

Acoustic Wave Sensors: Application to Biological Sensing in Liquid Environments

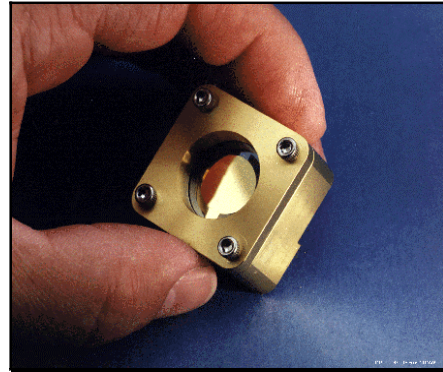
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1 Abstract

Acoustic wave devices have been successfully used for the sensing of various gases and vapors. A subset of these devices has the capability to interrogate liquids as well, making them attractive for biological sensing applications. The use of acoustic devices, in particular, the quartz crystal microbalance (QCM) and higher surface sensitivity devices such as shear horizontal surface acoustic wave (SH-SAW) are very useful sensors. These devices can be miniaturized while yielding the same or better results in short time intervals in comparison to bench-scale analytical instruments that can take hours to days. As the frequency of the acoustic wave device increases, the resolution of the device increases, offering sensitivity gains. Utilization and optimization of acoustic wave devices allows time sensitive results to be obtained more quickly, resulting in safer, more productive environments. Greater sensitivity coupled to easy integration with common electronics makes for a very versatile sensor that can be optimized and combined with other sensors to provide unparalleled performance at low costs. Results will be presented regarding initial testing for a unique method of detection of bacteria in municipal water supplies.

2 Introduction and Background

The quality of water intended for human consumption covers all aspects: drinking, cooking, bathing, and washing of devices that will come in contact with people. (WHO, 2003) Water is one of the most important substances required to sustain life. The average person requires approximately 20 liters of water each day (WHO, 2003). Bringing this fact to a larger scale, means for a population of a town or a city of 100,000 people, over two million liters of water is needed everyday. Obviously, with such large amounts required just to survive, water needs to be looked on as a precious commodity.



“Domestic water supplies are one of the fundamental requirements for human life. Without water, life cannot be sustained beyond a few days and the lack of access to adequate water supplies leads to the spread of disease... Diarrhoeal diseases attributed to poor water supply, sanitation and hygiene account for 1.73 million deaths each year and contribute over 54 million Disability Adjusted Life Years, a total equivalent to 3.7% of the global burden of disease (WHO, 2002). This places diarrhoeal disease due to unsafe water, sanitation and hygiene as the 6th highest burden of disease on a global scale, a health burden that is largely preventable... the Global Assessment of Water Supply and Sanitation data, describe reasonable access as being 'the availability of at least

20 litres per person per day from a source within one kilometre of the users dwelling' (WHO and UNICEF, 2000). However, it should be noted that this definition relates to primarily to access and should not necessarily be taken as evidence that 20 litres per capita per day is a recommended quantity of water for domestic use.” (WHO, 2003)

Table 1. Common bacteria found in water supplies as taken from WHO, 2003.

Bacteria	Health Significance	Persistence in water supplies¹	Resistance to Chlorine²	Relative infective dose³
<i>Campylobacter jejuni, C. coli</i>	High	up to one month	Low	Moderate
<i>Escherichia Coli</i>	High	up to one month	Low	High
<i>Salmonella typhi</i>	High	up to one month	Low	High
<i>Vibrio cholerae</i>	High	up to one week	Low	High

¹ Detection period for infective stage in water at 20°C

² During infective stage with normal chlorine doses.

³ Required amount to cause infection in 50% of adults tested.

The pathogens listed in Table 1 are more than just common problems; they should be considered as possible biological threats of terrorism. These already well known bacteria would pose a threat, as the impact on a population would be significant. “...each nation’s planning will have to take into account the laboratory capability required to minimize the impact and even to signal that an incident is occurring. The role of water testing will need to be re-evaluated...the HPC should be evaluated as a trigger for further investigation” (WHO, 2003)

It wasn’t until after the invention of the microscope in the 17th century that the ability to look at cells and the development of microbiology lead to conclusions that diseases were caused by microorganism. As technology progressed, more conclusive studies were done that better identified specific organisms as the causative agent. Finally in the late 19th century, a general test for the biological load of a water samples was developed, which is still used in the same fundamental method.

