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Sensitivity of Phage-Based Magnetostrictive Biosensors for Detecting *S*. Typhimurium on Fresh Produce

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Abstract: The magnetostrictive (ME) biosensors have been developed to detect pathogen rapidly and sensitively in fresh produce. The effects of concentration and incubated time of *S*. typhimurium enrichment on the detection sensitivity were investigated in this study. The concentrations ranging from 5×10^2 to 5×10^8 CFU/mL with incubated time of 22h, and incubated time ranging from 5h to 22h with the concentration of 5×10^2 CFU/mL were tested. The results showed that the higher concentration and longer incubated time, the more frequency changes due to the more bacteria binding on the ME biosensors. The micrographs by optical microscope were used to verify the bacteria binding on ME biosensors. The student t-test calculation was also used to analysis the difference between measurement and control sensors. The limit of detection (LOD) for phage-based magnetostrictive biosensors on fresh tomatoes was statistically determined to be lower than 5×10^2 CFU/mL for 5h enrichment with a confidence level of difference higher than 98% (p<0.05).

Keywords: Magnetostrictive, Biosensors, *S*. Typhimurium, Sensitivity, Detection

1. Introduction

Fresh produce safety is concerned by global people with the foodborne disease outbreaks, especially salmonellas [1]. Salmonella has been linked to the outbreaks of lettuce, tomatoes, jalapeno peppers and other fresh produce [2]. In the United States, there are 1.2 million cases of salmonellas every year, resulting in over 23,000 hospitalizations and 450 deaths [3]. Recently, a cucumber-associated outbreak of salmonella resulted in 418 cases and 2 deaths in 31 states in September, 2015.

Salmonella can be effectively detected and identified by the traditional methods, such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and some other methods [4]. However, these methods usually require approximately 5 days to complete the analysis [5]. Hence, a rapid and reliable detection method should be developed to prevent the salmonellas.

Wireless phage-based magnetostrictive (ME) biosensors have been developed to be a rapid detection method of salmonella in fresh produce. A ME biosensor consists of a magnetostricitive resonator platform as a transducer and phage coated as a bio-recognition element [6]. In this investigation, the resonator platform was coated with E2 phage for the specific binding of *S*. typhimurium [7]. In this investigation, according to the method described in the Bacteriological Analytical Manual (BAM) by the U.S. Food and Drug Administration (FDA), *S*. typhimurium was enriched to different concentrations in broth with different incubated time and rapidly detected by ME biosensors.

2. Materials and Methods

2.1. Fabrication of ME Resonators and Metal Deposition

Magnetostrictive resonator platforms were fabricated by METGLAS® 2826MB alloy (30-μm thick, Honeywell International, Inc., NJ, USA). The ribbon was diced into

1mm×0.2mm×0.028mm rectangular strip-shaped platforms using a computer controlled automatic micro-dicing saw (DAD 3220, Disco Crop, Tokyo, Japan), followed by cleaning in acetone and ethanol by ultrasonic cleaner, and drying in air. Afterwards, annealing was carried out at 220°C for 2h in vacuum ($\leq 10^{-3}$ Torr) to relieve the remained stress and correct defects from polishing and dicing. Finally, chromium (90 nm thick) and gold (150 nm thick) layers were deposited onto both sides of the platform surfaces at 2.5×10^{-6} Torr (Denton Vacuum, NJ, USA). The Cr coated layer used to protect the ME biosensors from corrosion, as well as an adhesive interface between the resonator and the gold layer. The Au layer acts as a protection of corrosion and immobilization of the phage bio-probe [8].

