determine the initial bacteria count in a food medium through a technique called Impedance Detection Time (IDT) [12-16]. The initial bacteria count obtained from IDT is then used to estimate the useful shelf life of a food product. However, the initial bacteria count does not always correlate well with the real shelf life due to improper storage conditions or post packaging food contamination. Furthermore, direct characterization of bacteria concentrations using impedance devices has some disadvantages such as penetration of the test chamber which introduces the potential for contamination, polarization of the probe electrode, and bubble formation at the electrode surface. An optimal way to monitor food quality is to remotely measure the bacteria population just prior to food consumption.

In this paper, we present a wireless, passive remote-query sensor for in-vivo monitoring of bacteria growth. The sensor, referred to as the LC sensor [17], is a series-connected interdigital capacitor and a spiral inductor printed on a thin plastic or paper substrate (see Fig. 1). A thin polyurethane layer is coated on the sensor to prevent the biological medium, which is electrical conductive, from shorting the capacitor electrodes. The sensor is immersed in the biological medium of interest, and the impedance spectrum of the sensor remotely detected by measuring the impedance across the terminals of a loop antenna used to monitor the sensor, see Fig. 2. The impedance spectra of the antenna is eliminated from the measurement by measuring the antenna impedance when the sensor is absent, and then subtracting that value from the impedance measurement of interest. Fig. 3 is a background-subtracted impedance spectrum of a sensor immersed in water. Two important parameters are determined from the plot: the resonant frequency f_0 which is defined as the frequency at the maximum of the real impedance (resistance), and the zero-reactance frequency f_z which is at the frequency where the imaginary impedance (reactance) goes to zero. From f_0 and f_z, the effective relative complex permittivity of the biological medium and polyurethane layer, $\varepsilon_{eff}' - j\varepsilon_{eff}''$, is given as [18]:

$$\varepsilon_{eff}' = \frac{1}{(2\pi f_0)^2 L\kappa\varepsilon_0} - \varepsilon_S \qquad \varepsilon_{eff}'' = \frac{\sqrt{f_0^2 - f_Z^2}}{4\pi^2 f_0^3 L\kappa\varepsilon_0} \tag{1}$$

where ε_0 is the free space permittivity ($\varepsilon_0 = 8.854\times10^{-12}$ Farads / meter), ε_S is the relative permittivity of the electrically lossless substrate (that is $\varepsilon_s = \varepsilon_s'$), κ is the cell constant of the interdigital capacitor, and L is the inductance of the spiral inductor in Henry's. The cell constant κ and inductance L are calculated from the sensor geometry using [19, 20]:

$$\kappa = \frac{\ell(N_C - 1)K\left[(1-(a/b)^2)^{1/2}\right]}{2K[a/b]} \tag{2}$$

$$L = 1.39 \times 10^{-6} (OD + ID) N_L^{5/3} \log_{10}\left(4\frac{OD + ID}{OD - ID}\right) \quad (3)$$

where a, b, ℓ, OD, and ID are the dimensions of the sensor defined in Fig. 1, N_c is the number of electrode fingers in the capacitor, N_L is the number of the inductor turns, and K is the elliptic integral of the first kind. For any given individual sensor, the thickness of the protective polyurethane coating affects the measurement accuracy. Hence to eliminate effects due to variation in the polyurethane coating thickness we first measure the complex permittivity of deionized water using the LC sensor and a strip-line cavity [21]. A correction factor is then calculated by normalizing the LC sensor measurement $\varepsilon_{cal}' - j\varepsilon_{cal}''$ to the strip-line measurement $\varepsilon_{ref}' - j\varepsilon_{ref}''$. The actual complex permittivity of the biological medium, $\varepsilon_r' - j\varepsilon_r''$, is then calculated by multiplying the measured effective permittivity by the correction factor:

$$\varepsilon_r' = \varepsilon_{eff}' \varepsilon_{ref}' / \varepsilon_{cal}' \qquad \varepsilon_r'' = \varepsilon_{eff}'' \varepsilon_{ref}'' / \varepsilon_{cal}'' \quad (4)$$

Experimentally, using the calibration technique we found the LC sensors to have permittivity measurement errors of less than 8% in comparison to stripline cavity measurments.

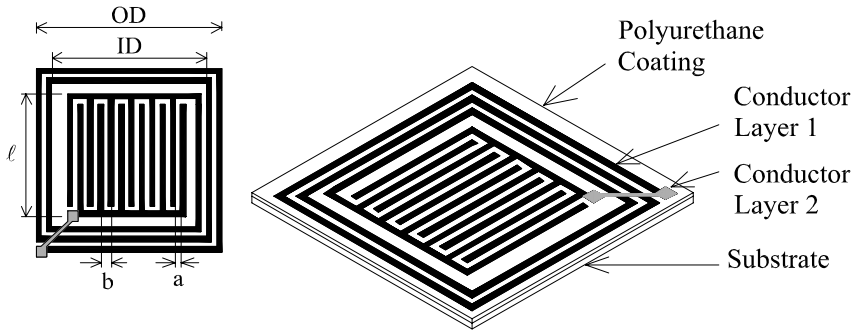

Figure 1. The LC sensor is comprised of a series-connected LC circuit printed on a plastic substrate. The sensor is covered with a layer of polyurethane to prevent the conductive biological medium from shorting the capacitor and damping the resonance.

