A QCM-D-based biosensor for *E. coli* O157:H7 highlighting the relevance of the dissipation slope as a transduction signal

Charles Poitras, Nathalie Tufenkji

Department of Chemical Engineering, McGill University, Montreal, Quebec H3A 2B2, Canada

**Abstract**

A biosensor for detection of viable *Escherichia coli* (*E. coli*) O157:H7 is developed using a quartz crystal microbalance with dissipation monitoring (QCM-D). The detection platform is based on the immobilization of affinity purified polyclonal antibodies onto gold-coated QCM-D quartz crystals via a cysteamine self-assembled monolayer. QCM-D measurements conducted over a broad range of bacterial cell concentrations show that the optimal biosensor response is the initial slope of the dissipation shift as a function of elapsed time (*D* slope). A highly log–log linear response in the initial *D* slope is obtained for detection of *E. coli* O157:H7 over a wide range of cell concentrations from $3 \times 10^5$ to $1 \times 10^9$ cells/mL. The prepared biosensor also exhibits a log–log linear working range from $10^7$ to $10^9$ cells/mL for *E. coli* K12 D21, a non-pathogenic model organism and further shows satisfactory selectivity using *Bacillus subtilis*. To our knowledge, this is the first study demonstrating the use of the initial *D* slope as a sensor response when using QCM-D technology.

1. Introduction

The contamination of drinking water by microbial pathogens is recognized as one of the most pressing water supply problems of our day. *Escherichia coli O157:H7* is a waterborne and foodborne bacterial pathogen that has been linked with several global disease outbreaks. As few as 100 cells of *E. coli* O157:H7 are needed to cause infection (Tuttle et al., 1999), highlighting the need for rapid and sensitive detection of this organism.

Conventional approaches for sensing of *E. coli* O157:H7 include culture-based methods and biochemical tests (Deisingh and Thompson, 2004). Although detection limits for these approaches are very low (i.e., a few colony forming units (CFU) per mL), the detection time can vary from 1 day to 1 week (Adams and Moss, 1995; Silk and Donnelly, 1997). Molecular techniques such as polymerase chain reaction (PCR), multiplex PCR and reverse transcriptase PCR have also been introduced as viable detection strategies for *E. coli* O157:H7 (Hu et al., 1999; Uyttendaele et al., 1999; Yaron and Matthews, 2002). Detection times for these methods vary between 2 and 24 h, while the detection limits reported are as low as $\sim 1$ CFU/g of sample.

Reliable detection methods are also needed for monitoring of non-pathogenic microbes found in engineered water treatment facilities (e.g., activated sludge systems or sequencing batch reactors). These biotreatment applications depend on active and complex microbial communities for removal of target contaminants from water matrices. On-line monitoring of biodegrading microorganisms in such treatment systems would allow for improved system operation and treatment efficacy (Carvalho et al., 2001). Hence, a real-time online method for detection of various bacteria in different water matrices is of interest.

Quartz crystal microbalance (QCM)-based biosensors are a viable alternative to the previously described methods due to their sensitive mass detection capabilities and their ability to monitor in real-time. The general operating principle of a QCM biosensor is a decrease in the crystal’s resonance frequency following binding of the target organism. When a rigid mass binds to the oscillating crystal surface, the shift in the resonance frequency ($\Delta f$) is proportional to the mass bound (Sauerbrey, 1959). Hence, the QCM can potentially be used to quantify the number of target cells in a sample. Species detected by microgravimetry include *Chlamydia* (Ben-Dov et al., 1997), *Salmonella* (Park and Kim, 1998), *Bacillus* (Vaughan et al., 2003), *Pseudomonas* (Kim et al., 2004) and *E. coli* (Su and Li, 2004). Recently, piezoelectric excited millimeter-sized cantilevers have been used to detect *E. coli* O157:H7 at a concentration as low as 1 cell/mL (Campbell and Mutharasan, 2007).

One advantage of QCM-D over the traditional QCM approach is that it gives insight into the viscoelastic properties of the adsorbed mass. The fundamental concept of the QCM-D technology is that a “soft” mass adhered to the crystal surface, such as bacterial cells, will not fully couple to the crystal’s oscillation and thus will dampen it. As a result, the mass adsorbed onto the crystal surface will be...
underestimated when using the classic Sauerbrey relation (Rodahl et al., 1997). Rodahl et al. (1995) developed a simple and fast method to have access to simultaneous measurements of frequency shift and the dissipation factor \( D \) defined as:

\[
D = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}} \tag{1}
\]

where \( E_{\text{dissipated}} \) is the energy that is lost during one oscillation period and \( E_{\text{stored}} \) is the energy stored in the oscillating system. \( D \) represents the sum of all processes that induce energy losses in the oscillating system (Hook, 1997).

The purpose of this study is to examine the use of QCM-D as a platform technology for detection of microorganisms in aqueous media. Polyclonal antibodies immobilized onto a gold-coated crystal were used for detection of viable \( E. coli \) \( O157:H7 \) and a model non-pathogenic strain, \( E. coli \) K12. Results of experiments conducted over a broad range of target cell concentrations demonstrate that the initial slope in the dissipation factor \( D_{\text{slope}} \) can be a more reliable and rapid biosensor response than the commonly used frequency shift.

