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A surface acoustic wave sensor modified from a wireless transmitter for the monitoring of the growth of bacteria

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Abstract

This work presents a novel surface acoustic wave (SAW) sensor for the measurement of the microbial count in a biological culture. The signal translation interface of the sensor was modified using a common commercial wireless SAW transmitter design. A pair of conductive electrodes was inserted in a 314.5 MHz SAW stabilized oscillator of the transmitter. Measurements were made by placing the electrodes within the culture solution of interest and measured the time required to identify readily detectable frequency changes (detection time, DT). The change of frequency was caused by the impedance change of the microbial metabolism. The calibration curve of detection times against density of *Escherichia coli* shows a linear correlation coefficient ($R^2 = 0.924$) over the range of 10^2 to 10^7 cells/mL. A sample that contains 10^2 cells/mL of *E. coli* required a detection times (DT) of 7 h, shorter than was required using instruments based on conventional conductance methods. The proposed ultra high frequency SAW sensor (314.5 MHz) shows a large total frequency change and gives a sharp inflexion at the DT but possesses the same stability compared with that of low frequency serial piezoelectric quartz crystal (PQC) sensor for the measurement of bacteria concentration. This sensor platform enabled real time monitoring of bacterial growth within a sealed opaque container. This sensor is potentially applicable to a remote query wireless measurement in hazardous environments when a suitable antenna device is adapted.

Keywords: Surface acoustic wave; SAW; *Escherichia coli*; Bacterial growth

1. Introduction

Conventional microbiological methods for determining bacteria counts are time-consuming, taking up to 2 days. Additionally, a large number of tests lead to great expense due to labor, material costs, and huge storage costs in the food industry. As a result, various rapid methods have been developed for the determination of bacteria counts in the past several decades, including ATP estimations [1], direct epifluorescent filter technique [2], amperometric biosensing method [3], immuno-biosensor assays [4], and electrical methods [5,6]. This work presents an ultra-high frequency surface acoustic wave (SAW; 314.5 MHz) sensor for the measurement of the microbial count in a biological culture. This type of acoustic biosensor could potentially offer a simple, low-cost and robust (repeatable) way to assay bacterial loads in complex liquids [7]. The availability of an ultra-high frequency also enables the design of wireless remote control functions. The ATP method and epifluorescent filter method are fast but complicated for handling. Strong electrochemical interference from oxidisable species in the media exposes a serious problem for the practical operation of amperometric biosensors. The immuno-biosensor assays encounter non-specific adsorption of protein during measurement. Electrical methods are simple but require expensive instruments. Impedance microbiology [8] was one of the earliest electrical methods for the detection of bacteria in foods, and has been developed as a rapid method that can detect bacteria within 24 h [9,10]. It is based on the measurement of changes in electrical impedance of a medium or a reaction solution resulting from the growth of bacteria. The growth of microbial populations in culture changes the electrical

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properties of the culture medium. Substrates in microbiological growth media are generally uncharged or weakly charged. They are normally transformed into highly charged end products following the organism's normal metabolic pathways. Thus the electrical properties of the test medium will change during the growth of bacteria [9,11]. Simple examples include the conversion of glucose from a non-ionized substrate to two molecules of lactic acid with a corresponding increase in conductivity. Typically, changes in the electrical properties of the culture medium resulting from bacterial growth become detectable when the microbial density reaches 10^6 to 10^7 cells/mL [12,13]. The time required to identify readily detectable changes (detection time, DT) provides an approximate measure of the number of microbial organisms in the initial inoculums [14].