The complex permittivity of a biological medium increases with bacteria concentration causing a change in the capacitance of the interdigital capacitor, and in turn shifting f_Z and f_0. The changes in f_Z and f_0 are used to calculate ε_r' and ε_r'' to determine the bacteria concentration in the biological medium. In this paper we present the results on *Bacillus subtilis*, *Escherichia coli* JM109, *Pseudomonas putida* grown in Luria Bertani medium. The results are compared to optical density measurements at 600 nm.

Due to its low unit cost and wireless detection, the LC sensor is suitable for commercialized food quality monitoring by measuring the bacteria concentration of the food before consumption. In this work we demonstrate application of the LC sensor technology for monitoring the quality of milk, meat, and beer. To measure bacteria concentration in a liquid such as milk or beer, the sensor is fully

immersed in the medium, while to measure bacteria concentration in a solid medium such as meat, the sensor is placed next to the object with the interdigital capacitor facing the object. Since the response of the passive LC sensor is remotely measured through a loop antenna, placing an autoclaved sensor within the food package prior to sealing would enable the quality of the food within the package to be determined at any time. Since the sensor is powered by the query field generated by the antenna, it does not require any internal batteries thus avoiding battery lifetime issues. The sensor is a simple resonant circuit consisting of conductor lines printed on a paper or plastic substrate, which allows it to be inexpensively fabricated and used on a disposable basis.

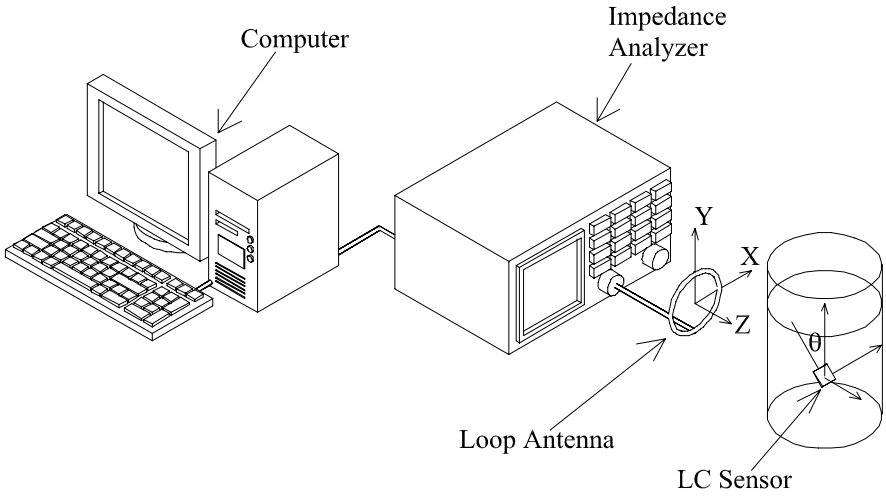

Figure 2. The general experimental setup for bacteria monitoring. An impedance analyzer is used to measure the response of the sensor. The coordinate system used to analyze sensor performance with orientation and location is also shown.

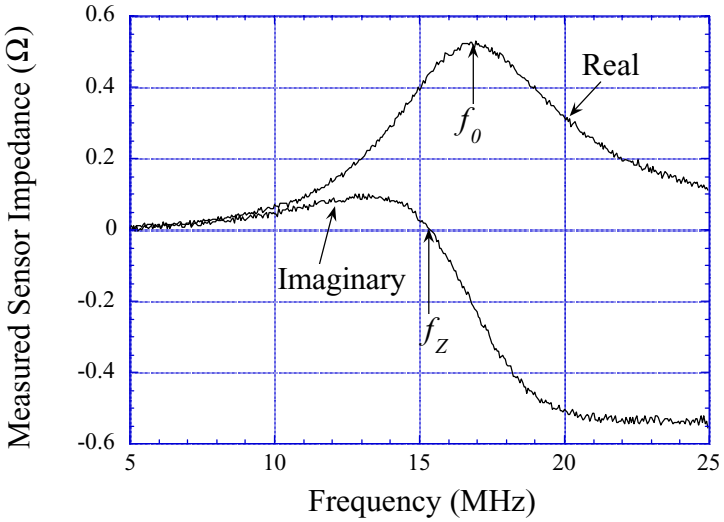

Figure 3. The impedance spectrum of the sensor after the antenna impedance is subtracted. The resonant frequency f_0 is defined as the maximum of the real impedance, while the zero-reactance frequency f_Z is the zero of the imaginary impedance. The complex permittivity of the medium is calculated from the measured values of f_0 and f_Z.

Experiments and Results

Bacteria Growth Monitoring

The growth of three bacteria strains, *Bacillus subtilis*, *Escherichia coli* JM109, and *Pseudomonas putida*, were monitored at room temperature (25 °C) as a function of time. The bacteria culture was continuously stirred at a constant temperature using a water bath throughout the duration of the experiment (see Fig. 4). The culture medium, sensor, and the container used for the experiment were autoclaved at 120 °C for 15 min prior to the experiment. All three bacteria strains were grown in Luria Bertani (LB) medium, obtained from Difco Laboratories (Detroit, MI), at a constant temperature of 25°C. The sensor was 4 cm × 4 cm and protected by 150 μm thick polyurethane layer. The antenna was a 6-turn loop antenna of 9 cm in diameter, placed at 8 cm away from the sensor with both the sensor and antenna basal planes in parallel. The impedance of the sensor is measured with a HP 4192A Impedance Analyzer.