2.2. Immobilization of E2 Phage and Bovine Serum Albumin (BSA) Surface Blocking

The filamentous E2 phage (1.01×10^{12}vir/mL) was prepared and provide by Dr. James M. Barbaree's lab in the Department of Biological Science at Auburn University. The E2 phage is specific for *S*. typhimurium [9]. The bare ME resonators were immersed in a 330µL tube with the phage suspension that was diluted to 5×10^{11}vir/mL with 1×Tris-Buffered Saline (TBS) solution and rotated for 1h at room temperature. After washing with distilled water twice to remove the unbound bio-probe, the resonators surface was adsorbed physical phagesuniformly. In order to prevent the nonspecific binding [10], the resonators were immersed in the tube (330µL) with 1mg/mL bovine serum albumin (BSA) and rotated for 45min, followed by dipping in DI water for 5 seconds to remove the debris.

2.3. *S. typhimurium* Cultures and Contamination on Tomatoes

The *S*. typhimurium was also provided by Dr. James M. Barbaree's labin the Department of Biological Science at Auburn University. Two tomatoes (purchased in a local grocery store) were placed in a sterile plastic bag, and the preparation of S. typhimurium in this work is conducted basically according to the general rules of BAM (FDA), though with few modifications. *S*. typhimurium solution of 10ml at concentration of 5×10^8, 5×10^6, 5×10^4, and 5×10^2 CFU

was applied on the tomato surfaces with a sterile adjustable Sprayer (Spray Anywhere, Fisher Scientific, Pittsburgh, PA) inside a sterile tote and incubated in LB broth (the weight ratio of tomato to broth is 1 to 2). The tomatoes with the broth were incubated at different time (5h, 6h, 7h, 22h) at 37°C after 1h pre-enrichment at room temperature. The incubated broth was concentrated to 2mL with filtered water washing. The measurement sensors (immobilized with phage) and control sensors were immersed in the *S*. typhimurium solutions in the PCR tubes with the volume of 330mL for 30min rotation.

2.4. The Principle of Detection and Resonance Frequency Measurements

ME biosensors made by METGLAS® 2826MB alloy are ferromagnetic materials with the property that the shape or the dimensions would be changed due to magnetization [11]. When subjected to an applied time-varying magnetic field, the ME biosensors can be placed into mechanical resonance [12]. The principle of the detection is based on the resonant frequency shifts of ME biosensors when the mass is changed after binding the pathogenic bacteria. The fundamental resonant frequency of a thin, freestanding, strip-shaped resonator under longitudinal vibration is given by [13–15]:

$$f = \frac{1}{2L}\sqrt{\frac{E}{\rho(1-\upsilon)}} \tag{1}$$

Where *f* is the initial resonant frequency, *L* is the length of the resonator, *E* is Young's modulus of elasticity, ρ is the density of the resonator material and *v* is the Poisson ratio.

When a small mass of the pathogenic bacteria attached to the ME biosensor, the increased mass causes a decrease of resonant frequency. The resonant frequency shift can be given by [16, 17]:

$$\Delta f \approx -\frac{f}{2}\cdot\frac{\Delta m}{M} \tag{2}$$

Where Δm, M ($>>\Delta m$), and Δf denote the mass change, the initial mass of the resonator, and the resonant frequency shift, respectively.
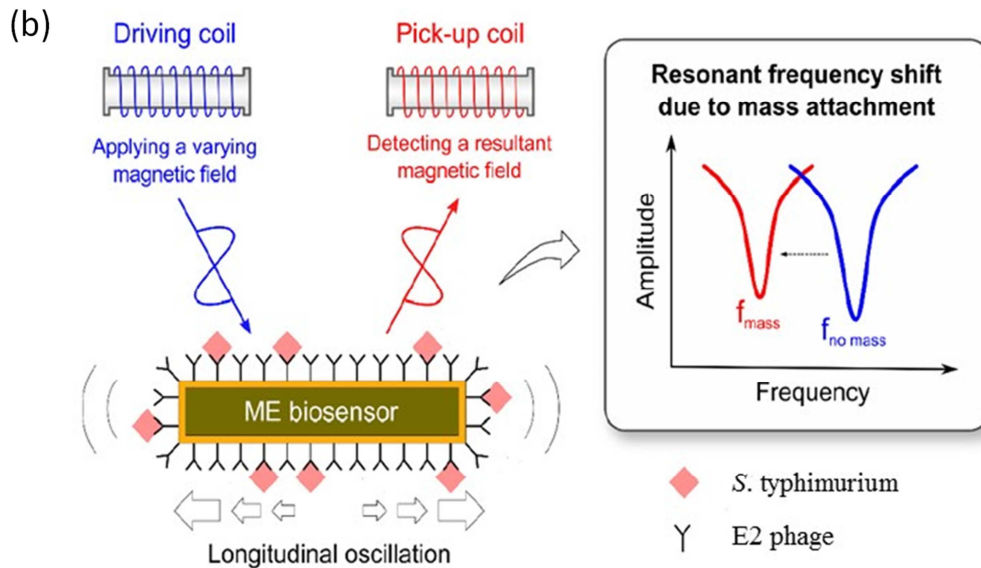
(a)
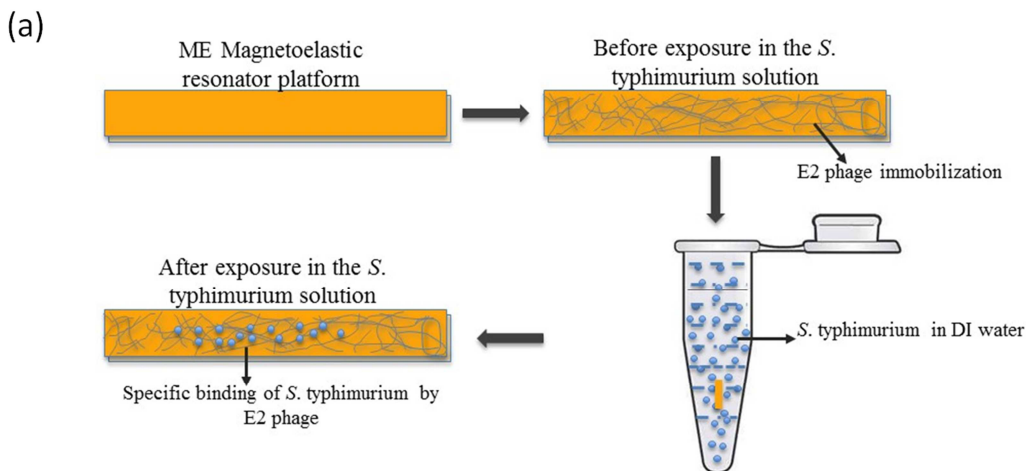
Figure 1. *Detection of principle of the ME biosensor.*

As shown in Figure 1, the initial and final resonant frequency (before and after immersing in the *S*. typhimurium solution) of measurement sensors and control sensors were measured by a single-layer solenoid coil [18, 19] connected with a network analyzer (HP/Agilent 8751A, Agilent Technologies, Inc., Santa Clara, CA, USA). The solenoid coil was made by glass capillary (1mm inner-diameter) with copper wire (0.1mm thick). The frequency shifts presented the mass change because of specific bindings on the ME biosensors.

2.5. Optical Microscope

The specific binding was observed and verified by optical microscope (50×, Olympus BX51, Tokyo, Japan). In order to clearly observe the cells, the ME biosensors were exposed in the crystal violet dye (5% in DI water) to absorb the color for 10min, and washed by distilled water to remove the remained dye on the surface prior to observing.

2.6. Statistical Analysis

Statistical analysis was conducted on the responsesof ME biosensors exposed to tomato surfaces spiked with differentconcentrations of *S*. typhimurium. For each concentration, a Student's t-test was performed to compare the difference between measurement and control sensors to get the confidence level of the experiment.