Two types of measurement techniques have proved useful in commercial applications: conductivity and impedance [5,15,16]. Devices based on these techniques monitor microbial metabolism in growth medium by immersing electrodes directly into the medium and measuring the permittivity and/or conductivity [17]. The impedance method was approved as an official method for the detection of *Salmonella* in foods by the Association of Analytical Communities (AOAC) [6]. Despite their widespread application, these techniques have many disadvantages including polarization of the probe electrodes, decreased sensitivity of the device in more conductive media and the high cost of the instruments [18]. Alternatively, surface acoustic wave (SAW) resonator impedance systems appear very attractive because they work at very high frequency [19], and thus electrode polarization is eliminated. Surface acoustic wave (SAW) resonator transmitters have been widely used for unlicensed applications for more than a decade. Applications include: automotive keyless entry, door and gate openers, wireless alarm sensors, bar code reader, and many others in wireless remote control, security and transmission areas. The SAW resonator stabilized oscillator provides a very stable fundamental mode frequency source at ultra-high frequency.

Yoa et al. developed a system consisting of a SAW resonator (61 MHz) and a pair of conductive electrodes in the feedback circuit with a radio frequency amplifier [20,21]. Designs using SAW impedance systems have been applied to various bioanalytical fields concerning the (i) total salt concentration in serum [22], (ii) determination of urea [23], and (iii) pancreatic lipase [24]. The system can respond to changes in the capacitance and the conductance of the solution between the two electrodes, but its use to monitor bacterial growth has not been reported. Furthermore, there have been no studies of SAW impedance system using an ultra-high frequency resonator signal, which could improve sensitivity [25]. The availability of an ultra-high frequency also enables the design of wireless remote control functions [26].

This article describes the development of a sensor with a signal translation circuit, modified using a common commercial wireless SAW transmitter. The device consists of a pair of conductive electrodes inserted into a 314.5 MHz SAW stabilized oscillator. The oscillator is characterized by its low cost, small size and energy-efficiency. We describe the application of this sensor for measurement of the number of microbes in a biological culture. The stability and sensitivity of proposed SAW sensor was also compared with that of low frequency PQC (8.0 MHz) sensor [27].

2. Experimental

2.1. Reagents

The 314.5 MHz one-port, two terminal SAW resonator (RO2113A) used in this study was obtained from the Radio Frequency Monolithic Company (RFM, USA). The AT-cut AT-cut piezoelectric crystal with 8 MHz frequency used in this study was obtained from the Tai Tien Electric Co., Ltd., Taiwan. *Escherichia coli* (ATCC 43886) obtained from the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan) was inoculated into nutrient broth (Difco) and incubated at 30 °C overnight. The home-made reaction cell was constructed from a Teflon cup (i.d. = 5 mm, depth = 20 mm) and a teflon cover which held two gold electrodes (o.d. = 1 mm, L = 10 mm). L-Glycine, L-cysteine, L-leucine, L-tyrosine, L-methionine, L-histidine, L-aspartate, L-lysine and L-arginine were purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Experimental set-up

Fig. 1 shows the experimental set-up. The sensor was constructed with a SAW-stabilized oscillator [26]. A pair of gold electrodes was connected to the SAW resonator in series. The oscillation signal was fed to a frequency counter with a coaxial cable (Fig. 1A) (HP 53131A, Hewlett-Packard, USA).

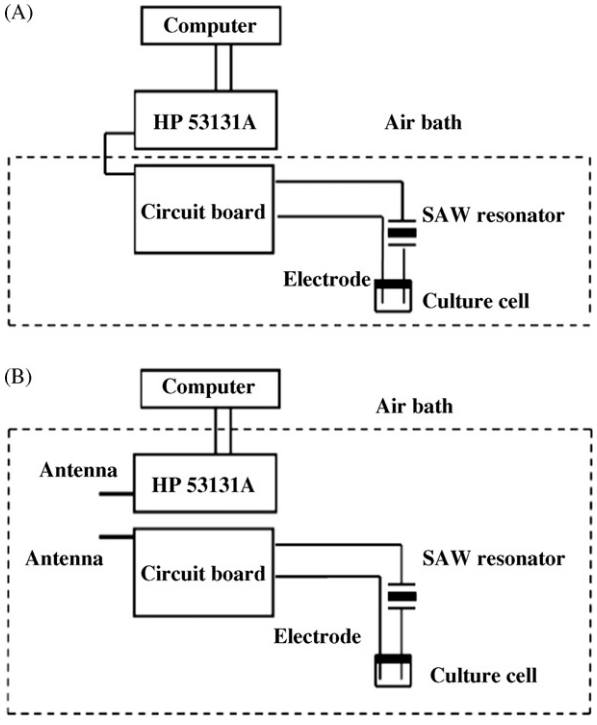

Fig. 1. Schematic diagram of the experimental set-up where (A) signal is fed to a frequency counter via a coaxial cable and (B) signal is transmitted wirelessly.