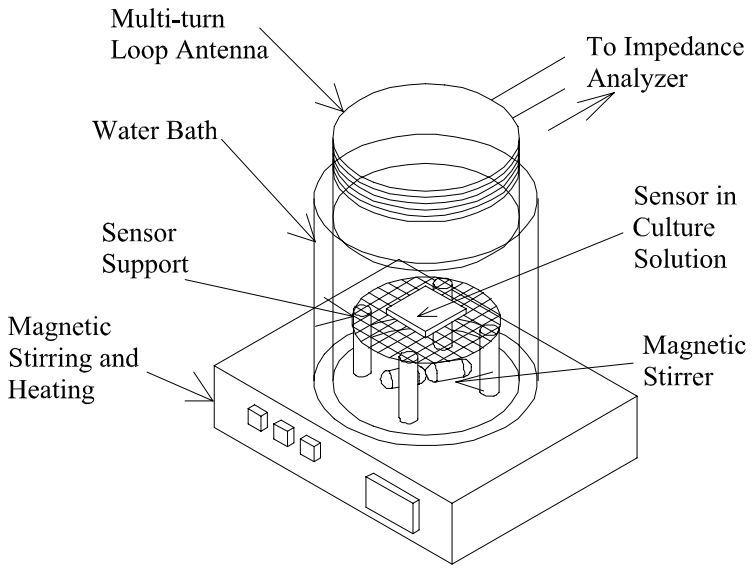

Figure 4. The experimental setup that provides a constant stirring and temperature for the bacteria culture.

While the polyurethane coating protects the electrical integrity of the sensor, when immersing the sensor in a liquid medium water molecules are initially absorbed into the polyurethane layer increasing its complex permittivity. However, this absorption process stops within a few hours after the polyurethane pores become saturated. Hence to eliminate the effect of water absorption on liquid immersed sensor experiments the sensors were initially soaked in water for four hours prior to use.

Optical density measurements were taken while the permittivity of the medium was measured. Fig. 5 shows a linear correlation between the optical density and the change in the permittivity magnitude $|\Delta\varepsilon_r|$ for both *E. coli* and *Pseudomonas* cultures. The optical density measurements require more than one hour between two consecutive measurements to detect any change. In contrast, the LC

sensor can resolve changes in $|\varepsilon_r|$ of less than 0.01, corresponding to a resolvable continuous monitoring time period of approximately 5 s.

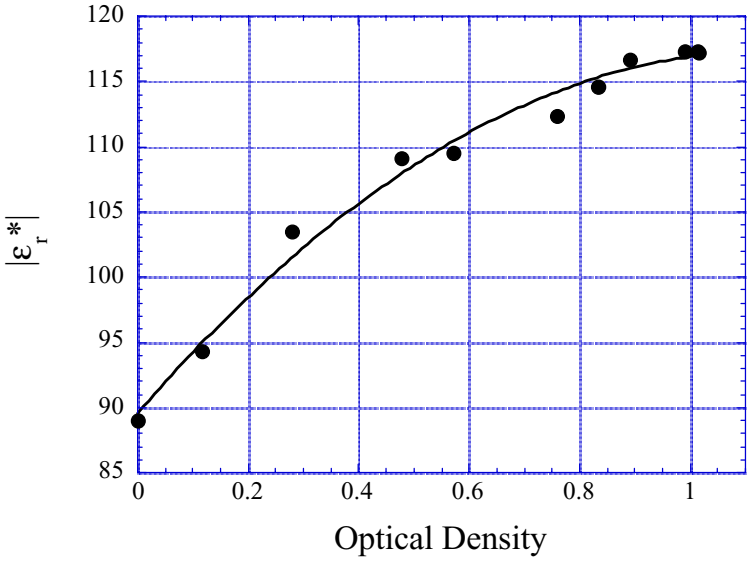

Figure 5. Correlation between LC sensor and optical density measurements of a bacteria laden solution.

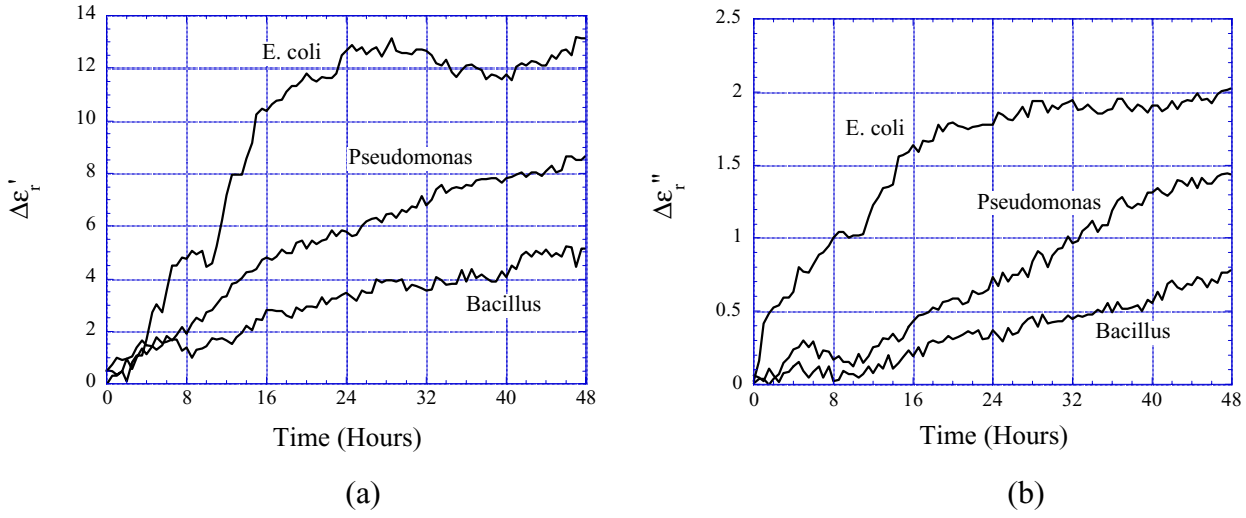

Figure 6. The change in (a) $\varepsilon_{r'}$ and (b) $\varepsilon_{r''}$ of the Pseudomonas, E. coli and Bacillus cultures grown at 25 °C as a function of time.