In 1883, Robert Koch published the first paper on methods for detection of microorganisms in water. Following publication, Koch went on to do a number of studies on water quality. One of the most notable was performed on the Altona waterworks. This facility drew in contaminated water from the Elbe River and filtered it through sand. The results demonstrated by Koch were achieved by filtering the water and having bacterial count less than 100 colonies per milliliter, outbreaks of typhoid and cholera could be avoided. If the levels reached 1,000 colonies per milliliter due to filtration problems an outbreak occurred. (WHO, 2003)

Since the time of Koch, science has advanced considerably; however, the majority of techniques today used for the detection of microorganisms very closely resemble that of Koch's first work. (WHO, 2003) There have only been modifications to the original method to enhance the results and specify particular organisms. Other improvements have been made by companies such as 3M™ that facilitate computer-assisted analysis of water samples. The major drawback of even the latest techniques is the need to incubate a sample from 24 hours to a week in time.

Heterotrophic plate count (HPC) is a method that allows for the enumeration of live heterotrophic bacteria in water. This type of bacteria is a very general classification for organisms that require organic nutrients for growth. Heterotrophic plate counts typically are performed using special nutrients to enhance the growth of one particular microbe over another to try limiting the many different species that are grown. (Edberg and Allen, 2004) "In general, HPC monitoring is used as a tool to gain information on the water treatment process and the general bacteriological quality of the water leaving the water treatment plant and within the distribution system." (WHO, 2003) HPC has advantages over simple coliform tests since not all pathogenic bacteria are a member of the coliform group (Deninger, 2001). It has numerous applications such as: process water monitoring, swimming pool quality monitoring, finished water and raw water monitoring for drinking water systems. The method itself is relatively simple; a sample is applied to a nutrient enriched media that is then incubated at an elevated temperature for at least 24 hours. The media is then analyzed by counting the number of colonies present per unit area. This number is referenced back to the volume of sample originally used to generate a colony forming unit (CFU) per milliliter. A colony is essentially a dot on the surface of the media, where an organism was deposited and multiplied sufficiently that it can be observed visually (represents 10^6 - 10^9 individual cells).

Performance of this test can estimate the bacterial load on the water. Different countries give varying numbers for CFU, so it is difficult to state a definitive number. The EPA has avoided setting standards for plate counts most likely due to local variations in the bacterial densities observed (DuZuane, 1990). In the USA, guidelines for filtration systems state that a load of under 100 cfu/mL is achievable, 100-500 cfu/mL is a mediocre value and implies a seasonal fluctuation requiring attention, and above 500 cfu/mL is water of poor quality. All of this results in the typical output at the top of municipal water having less than 500 cfu/mL. (WHO)



HPC was originally used as a measure of the performance of filtration systems; today it still finds use as an indicator of water treatment effectiveness, a measure of regrowth organisms, and as a measure of possible interference with coliform measurements. Even with all of the uses of HPC, it is still not a sole indicator that a health risk is present. It is just one of many necessary measures to verify the content and safety of water. HPC is useful in terms of process monitoring and control for determining:

- Efficiency of filtration

- A possible state change within a system
- Efficiency of disinfections
- Indication of bio-film sloughing which can have multiple causes
- Predicting nitrification
- Bacteria levels in areas of possible high contamination
- Bio-stability of household water

Acoustic wave devices have been widely used primarily in the electronics industry to create stable frequencies for radio type devices. The fundamental principle of all acoustic wave devices is the same: An electrical signal from an electrode stimulates a piezoelectric material and in turn creates a mechanical wave which is picked up by an electrode. This acoustic signal is then converted back into an electrical signal which can be compared to the input signal for frequency shift and power losses.