The signal from the frequency counter was coupled to a PC (Pentium 600 MHz) using a PCMCIA-GPIB interface (GPIB National Instruments, Austin, TX, USA). Data acquisition and display were programmed using the LabVIEW 5.1 software package (National Instruments, Austin, TX, USA). Fig. 1B depicts the wireless experimental set-up. The design of the oscillator was the same as shown in Fig. 1A except that the signal from the oscillator was transmitted via the antenna to the frequency counter. The counter was separated from the sensor by approximately 20 cm and was used to receive the wireless signal. The series piezoelectric quartz crystal (PQC) sensor was constructed by connecting an AT-cut piezoelectric crystal with 8 MHz frequency and a pair of gold electrodes in series. A homemade TTL oscillator was employed to drive the crystal.

2.3. Procedure

To eliminate cross-contamination during bacteria concentration monitoring experiments, the Teflon reaction cell and electrodes were washed and rinsed with distilled water and sterilized at 121 °C for 15 min before each incubation and prior to immersion in any of the solutions. Then, a volume of 0.1 mL milk sample mixed with 0.1 mL sterilized nutrient broth was added to the cell culture which was then fitted with the electrodes. Unless otherwise stated, the total volume was ca. 0.2 mL for each experiment. The conductive electrodes were immersed in the cell culture and connected to a 314.5 MHz SAW resonator. The sensor was placed in an air bath at 30 °C with precise temperature control within ± 0.2 °C. Unless otherwise stated, the measurements were taken half an hour after each inoculation of the cell cultures. The frequency was displayed and recorded by the computer until the incubation was completed. When a test is initially set up the user defines the detection criteria, normally −10 Hz for most applications and when the rate of change of frequency exceed this pre-determined value the system will detect growth. The time required to reach the point of detection is referred to as detection time (DT) and is a function of the number of initial microbial population. Pour plates were prepared from these samples and incubated at 30 °C for 24 h. All colonies were counted 24 h after they were incubated. The detection times were plotted against the responses determined by the plate count method for the solutions in a calibration graph. For the determination of growth curve, a concentration of *E. coli* of ca. 10^8 cells per milliliter (cells/mL) in the nutrient broth was used to prepare ca. 10^1 to 10^2 cells/mL *E. coli* in the aseptic nutrient broth. The concentration of *E. coli* in the dilution was confirmed by both plate counting method and SAW method. The growth curve of *E. coli* in this commercial aseptic nutrient broth at 30 °C was obtained by determining the bacterial counts of sample every 2–3 h.

2.4. Principle

The SAW resonator was used to control the frequency of a SAW resonator-stabilized oscillator. Fig. 2 shows the equivalent circuit model of a SAW resonator and its reactance characteristics with frequency. The circuit components C_m, L_m, R_m and C_o are the motional capacitance, motional inductance, motional resistance and static capacitance, respectively, of the SAW resonator. The components C_m and L_m determine the resonance frequency of the SAW resonator. When a load capacitor (C_L) is connected with the resonator in series, the frequency of the oscillator is changed by a frequency Df, where Df is $C_m/2(C_0 + C_L)$. The frequency is decreased with the increase of the C_L.