3. Results and Discussion

3.1. The Effects of *S*. Typhimurium Concentrations

S. typhimurium with four different concentrations ranging from 5×10^2 to 5×10^8 CFU/mL was spiked on tomatoes and incubated in LB broth for 22 hours. The incubated broth with the volume of 3mL was taken to concentrate to 1mL. Figure 2 shows the frequency changes of the measurement sensors and control sensors after exposure in bacteria with the different concentrations. As can be seen, the frequency shifts of measurement sensors have big difference with those of control sensors, which illustrates that the measurement sensors have much more mass changes because of the bacteria cells binding. And it also shows that at the same incubated time, the higher bacteria concentration spiked on tomatoes, the more resonant frequency shifts due to more bacteria capturing. Meanwhile, the student t-test calculation was used to analysize the differencebetween the measurement and control sensors, and it is found that the confidence level of the difference is higher than 99% (p<0.05), thus it can be concluded that the detection limit is lower than 5×10^2 CFU/mL for 22h enrichment.
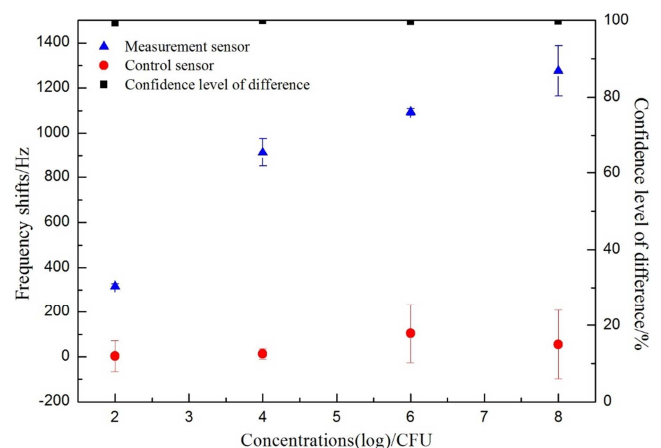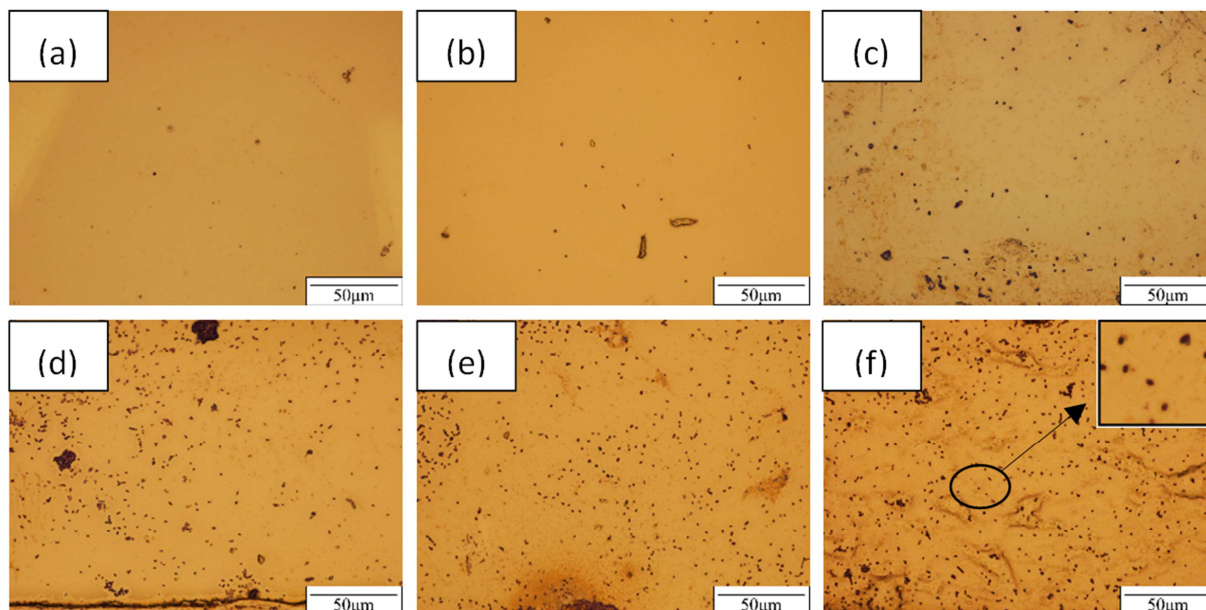