Other names for a QCM include a single or one port resonator or a thickness shear mode device (TSM). These names are not arbitrary, but describe how the device functions. The reasoning is only one port, or one coaxial cable, is used to both transmit and receive the electrical signal. As shown in the figure below, the signal is transmitted from one electrode to the one on the opposing side, where it is reflected. The transmission and reflection of the signal keeps reoccurring creating a standing resonance due to the physical properties of the substrate. The resulting signal can then be integrated with common electronics to determine the optimum frequency, where the majority of the signal is reflected, referred to as the center or fundamental frequency. (Ballantine et al, 1997)

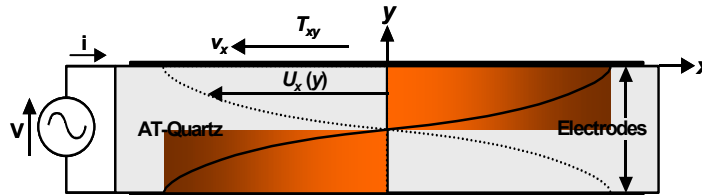


Figure 3. Schematic of QCM with resonating wave.

The general concept of a QCM can be developed into a very complicated system of equations. However, the Sauerbrey equation is typically used as given by equation 1, where a simplified solution to the general equations of motion, piezoelectric and electric properties are employed. From these general equations, simple relations between the overall dimensions, material properties, and center operating frequency are easily developed.

Sauerbrey, who is considered one of the founders of acoustic wave sensors, did the essential first derivational work and developed a series of equations that are now seen as the fundamentals in this field. The variation of the equation that was used in this project is as follows:

$$\Delta f = -\frac{2f_1^2 \rho_s}{(\mu_q \rho_q)^{\frac{1}{2}}} \quad \text{Equation 1}$$

where f_1 is the fundamental mode oscillation frequency, ρ_s is the surface mass density, μ_q is the quartz shear stiffness, ρ_q is the quartz mass density and Δf is the expected frequency shift due to changes in the one variable, ρ_s . (Ballantine et al, 1997) The Sauerbrey equation does not hold true for every situation, but provides an initial estimation that is typically sufficient for simple cases. Researchers have developed refined equations of higher accuracy. However, as shown by figure 4 calculations fit well with the experimental sensitivity determination data.

The second important relation that is needed to complete the discussion of TSM sensors is the sensitivity of the device as given by equation 2:

$$S = \frac{df}{d\rho_s} = -\frac{f_1}{(\rho_q h_s)} \quad \text{Equation 2}$$

where h_s is the thickness of the quartz. For a typical 5 MHz QCM, the overall theoretical sensitivity (which is the total response to equal loading on both sides of the device) is approximately $58 \text{ Hz}\cdot\text{cm}^2/\mu\text{g}$. Acoustic sensors today have long surpassed the simplistic QCM's of yesterday by operating in a significantly higher frequency range of 5 – 150 MHz. These newer devices operate on the same fundamental principles, but are many times more sensitive, due to the decreased thickness of the quartz, h_s , and the ability of circuits to lock onto third and fifth overtone resonant frequencies. With increased sensitivity, acoustic wave sensors are now used quite extensively for a number of different applications that include the sensing of fundamental gas properties, gas and vapor compositions, viscosity of liquids, and properties of solids (Ballantine et al, 1997). Their uses are not limited to the research purposes, but are widely available in consumer end products that require high precision, easy configuration, and low power operation. One such configuration is for a mass deposition counter. A good representation of this is sold by MaxTek Inc. who has optimized both crystal and oscillator circuit design for easy consumer operation.