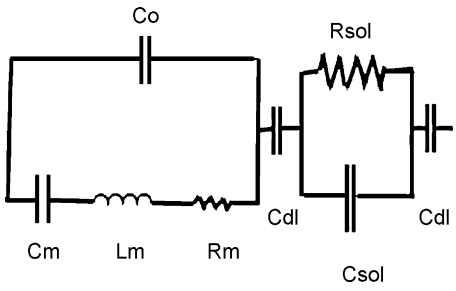

Fig. 2. The equivalent circuit of a SAW resonator. C_m, L_m, R_m and C_o are the motional capacitance, motional inductance, motional resistance and static capacitance, respectively, of the SAW resonator. R_{sol} is the reciprocal of the solution conductance, C_{sol} is the permittivity of the solution, and C_{dl} is the double layer capacitance between the electrodes and the fluid establishing contact with the electrode.

Yoa et al. reviewed the characteristics of electrode–electrolyte interfacial impedances when a pair of electrodes was connected with a SAW resonator in series [20]. For sinusoidal inputs, the terminal characteristics of an electrode have both a resistive (R_{sol}) and a reactive (C_{sol} and C_{dl}) component, where R_{sol} is the reciprocal of the solution conductance, C_{sol} is the permittivity of the solution, and C_{dl} is the double layer capacitance between the electrodes and the fluid establishing contact with the electrode [8,28]. When a pair of electrodes was connected to the SAW resonator in series and was placed within the bacterial culture solution of interest, microbial metabolism typically decreased both the resistance and the capacitance of the culture solution [5], causing a variation of the C_{sol}, C_{dl} and R_{sol} of the circuit loop and thereby altering the frequency of the oscillator. These features permit the SAW resonator to be used for the real-time monitoring of bacterial growth within a sealed opaque container.

3. Results and discussion

3.1. Frequency profile of the saw-stabilized oscillator

The frequency profile of the SAW-stabilized oscillator is shown in Fig. 3. The oscillation signal was fed to a universal frequency counter by an axial cable (HP 53131A, USA). The noise level of the oscillator is ±5 Hz (Fig. 3A). The relative frequency stability was 3.2×10^{-8} based on the observed noise to oscillation frequency ratio over an average period of 20 min. Value of frequency stability was the noise level divide by the frequency of the SAW device (314.5 MHz). Fig. 1B shows the wireless design of the oscillator, where the signal is transmitted by antenna to the frequency counter. The frequency profile obtained by the wireless transmission and the non-wireless transmission were almost identical to each other, except for the effects of EMI. A

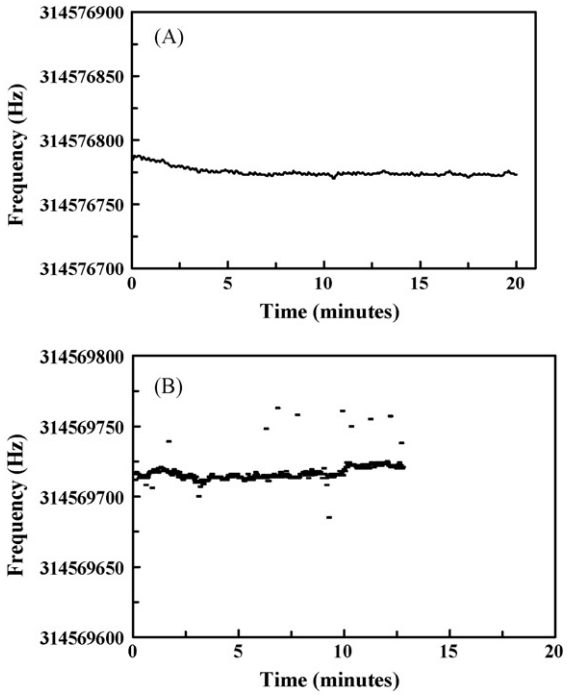

Fig. 3. Frequency profile of (A) the coaxial cable design and (B) the wireless design of the transmitter.

signal free from the effects of EMI is obtainable when the signal was fed into the counter via a coaxial cable. These findings indicate that the proposed SAW system has potential application for remote wireless monitoring of bacterial growth when the noise of EMI has been filtered.

The effect of temperature on the frequency of the oscillator was also studied. The frequency of the oscillator was steady within a temperature range from 25 to 40 °C (Fig. 4), but became unstable when the temperature exceeded 40 °C. Based on the above results, a temperature of 30 °C was selected for the following tests.