Figure 2. *The frequency shifts of ME biosensors immersed in different concentration of S. typhimurium incubated in broth for 22 hours with the contaminated tomatoes.*

The micrographs shown in Figure 3 visually confirmed that a large amount of bacteria were captured on measurement biosensors, while few bacteria on control sensors. Meanwhile, it can be obviously seen that the amount of bacteria captured by ME biosensor increases with the concentration of *S*. typhimurium spiked on the tomatoes.
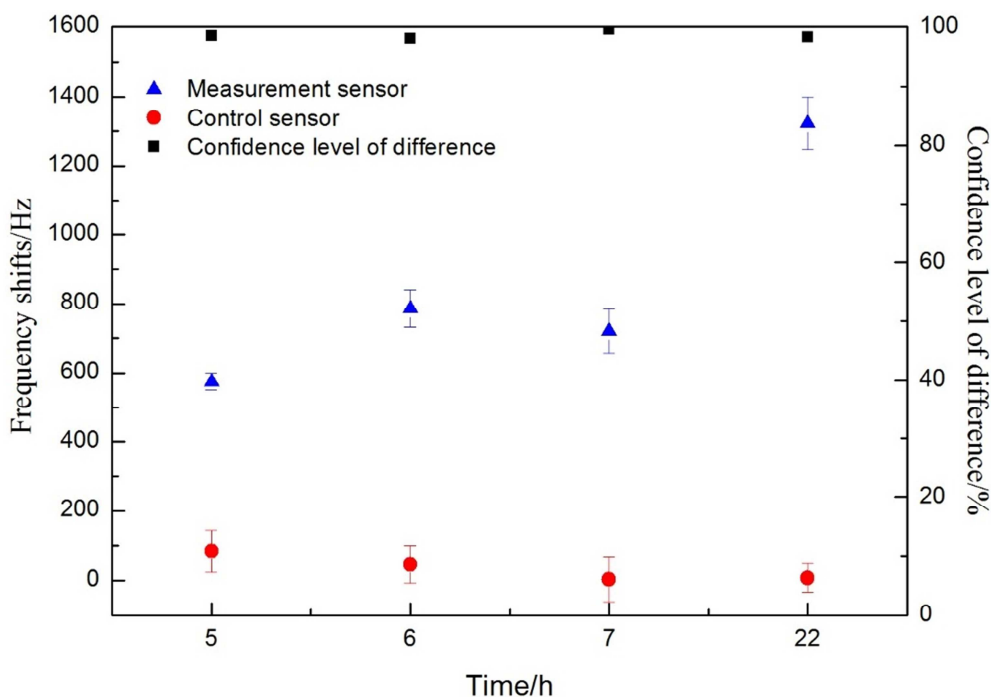
Figure 3. *Micrographs of ME biosensors: (a) bare sensor; (b) control sensor; measurement sensors after exposure in S. typhimurium incubated with different concentrations: (c) 10^2 CFU; (d) 10^4 CFU; (e) 10^6 CFU; and (f) 10^8 CFU.*

3.2. The Effects of Incubated Time

10mL of *S. typhimurium* solution with the concentration of 10^2 CFU was spiked on tomatoes, and then incubated in LB broth for different time ranging from 5h to 22h. All the incubated broth was concentrated to 2mL, and washed by DI water. Then the salmonella in DI water was concentrated from 2mL to 1mL. Figure 4 shows the frequency shifts of ME biosensors immersed in *S. typhimurium* with the concentration of 10^2 CFU incubated in broth for different time. It presents that the frequency shifts of measurement sensors are much bigger than those of control sensors, and it also shows the tendency that the frequency shifts increase with the incubating time, due to more *S. typhimurium* captured by ME biosensor, which was confirmed by micrographs shown in Figure 5.