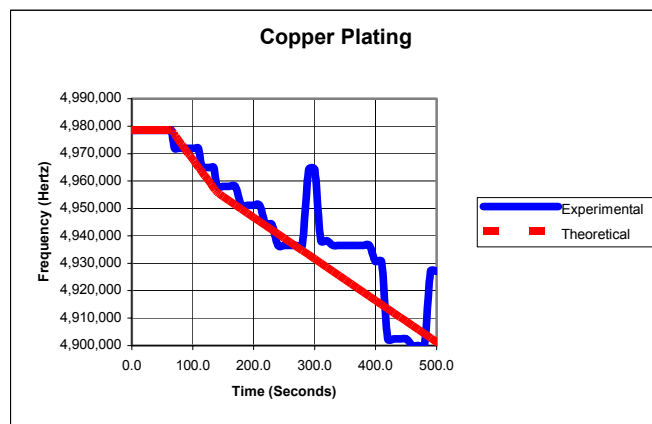


Figure 4. Frequency response to electro-deposition of copper onto one of the QCM's electrodes.

3 Experimental

Experiments were conducted at room temperature and 37°C with GPIB connectivity to a PC for data acquisition via custom Lab View programs. Measurements were performed using a Hewlett Packard P5384A frequency counter, an Agilent 34970A Data Acquisition / switch unit equipped with voltage metering and 50 ohm RF switching capabilities connected to a specially configured Lever oscillator as shown in Figure 5 (Martin, 1997). Additional measurements were made using a Hewlett Packard 8751A Network Analyzer. The QCM and oscillator circuit were enclosed in fixtures designed to

minimize the loss of signal between the crystal and oscillator circuit and minimize the environmental interference. Additional care was taken in cleaning of all equipment to prevent unwanted spreading of bacterial growth through the use of an autoclave and various disinfectants. Only one side of the crystal was exposed to the analyte environment in these experiments.

Monitoring and recording the data from the sensor, several LabView 6.1 programs were written to incorporate all of the instruments and measurements. The program was designed in such a fashion that prior to recording the data; it was passed through a subroutine based on the Sauerbrey equations to verify the data was in the proper range of the QCM's linear response capabilities. Time, frequency, damping voltage, and relative change in each was recorded from the various monitoring equipment, processed, and recorded in an ASCII tab delimited file.

In the scope of this research, we solely operated with liquids or gels on the surface of the crystals, so special attention had to be paid to the circuitry. As with all sensors, they are insignificant devices if one is unable to connect them to a robust system for data monitoring and recording. QCM's are most often operated in hermetically sealed packages and used for frequency generators, where the damping effects of the environment are not a factor, and thus a Pierce or Culpitts oscillator style circuits can be used with no problems. (Martin et al, 1997) Minor damping effects are also the case when using the QCM's to sense gases or light vapors, as the loaded film on the surface of the crystal is not enough to damp out the acoustic wave beyond its optimal linear range. In the case of using a QCM to sense liquids or in this particular case a gel, the regular circuitry is not capable of handling the lossy damping environment. As a result of this known issue, a Lever oscillator design was employed. The Lever oscillator uses a negative feedback to leverage the resonators impedance to maintain the gain and phase of the loop. To allow the circuit to maintain its phase and gain, it was necessary to have added extra inductance to the circuit to help compensate for the excessive load on the crystal, from such a viscous environment. (Martin et al, 1997)

Calibration of the QCM sensors was performed through an electro-deposition process of copper metal from a copper sulfate solution, onto the ground electrode of the QCM. Sixteen milli-amperes and 0.17 volts were delivered through the layer for 640 seconds, resulting in a deposition of 3.4 mg of copper. The result was the determination of the sensitivity to be $58.85 \text{ Hz}\cdot\text{cm}^2/\mu\text{g}$. This agrees well with the theoretical sensitivity calculated using equation 2 with the parameters of thickness being 0.033 cm and active electrode area of 0.39 cm^2 ; resulting in a theoretical value of $57.15 \text{ Hz}\cdot\text{cm}^2/\mu\text{g}$.