Fig. 6a and 6b plot the changes in medium complex permittivity, $\Delta\varepsilon_r'$ and $\Delta\varepsilon_r''$, of *E. coli*, *Pseudomonas*, and *Bacillus* cultures over a 48 hr period, with the upward trend in permittivity indicating culture growth. The initial complex permittivities of the three bacteria are different, with ε_r' ranging from 118 to 137 and ε_r'' ranging from 19 to 37, and so for comparison starting values have been normalized to zero. As can be seen from Fig. 6 *E. coli* has the fastest growth rate with an increase of 13.1 in ε_r' and 2.0 in ε_r'' over 48 hr, while *Bacillus* has the slowest growth rate with a 5.1 increase in ε_r' and 0.8 in ε_r'' over 48 hr. Also notice a well-defined growth step in the *E. coli* $\Delta\varepsilon_r'$ spectral

indicating synchronized growth, whereas the $\Delta\varepsilon_r'$ spectra of both *Bacillus* and *Pseudomonas* are continuous.

Food Quality Monitoring

The LC sensor has been used for monitoring the quality of milk, meat, and beer by determining the bacteria populations as a function of time. All sensors in our experiment were $4 \times 4 \times 0.05$ cm in size except the sensor used in the meat package, which was $2.5 \times 2.5 \times 0.05$ cm. A polyurethane layer, approximately 150 μm-thick, was sprayed on the sensor, and the sensor baked at 120°C for 2 hrs. Before starting the experiment, all sensors were autoclaved at 120 °C for 15 mins. The LC sensor measurements were correlated with standard plate counts (SPC) for the milk and meat monitoring experiments.

Milk Quality Monitoring

The milk sample used in our experiment was 1%-lowfat skim milk purchased from a local grocery store. A portion of the milk (500 mL) was poured into a beaker and then autoclaved at 120 °C for 10 mins and used as a control sample. Another 500 mL of milk was poured into another beaker. A sensor was inserted into each beaker; the autoclaved milk was aseptically sealed, and the regular milk loosely covered with a plastic wrap. The experimental setup of the milk is similar to the one shown in Fig. 2, except that two single-turn 8 cm-diameter loop antennas were used to simultaneously measure both milk samples. Both samples were kept at room temperature and monitored for 24 hours. To correlate the complex permittivity with the bacteria count samples were taken from the open milk culture every 2 hrs with the bacteria population determined using standard plate count (SPC). The autoclaved milk sample remained unopened until the termination of the experiment.

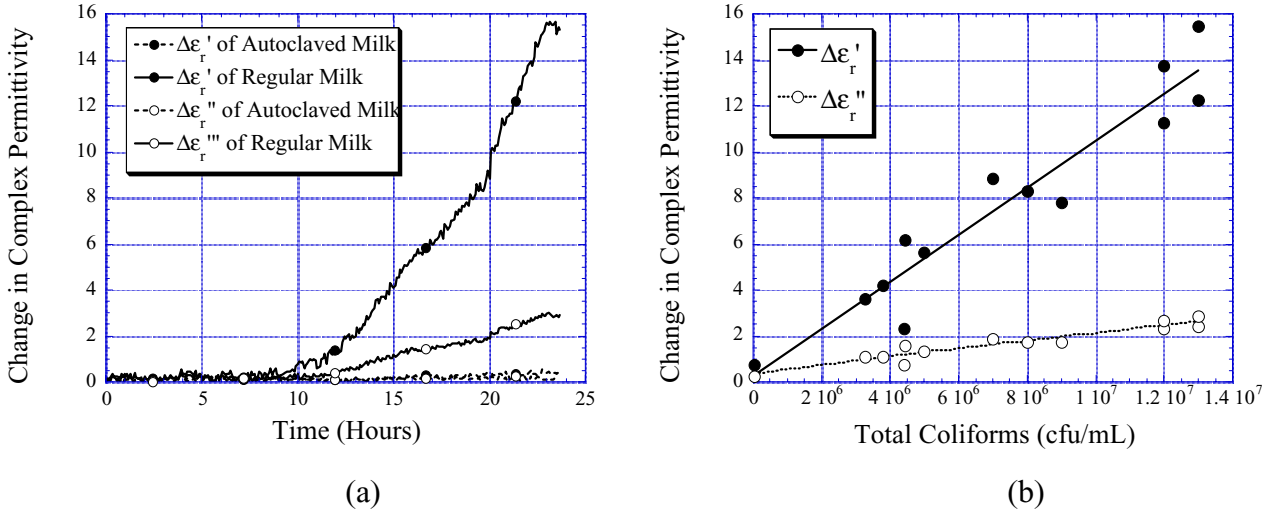

Figure 7. (a) The complex permittivity of the sensor immersed in the regular milk increases as the bacteria count increases, while the complex permittivity of the sensor immersed in the autoclaved, bacteria-free milk remains constant over time. (b) The correlation between the change in complex permittivity and bacteria plate count for the regular milk.