The student t-test calculation was also used to analysis the difference between measurement and control sensors. And the confidence level of difference higher than 98% ($p<0.05$) was obtained. Therefore, it can be concluded that the detection limit is lower than 5×10^2 CFU/mL for 5h enrichment as well.

Figure 4. *The frequency shifts of ME biosensors immersed in S. typhimurium incubated in broth with the contaminated tomatoes for different time.*
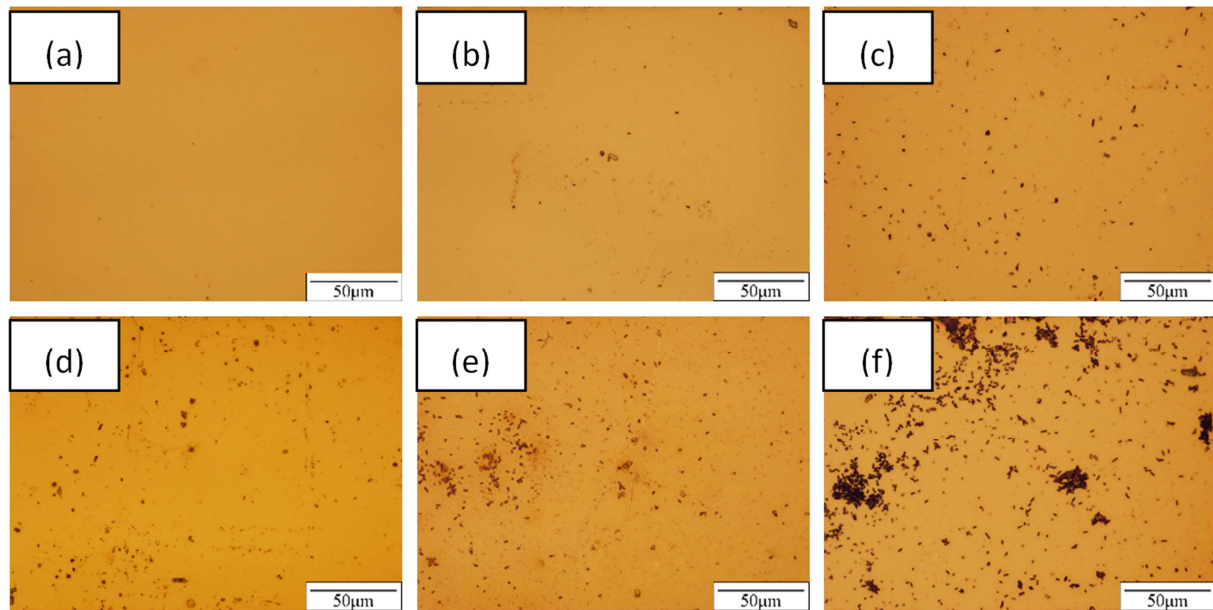
Figure 5. *Micrographs of ME biosensors: (a) bare sensor; (b) control sensor; measurement sensors after exposure in S. typhimurium incubated for different time: (c) 5h; (d) 6h; (e) 7h; and (f) 22h.*

4. Conclusions

The effects of *S.* typhimurium concentrations and incubated time on the detection sensitivity of ME biosensors were studied. Phage based biosensors were used to specifically bind *S.* typhimurium and the bound bacteria were verified by optical microscope. The student t-test calculation was also used to analysis the difference between measurement and control sensors. Both the experiments and statistical analysis results illustrated that the limit of detection (LOD) was determined to be lower than 5×10^2 CFU/mL for 5h enrichment with a confidence level of difference higher than 98% ($p < 0.05$).

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