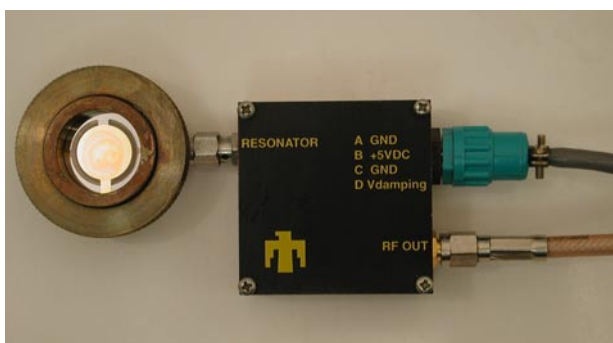


Figure 5. Sandia National Laboratories' Oscillator circuit and QCM fixture.

Additional calibration was necessary to determine and factor in the evaporative loss of the thin film coatings on the sensors, since the agar films were largely composed of water, nearly 98% in some instances. In order to monitor the loss, both frequency and mass were monitored immediately after coatings for a set period of time, as shown in figure 6. Typical shifts of 2 KHz were observed over a period of 2 hours.

Various formulations and concentrations of agar solutions were tested for their ability to grow bacteria and to allow the sensor to function within its optimum range. Spin coating was the method of choice for coating the QCM's with the multiple agar solutions; although a spraying alternative could provide similar results. The optimized process of spin coating was to take the agar solution from just under a light boil and spin coat the QCM within 2 minutes of removal from the heat. Speed was important to prevent solidification of the solution. The approximate settings for spin coating were 2,500 revolutions per minute for a time of 5 seconds. Variation in the time and speed allow for a fine-tuning of the film properties while using the spin coat method. Once the sensors were coated, thickness measurements were verified using a Dektak profilometer. A sample data print out is shown in figure 7. The large step shown in the figure as achieved by masking the crystal with transparent tape prior to spin coating with agar. The tape was removed to generate a sharp edge to indicate the thickness of the film. Films were typically less than 1 um thick to prevent overloading of the crystal.

Due to the thinness of the agar film on the surface of the QCM, it is necessary to enclose the coated crystal in a high humidity chamber to prevent evaporative loss of water from the film. In performing the experiments, temperatures of 25°C and 38°C were used in order to verify against known growth values. The elevated temperature of 38°C was necessary for us to compare our result to that of known literature values for the growth rate of bacteria. As an example, growth of *Escherichia coli* is documented in textbooks to approximately double every 20 minutes at 38°C.

The strain of *e. coli* that was selected was ATCC 11229, as this is a common and well known strand. An initial broth was made and cultured with a count of 630 million organisms. From this primary broth dilutions were made to give concentrations of 630,000, 6,300, and 630 bacteria per milliliter.