Fig. 7a plots the change in complex permittivity over time for the sensors immersed in the autoclaved milk and in the regular 1%-lowfat milk. As can be seen from the plot, both the real and imaginary permittivity values of the sensor within the autoclaved milk are almost constant over time, indicating no significant bacteria growth. Conversely, the real permittivity of the 1%-skim milk increases by 16 in 24 hrs while the imaginary permittivity increases by 3. Fig. 7b plots the change in the real and imaginary permittivity of the skim milk versus the bacteria count. Correlating Fig. 7a to Fig. 7b, the complex permittivity can be readily used to indicate if the milk is spoiled. For example, if a bacteria count of 1.2×10^7 cfu/ml is considered unsafe for human consumption, the milk in our experiment should be discarded when the $\Delta\varepsilon_r'$ reaches 12 and $\Delta\varepsilon_r''$ reaches 2.5, which is about 21 hrs after the experiment began.

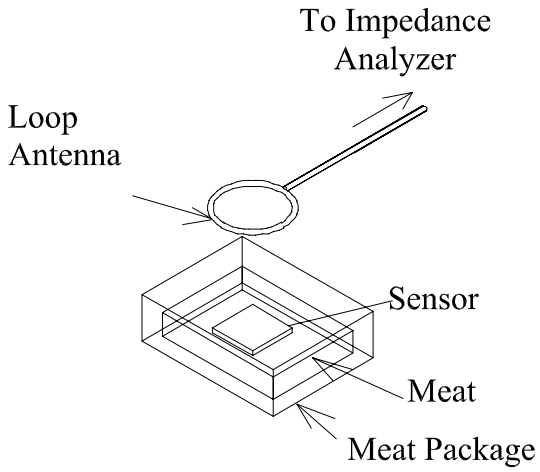

Figure 8. The experimental setup for meat quality monitoring. The sensor is placed between the meat and the wall of the Styrofoam package container.

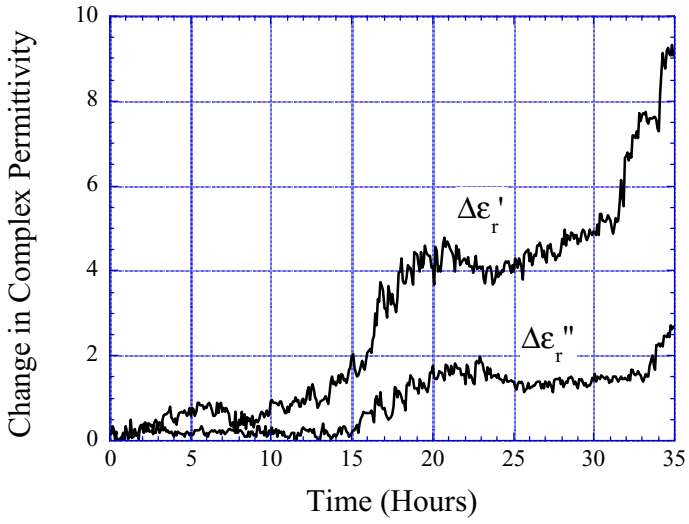

Figure 9. The experimental setup for meat quality monitoring. The sensor is placed between the meat and the wall of the Styrofoam package container.

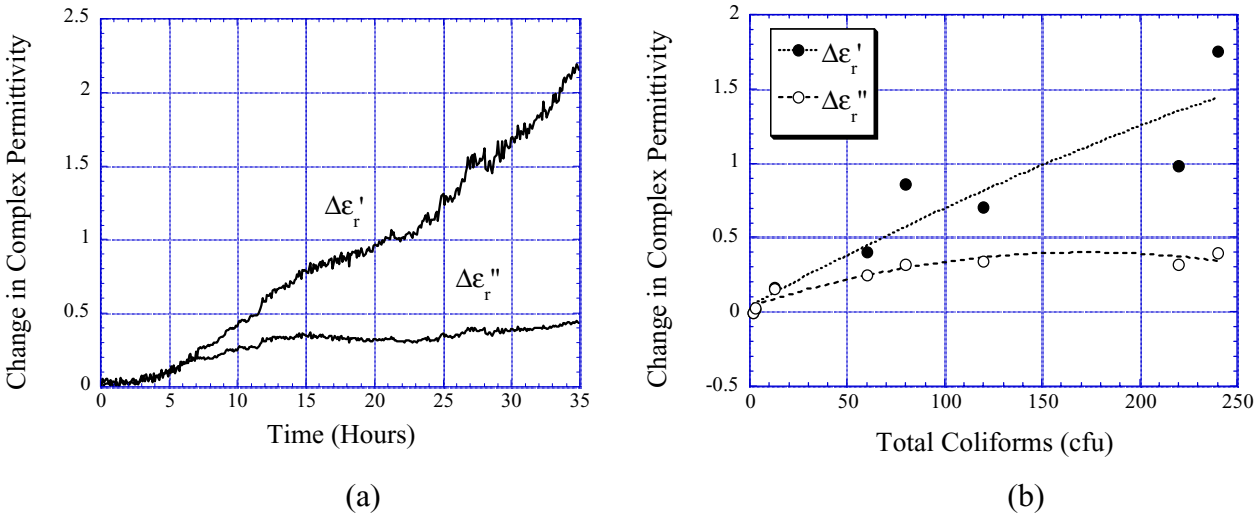

Figure 10. (a) The change in the complex permittivity of the LB-meat culture increases over time as the bacteria concentration increases. (b) The correlation between the change in complex permittivity and the bacteria count in the LB-meat culture.