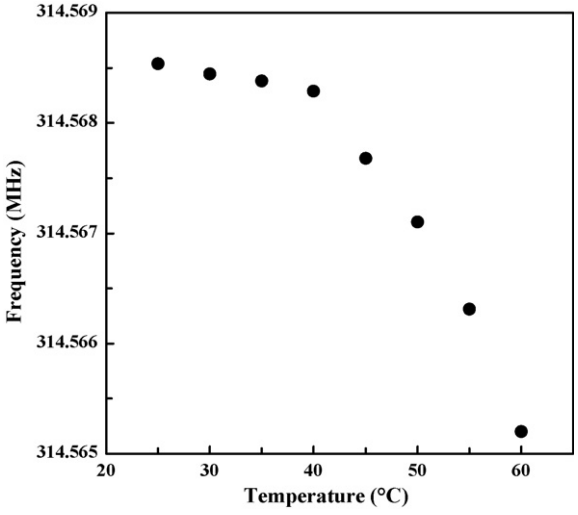

Fig. 4. Effect of temperature on the frequency of the SAW oscillator.

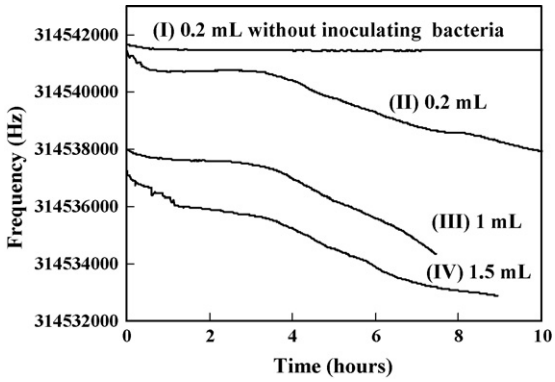

Fig. 5. Effect of sample volume on the response of the SAW sensor. The sample is a nutrient broth (pH 7.0) inoculated with ca. 10^5 cells/mL *E. coli*.

3.2. Optimization of the bacterial growth monitoring system

Fig. 5 (line I) shows the frequency profile of the SAW sensor when the electrodes were immersed in a reference culture cell containing sterile nutrient broth. The sensor response remained stable for more than 10 h. The noise level of the sensor was ±10 Hz and the relative frequency stability was 6.36×10^{-8} based on the observed noise-to-oscillation frequency ratio over an average period of 10 h. This result indicates that the physical and/or chemical factors unrelated to microbial growth of the media only affect the frequency of the sensor at a negligible level. On the contrary, the frequency decreases the frequency decreased slowly in the following 1 h when the electrodes were immersed in a cell culture containing bacteria inoculums. This may due to the bacteria and protein adsorbing on the electrode leading to an increase in the double layer capacitance (C_{dl}), resulting in the frequency decreased [6]. After equilibrium, the sensor response remained stable for a period of time hours (Fig. 5, line I). The stable time depend on the initial concentration of bacteria in the cell culture (Fig. 5, lines II and III).

A further study was carried out to investigate the effect of sample volume on the response of the sensor. The detection time of 0.2 and 1.0 mL sample volume were 2.7 and 3.0 h, respectively (Fig. 5, lines II and III). Both lines have the same frequency profile model. A sample of 1.0 mL or larger than 1.0 mL required a longer time for the sensor to reach a plateau (Fig. 5, line IV), A sample volume less than 0.2 mL, however, was insufficient to perform the operation. The sample volume was selected as 0.2 mL for the following experiments.