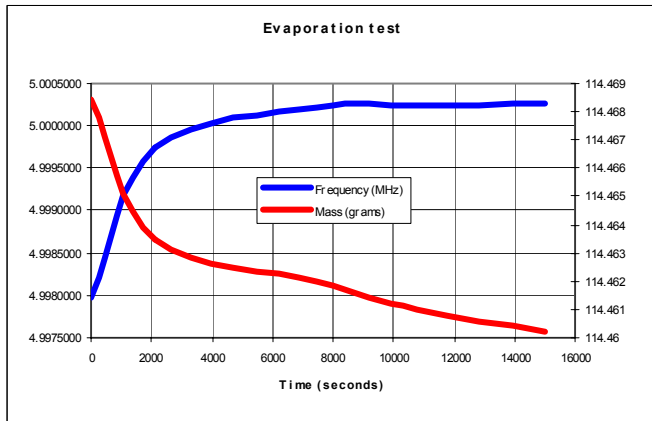
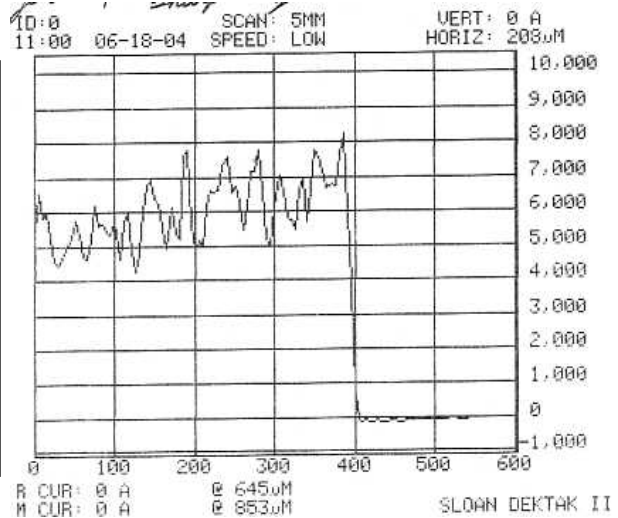
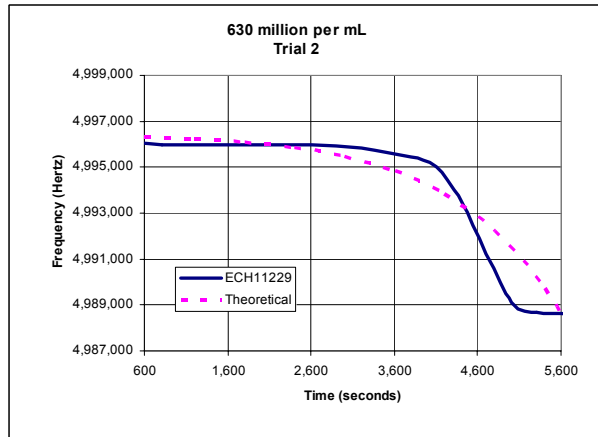
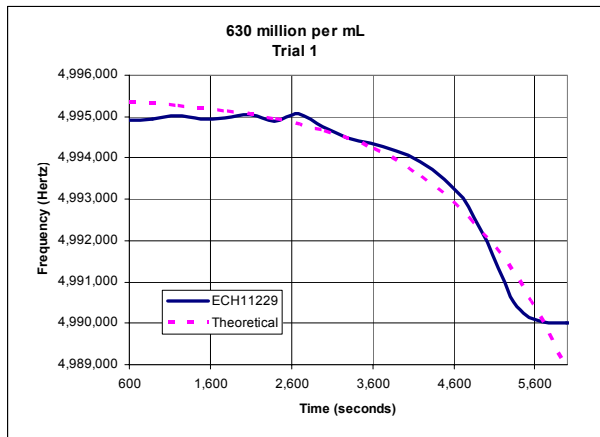


Figure 6. Experimental data of the evaporative loss of water in terms of frequency and mass.



4 Results

For the results of the growth of bacteria, two trials were made using a high concentration of approximately 630 million units per milliliter of e. coli 11229 as determined through a standard culture and counting techniques over a 48 hour period.



Figures 8 and 9. Frequency response to 630 million unit concentration of e. coli growth on QCM with agar thin film.

Eosine Methylene blue agar was used to provide a selective growth medium for these experiments. A sample of 10 µL from the same solution was used to coat a QCM. Therefore, 6.3 million bacteria were deposited onto the surface of the agar to begin with. Through basic curve fitting procedures using the above equations and an Arrhenius rate law, the result indicate that bacteria with a mass of 1 pico-gram per unit show a count of approximately 700 million units per milliliter with a doubling time of approximately 17 minutes. The measured doubling time corresponds well to literature values of 20 minutes. These results indicate that at high levels of contamination results can be

generated in less than an hour. The ½ way point (between starting and maximum response) occurs at an average of the two at ~4900 sec or 1 hr 20 minutes. Following this experiment additional trials were performed with decreasing concentrations to further evaluate the validity of using a QCM to monitor bacteria load in water. The frequency response to a concentration of 630,000 bacteria per milliliter is illustrated in figure 10. The ½ way point (between starting and maximum response) occurs at ~3800 sec or just over an hour.