Meat Quality Monitoring

A 90%-lean 0.5 kg ground beef package was purchased from a local grocery store. The sensor was inserted between the beef and the Styrofoam container, as shown in Fig. 8, with the package then covered, but not hermetically sealed, with a plastic wrap. The complex permittivity of the meat, kept at room temperature, was measured for 1.5 days with the results plotted in Fig. 9. Plate counts were not performed on the meat sample due to an inadequate volume of liquid inside the meat package, instead another experiment was conducted to correlate the changes in the meat permittivity to bacteria growth. In this experiment, 100 mL of Luria Bertani (LB) media were inoculated with 30 μL of an overnight bacterial culture grown from 2 g of meat. The LB-meat mixture was used to represent the bacteria populations typically found in ground beef. The sensor was presoaked in the LB media for 8 hours before the culture was added, and the complex permittivity of the LB culture monitored as a function of time. Samples were taken from the LB culture every 4 hrs to perform SPC. Fig. 10a plots the changes in the LB complex permittivity that had been inoculated with bacteria from a meat sample. Fig 10b plots the correlation between the complex permittivity of the LB-meat medium and the plate count of the medium, showing that the increasing trend of the complex permittivity in Fig. 10a is due to bacteria growth in the medium.

Beer Fermentation Monitoring

The LC sensor can also be used to monitor the fermentation processes *in-situ*, without opening the container, thus preventing unwanted contamination. Here, we demonstrate the monitoring of the fermentation of a beer culture consisting of 1/2 cup of sugar, 1 cup of oatmeal, 2 cups of carbon-filtered tap water, and 7g of Fleischman's Rapidrise™ yeast. The beer container was sealed, and the response of the sensor was monitored for 4 days. The complex permittivity of the yeast culture, calculated from the sensor f_0 and f_z, was determined as a function of time and plotted in Fig. 11. The

plot in Fig. 11 shows a step-wise change in $\Delta\varepsilon_r'$ and a continuous change in $\Delta\varepsilon_r''$ due to the yeast growth.

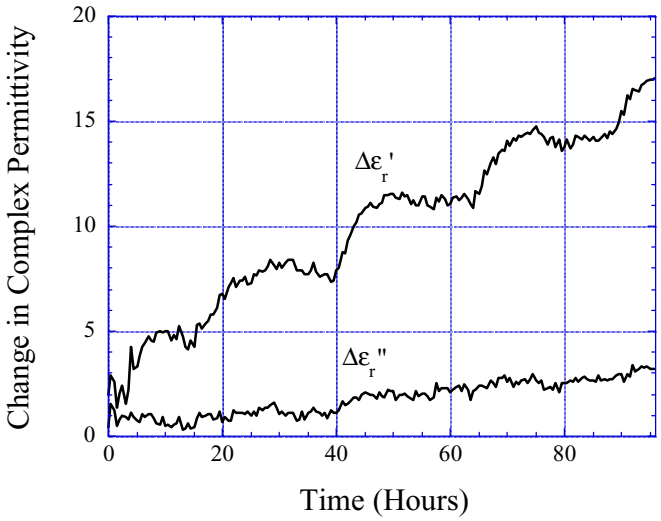

Figure 11. The change in the measured complex permittivity of the beer culture as a function of time, as measured with a LC sensor.

Performance and Limitations

Effects of Temperature and Coating Thickness

In our experiments the effects of the polyurethane coating were calibrated using Eq. (4), with the calibration process specific to the polyurethane coating thickness. Temperature also has a significant effect on the response of the sensor. The resonant frequency of a 4 cm square sensor made on a polystyrene substrate shows a change of 6.4 kHz change in resonant frequency for every degree °C change, which causes a decrease of about $0.7 - j\,0.4$ in the measured complex permittivity of LB medium. Hence for practical applications the LC sensor has to be made upon a more thermally stable substrate, or used in conjunction with a second LC sensor having a different temperature dependency to enable elimination of temperature effects through cross-correlation.

Effects of Sensor Location

The frequency response of the sensor experiences a small shift when the location and orientation of the sensor vary. However the sensor can operate with small error tolerance within a well-defined region. To determine the sensor operating region the zero-reactance frequency f_Z of a 4 cm sensor interrogated with an 8 cm-diameter loop antenna was measured as a function of the sensor displacement in the x and z directions, and the tilt angle θ between the basal planes of the sensor and the antenna (see the coordinate system defined in Fig. 2). The results, shown in Fig. 12, indicate that within a region (0 cm $< x <$ 3 cm, 3 cm $< z <$ 6 cm) the variation in f_Z was less than 5 kHz, or about 0.25% error in the measurement. To maintain the 5 kHz error tolerance, the tilt angle θ must also be less than 30°. There is a minimum separation distance in the z direction between the sensor and the

antenna due to the near-field inductance coupling between the sensor inductor and the antenna that causes a change in f_0 and f_z. The maximum separation distance between the sensor and the antenna is due to the errors from the electronic instrumental noise when the sensor signal becomes too small to be detectable at large distance.