The effect of peptone and/or glucose enrichment on the growth of *E. coli* was also studied. Peptone enrichment was effective to have a shorter detection time of *E. coli* culture. On the contrary, the glucose enrichment showed no effect. As the result of our experiment, the breakdown of peptone by microbial metabolism was responsible for the frequency shift. Table 1 shows the effect of various amino acids on the frequency shift of the SAW sensor. The amino acids with acid or basic residues (e.g. L-aspartic acid, L-lysine, and L-arginine) could lead to a great change in the conductivity of the media, thereby the frequency shift notably, whereas the neutral amino acids (e.g. L-glycine,

Table 1
The effect of the concentration of different amino acid on the frequency of the SAW sensor

Amino acid	Frequency[a] (Hz)		Frequency change (Hz)
	0 mM[b]	10 mM[b]	
L-Glycine	314.564737	314.564532	205
L-Cysteine	314.564157	314.563890	267
L-Leucine	314.565694	314.565502	192
L-Tyrosine	314.565700	314.565501	199
L-Methionine	314.564930	314.564807	123
L-Histidine	314.565010	314.564750	260
L-Aspartate	314.565958	314.564010	1948
L-Lysine	314.564312	314.563072	1240
L-Arginine	314.564375	314.563849	526

[a] The sample volume was 0.2 mL and the sensor was placed in an incubator at 30 °C with temperature precisely controlled at ±0.2 °C. Each data point is the mean value of three measurements.
[b] Amino acid concentration.

L-cysteine, and L-leucine) caused relatively low frequency shift. Fig. 6 shows the effect of pH on the frequency response curves of *E. coli* in nutrient broth at 30 °C. The SAW sensor was used at an inoculums level of 10^4 cells. Notably, detection time did not occur if the media was adjusted to pH 5.5 by adding HCl. This phenomenon can be explained by the following arguments. First, as described in a previous article [21], in high conductive media, the relative changes in conductance in the solution were smaller than in a low conductive media. The media with pH 7.0 could be classified as a low electrolytic conductivity medium. Thus, the relative changes in conductance in the solution were significant, thereby producing a decrease in the frequency response after several hours' incubation. However, the electrolytic conductivity of the media with pH 5.5 could be classified as high. Therefore, it should not be expected that a frequency decrease will be found in response to the growth of bacteria at pH 5.5 media. Second, the growth rate of *E. coli* in pH 5.5 was slower than that at pH 7.0 (data not shown). Interestingly, comparison of the growth rate of the bacteria in pH 8.5 with that of pH 7.0 revealed that both had identical grow curve (data not shown). However, the DT was delayed when the pH of culture media was increased from pH 7.0 to 8.5. It is possible that the major

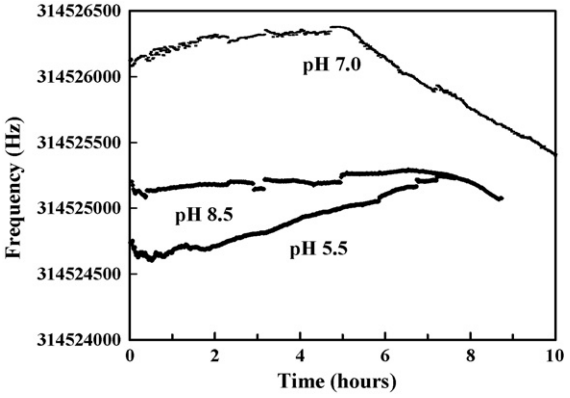

Fig. 6. Effect of pH on the response of the sensor at 30 °C. The sample is 0.2 mL nutrient broth inoculated with 10^4 cells/mL *E. coli*.

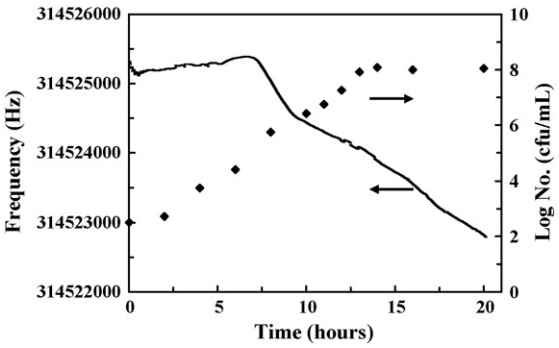

Fig. 7. Growth and frequency response curves of *E. coli* in nutrient broth (pH 7.0) at 30 °C using the sensor with inoculums with 10^2 cells/mL. The sample volume was 0.2 mL.

protease produced by the *E. coli* in the present study is not alkaline protease. The protein breakdown by microbial metabolism was slow at high pH. Thus, the changes in conductance in the solution were less significant. Therefore, pH 7.0 of the culture medium was selected for the following tests.