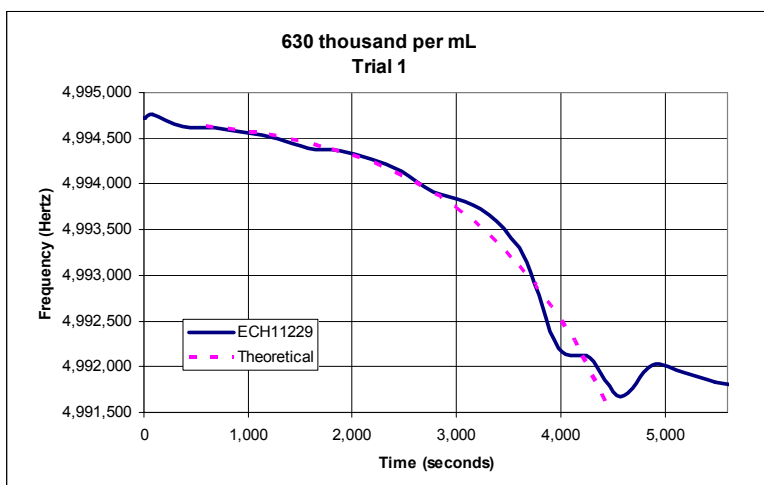


Figure 10. Frequency response to 630,000 unit concentration of e. coli growth on QCM with agar thin film.

5 Conclusions

This work has shown that organisms can be cultured and detected on a QCM using standard growth media. Future work needs to be performed to demonstrate that appropriate detection limits can be achieved (for the water industry) by employing lower density starting solutions (1000-100 CFU/mL) and longer integration/culturing times (6-8 hrs). It is interesting to note (and the experiment bears repeating) that a lower starting

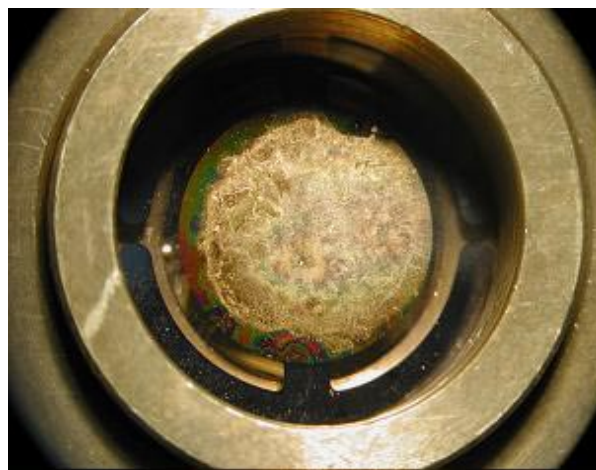
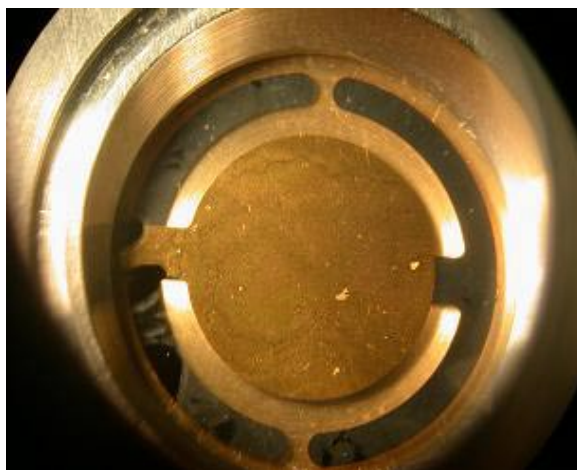


Figure 11 and 12. Before and after images of e. coli growth on QCM.

concentration had a faster time interval to maximum response on the device. This could be an indication that the organisms are in a severely overcrowded state at high deposited concentrations where there are less total available nutrients to be used for reproduction (and subsequently a sensor response). Looking at the shapes of the curves it appears that the lower concentration deposition started changing very quickly after deposition whereas the higher concentrations had longer lag times before

significant changes were observed which would support this hypothesis. From this preliminary study, it was shown that there is merit to measuring the bacteria load in water using acoustic wave sensors. The determination of the number of organisms can be found in a fraction of the time needed for normal plating techniques. Further experiments are required to reinforce the initial data and to develop a scheme for a more selective sensor in water bacteria load monitoring. The continuation of the project to incorporate higher frequency sensors to provide an even lower detection limit is necessary as well.

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