Effects of Water Absorption in the Sensor Surface

When the LC sensor is immersed in a liquid, water molecules are absorbed into the polyurethane layer, decreasing f_0 and f_z and causing a shift in measured complex permittivity. However, the absorption process stops in a few hours after the polyurethane layer saturates. Fig. 13 shows f_z of a sensor coated with 150 μm-thick polyurethane layer, subsequently baked at 120 °C for 2 hrs, and then immersed in water. As can be seen from Fig. 13, the saturation time is ≈ 4 hrs. The saturation time of the water absorption process depends upon the polyurethane thickness, and the pore size which is a function of the curing temperature. Experimentally we found that the saturation time increases to 15 hrs when the curing temperature is 80°C, and 28 hrs when the curing temperature is 60°C. The saturation time also increases from 4 hrs to 14 hrs when the polyurethane thickness increases from 150 μm to 400 μm. In summary, the saturation time decreases with increasing baking time and temperature, and decreasing coating thickness.

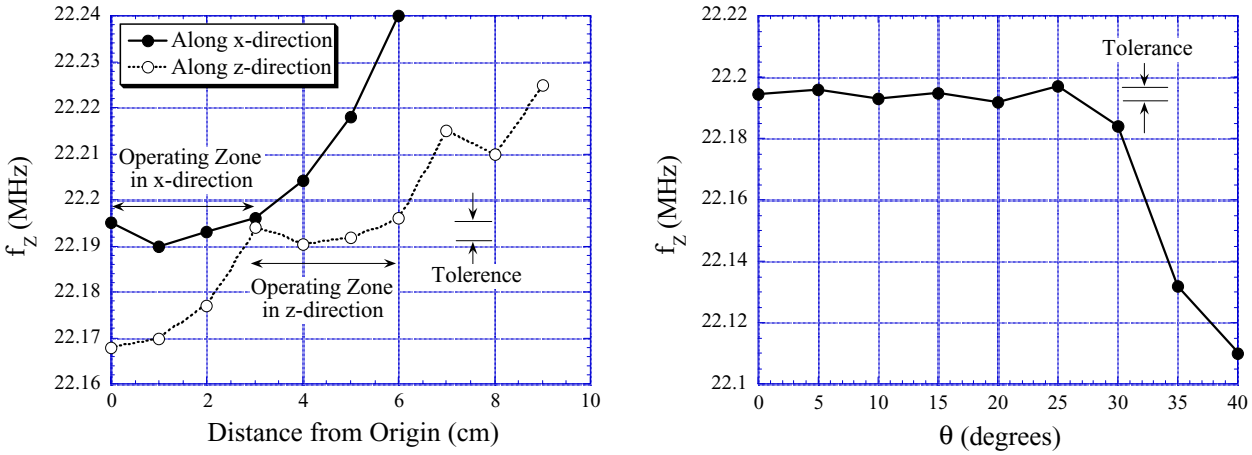

Figure 12. (a) The shift in f_z of the sensor in the x direction (solid line) when the sensor is 3 cm from the xy-plane, and the shift in f_z in the z direction (dashed line) when the sensor is on the z-axis. (b) The shift in sensor f_z as a function of θ when the sensor is on the z-axis, 4 cm away from the origin.

Long-term exposure to a bacteria laden medium will damage the protective polyurethane coating. The surface of an as-made sensor is shown in Fig 14(a), and the surface of a sensor immersed in distilled water for 4 days is shown in Fig 14(b). Fig. 14(c) shows the sensor surface after immersion in a bacteria culture for 2 days, and 14(d) the sensor surface after immersion in a bacteria for culture for 4 days. The lifetime of a polyurethane-coated sensor in bacteria laden solution is about one week, after which the polyurethane layer peels away from the sensor.

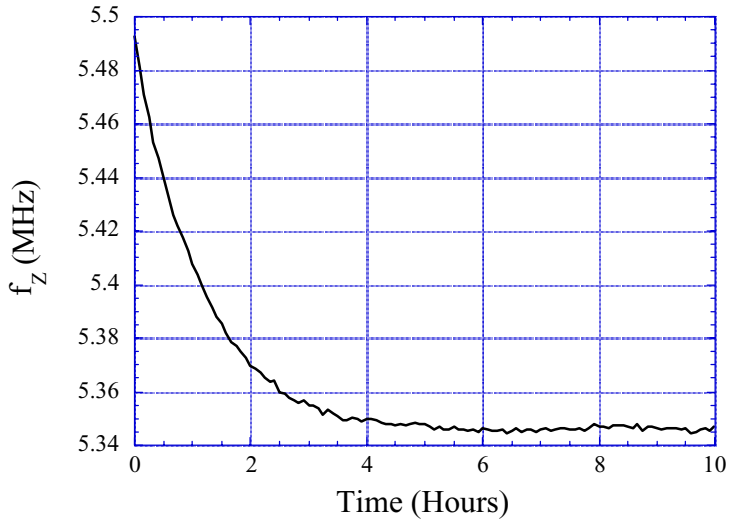

Figure 13. The change in f_z of an LC sensor immersed in water, as a function of time, due to water absorption in the 150 µm thick polyurethane layer.