3.3. Detection time (DT) versus standard plate count

The change in frequency of the oscillator when the electrodes were immersed in the culture was measured to monitor the growth of bacteria. Fig. 7 plots the growth and the frequency response against time after *E. coli* inoculation. A lag phase, a period of exponential growth, and a stationary phase were observed. The initial small change in frequency occurred in the seventh hour when the cell concentration reached 10^5 to 10^6 cells/mL. The microbial level associated with this change in frequency for such an impedance system is called the microbial threshold level. The microbial threshold level for our sensor was less than the conventional impedance method which shows a threshold level of 10^6 to 10^7 cells/mL [12,13].

Fig. 8 shows the frequency response curves of the sensor with 10^2, 10^4 and 10^6 cells/mL inoculums size of *E. coli* in nutrient broth at 30 °C. The detection time was found exponentially

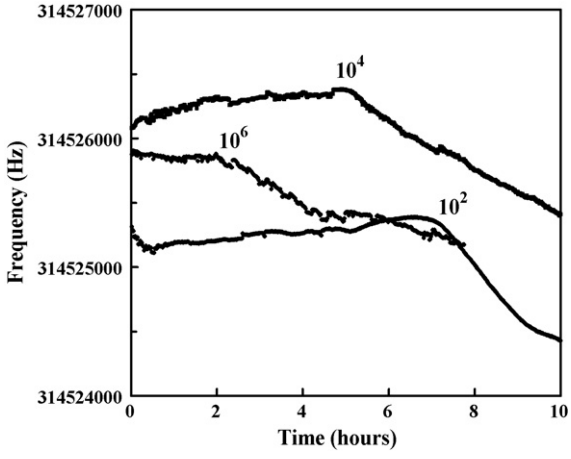

Fig. 8. The response curves of the sensor at 30 °C in 0.2 mL nutrient broth (pH 7.0) inoculated with 10^2,10^4 and 10^6 cells/mL *E. coli*.

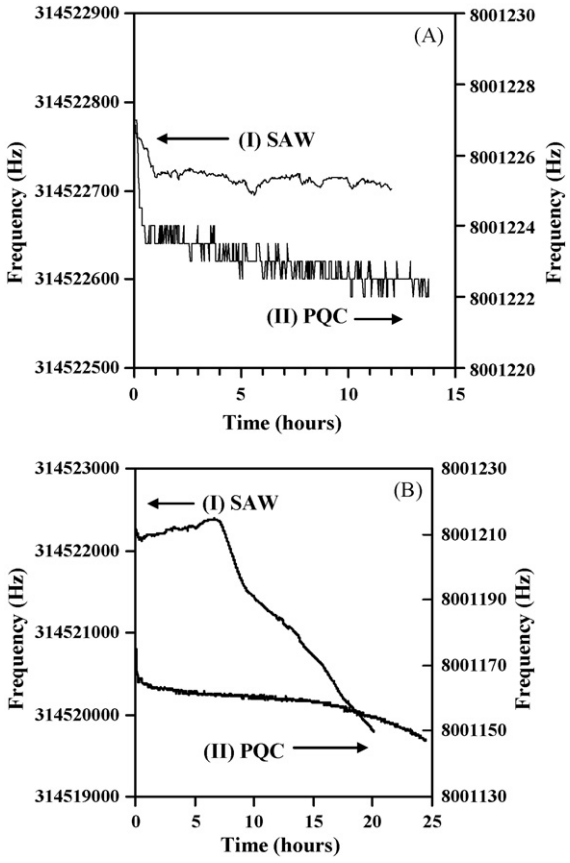

Fig. 9. (A) Noise trace of a SAW sensor (I) and a PQC sensor (II) in the sterile media. (B) Frequency response curves of SAW sensor (I) and SPQC sensor (II) of *E. coli* in nutrient broth with inoculums with 10^2 cells/mL.