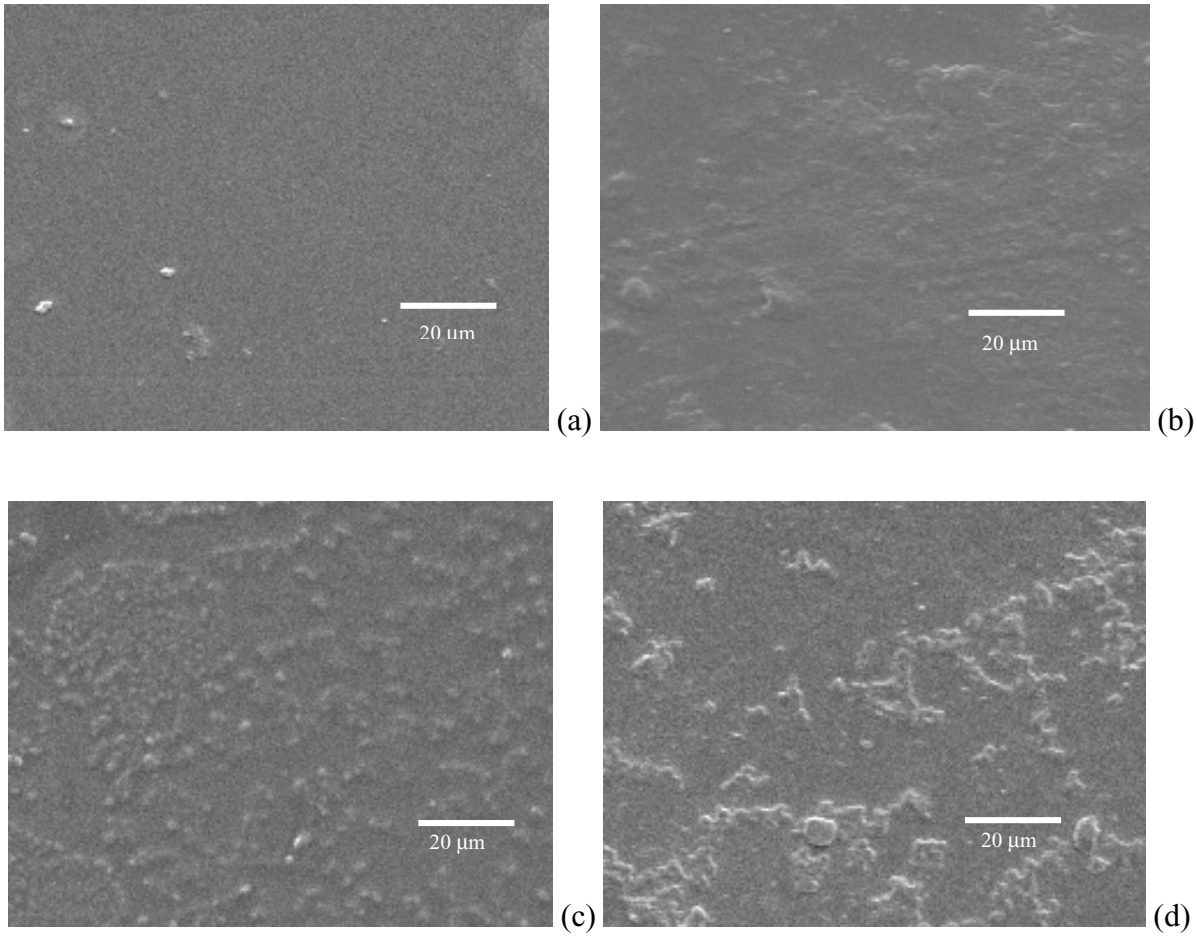

Figure 14. SEM images of the polyurethane layer surfaces for (a) the sensor in air, (b) the sensor in water for 4 days, (c) the sensor in a bacteria culture for 2 days, and (d) the sensor in a bacteria culture for 4 days.

Conclusions

A wireless, passive remote-query sensor technology is presented for monitoring bacteria growth. The sensor is a planar, printed or photolithographically defined resonant circuit, the response of which is remotely detected by a loop antenna. The substrates upon which the resonant circuits are defined can be flexible (e.g. paper or plastic film) or rigid (e.g. ceramic, plastic, Plexiglas) depending upon the desired application. The sensor is immersed in the medium of interest, and the complex permittivity of the medium calculated from the electromagnetic response of the sensor. Our results show the sensor can measure complex permittivity changes in bacteria cultures consisting of *E. coli*, *Bacillus*, and *Pseudomonas* with greater discernable measurement sensitivity than optical density measurements.

The application of the LC sensor technology to monitoring milk, meat, and beer quality was illustrated. Experimental results show the complex permittivity of the autoclaved milk was constant over time, while the complex permittivity of the 1%-fat skim milk increased over time as the bacteria population increased. The sensor technology was also used to measure the increase in complex permittivity of a meat sample, with time, as the meat spoiled. By correlating the complex permittivity shift with the bacteria count the sensor can be used to indicate if a food product is safe for human consumption. In the case of a dry food product, such as cereal or grain, the presence of humidity due to package failure would readily change the measured impedance of the sensor. Additionally, the LC sensor was used to monitor the fermentation process of a beer culture during fermentation.

Since the LC-sensor monitoring electronics could be reduced to a relatively inexpensive small-scale portable package, the LC sensor technology presented here shows promising potential for commercial food quality monitoring in a grocery store or even at home. Each sensor costs a fraction of a penny, so it can readily be used on a disposable basis. Furthermore the LC sensor is relatively insensitive to location and orientation within its operation range so untrained users can operate it.

Acknowledgements

Support of this work by NSF grants ECS-985104 and ECS-9988598, and NASA grant NAG-1-01036 is gratefully acknowledged.

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Sample Availability: Available from the authors.