related to the inoculums size. The scattergrams of the detection time against the logarithm of the inoculums size of *E. coli* from 10^2 to 10^7 cells/mL showed a linear curve. The regression equation was $Y = 6.7692 - 0.6531X$ ($n = 10$) and the correlation coefficient (R^2) was 0.924. The relationship between the detection time and the concentration of *E. coli* became non-linear or undetectable when the inoculums concentration of *E. coli* was lower than 10^2 cells/mL (data not shown). Therefore, the detection limit of 10^2 cells/mL was determined. This finding is in agreement with previous reports using conventional impedance systems [12,13,27,29].

Further studies involving pre-incubation of the sample before testing for lower bacterial concentrations may exploit whether these sensitivity limitations can be overcome [8].

3.4. Comparison of SAW sensor with the PQC sensor for the determination of bacteria concentration

The stability and sensitivity of proposed SAW sensor was compared with the PQC sensor (Fig. 9A). The SAW sensor and the PQC sensor have the same stability when the electrodes were immersed in a reference culture cell containing sterile nutrient broth. The noise level of the SAW sensor and PQC sensor were ± 10 Hz (relative stability of frequency $= 6.36 \times 10^{-8}$) and ± 0.25 Hz (relative stability of frequency $= 6.25 \times 10^{-8}$), respectively. The relative stability of frequency was based on the observed noise-to-oscillation frequency ratio over an average period of 10 h. After the culture cell inoculated with *E. coli*, the SAW sensor shows a rapid initial rate change of the frequency signal (400 Hz/h), with a large total frequency change and give a sharp inflexion at the DT, whereas the PQC sensor only shows a slow and shallow response (0.25 Hz/h) (Fig. 9B). This result reveals that the SAW sensor is superior to PQC sensor in determination of bacteria concentration. The noise level is the same for both SAW and PQC sensor. However, the resolution is higher for the SAW sensor due to its ultra high frequency. Furthermore, the SAW sensor was more sensitive than the PQC sensor based on the signal to noise ratio. When compared with conventional bacterial plating methods, this technique is relatively simple and, therefore, does not require the expertise of highly skilled technicians. Nevertheless, the detection limit of our sensor for *E. coli* was only 10^2 cells/mL. Further investigation on the effect of pre-incubation of the sample may determine whether the sensor can be used in testing for lower bacterial concentrations. Another important feature of our SAW impedance system is the possibility of wireless transmissions of the sensor signal, which may enable the development of distinct application from conventional systems, such as in aseptic environments, which would be contaminated by a connecting wire or in hazardous environments. The wireless method may improve safety.

4. Conclusions

This study presents a novel design for a SAW impedance sensor. This device was used to detect the growth of *E. coli*. The impedance signal from the interface of the electrode in the bacterial culture solution was translated into a frequency signal by our SAW impedance sensor. An important advantage of our sensor is its lower microbial detection threshold number than traditional impedance methods. The microbial detection threshold in this study was 10^5 to 10^6 cells/mL. Hence, the sensor described in this paper is faster than instruments employing traditional impedance methods (10^6 to 10^7 cells/mL). Although the proposed SAW sensor work at ultra high frequency, it possesses the same stability compare with that of lower frequency PQC sensor. Furthermore, the SAW sensor was more sensitive than the PQC sensor base on the signal to noise ratio. When compared with conventional bacterial plating methods, this technique is relatively simple and, therefore, does not require the expertise of highly skilled technicians. Nevertheless, the detection limit of our sensor for *E. coli* was only 10^2 cells/mL. Further investigation on the effect of pre-incubation of the sample may determine whether the sensor can be used in testing for lower bacterial concentrations. Another important feature of our SAW impedance system is the possibility of wireless transmissions of the sensor signal, which may enable the development of distinct application from conventional systems, such as in aseptic environments, which would be contaminated by a connecting wire or in hazardous environments. The wireless method may improve safety.

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