2. Experimental

2.1. Reagents and materials

Affinity purified polyclonal antibodies, specific to \( E. coli \) \( O157:H7 \), were obtained from Kirkegaard and Perry Laboratories. Cysteamine (Sigma–Aldrich), bovine serum albumin (BSA) (Sigma–Aldrich), glutaraldehyde (Fisher), KCl (Fisher), NaCl (Fisher), \( Na_2HPO_4 \) (Sigma–Aldrich), \( KH_2PO_4 \) (Sigma–Aldrich) were used to prepare the immunosensing surface. Hellmanex (Helma), hydrogen peroxide (Fisher), and ammonia (Lab Chem) were used to clean the crystal surfaces after each experiment. Ultrapure deionized (DI) water (Biolab) was used to prepare all solutions.

2.2. Antibody immobilization procedure

Clean gold-coated QCM crystals (5 MHz, AT-cut, Q-Sense AB) were immersed in a 10 mM cysteamine solution overnight at room temperature with ultra-high purity (UHP) \( N_2 \) bubbling through the solution. After rinsing with DI water and drying with UHP \( N_2 \), the crystals were mounted inside QCM-D flow modules. Degassed phosphate buffered saline (PBS) at pH 7.4 was then injected into the flow modules at 50 \( \mu \)L/min for 10 min using a peristaltic pump (Reglo-Digital IPC-N4, Ismatec) until stable baseline \( f \) and \( D \) shifts were achieved (i.e., signal drift lest 2 Hz/h for the \( f \) shift and 0.2 \( \times \) 10\(^{-6}\) dissipation units/h for the \( D \) shift). Next, a 2% degassed solution of glutaraldehyde in PBS, which acts as a cross-linker molecule, was injected into the flow modules at 50 \( \mu \)L/min for 30 min. This was followed by a 20 min rinse with degassed PBS to remove any unreacted glutaraldehyde from the crystal surface. Then, a solution of anti-\( E. coli \) \( O157:H7 \) antibodies (10 \( \mu g/mL \)) in PBS was injected into each flow chamber for a duration of 20 min (50 \( \mu \)L/min). The pump was then stopped for a duration of 60 min to allow time for antibody reaction with the glutaraldehyde. This step was followed by a 20 min PBS rinse to remove unbound antibody. Next, a BSA solution in PBS (10 mg/mL) was injected for 20 min to block unreacted sites, and the pump was stopped for 60 min before a final PBS rinse (20 min) to remove unbound BSA. The prepared sensors were then ready for detection of the target bacteria (Fig. 1).

2.3. Bacterial cell preparation

\( E. coli \) \( O157:H7 \) ATCC 700927 and \( E. coli \) K12 D21 were used as the target bacteria. \( Bacillus subtilis \) (\( B. subtilis \) ATCC 6633) was utilized to verify selectivity of the biosensor. \( E. coli \) \( O157:H7 \) is a Gram-negative bacterium that is positive for both Shiga-toxin producing genes. \( E. coli \) K12 D21 (obtained from the \( E. coli \) Genetic Stock Center at Yale University) is a mutant of the well-characterized model laboratory organism \( E. coli \) K12. \( B. subtilis \) is a model Gram-positive organism commonly found in natural environments.

Pure cultures were maintained at \(-80^\circ \)C in Luria-Burtani (LB) Lennox broth (Fisher) (20 g/L) containing 15% glycerol. Liquid cultures were incubated at 37 °C for 24 h. Prior to each experiment, colonies from a fresh plate were used to inoculate 150 mL of LB broth (in a 500 mL baffled flask). Pure cultures were then streaked onto LB agar and subsequently incubated at 37 °C for 24 h. Prior to each experiment, colonies from a fresh plate were used to inoculate 150 mL of LB broth (in a 500 mL baffled flask). Liquid cultures were incubated at 37 °C for 18 h at 200 rpm. The described growth protocol does not result in expression of Shiga-toxins for \( E. coli \) \( O157:H7 \). The bacterial suspension was harvested by centrifugation (Sorvall RC6) at 5860 g in a S34 rotor (Kendro) at 4 °C. The supernatant was decanted and the pellet was resuspended in PBS at pH 7.4. The centrifugation and re-suspension steps were performed one additional time to remove all traces of the growth medium. The resulting bacterial cell concentration was determined using a calibration curve relating cell concentration (CFU/mL) to \( A_{600 \text{nm}} \) using a UV–vis spectrophotometer (Hewlett-Packard model 8453).

Purification treatments were then diluted to the desired target concentration between 10\(^5\) and 10\(^9\) cells/mL in PBS before being injected into the biosensor flow modules.

2.4. QCM-D measurements

A QCM-D (E4, Q-Sense AB) was used to monitor binding of bacteria to the functionalized gold crystal surface. The E4 unit consists of a measurement platform that can hold four sensor flow chambers. Each flow module was mounted with a cysteamine functionalized gold crystal and the antibody immobilization procedure described in Section 2.2 was followed while the flow chambers were maintained at 20 °C. A suspension of live bacteria in PBS (between \( 10^5 \) and \( 10^9 \) cells/mL) was then drawn through the chamber for a duration of 2 h (50 \( \mu \)L/min), followed by a 20 min PBS rinse (50 \( \mu \)L/min). Shifts in the crystal frequency and dissipation for the first overtone were continuously monitored using the Q-Sof software (Q-Sense AB).

3. Results and discussion

3.1. Characterization of biosensing surface using QCM-D

The different steps of the biosensor preparation were characterized using the QCM-D instrument, with the exception of the cysteamine monolayer formation which was performed ex-situ. Representative measured \( f \) and \( D \) shifts during each step of the biosensor preparation protocol and the \( E. coli \) \( O157:H7 \) detection step are presented in Fig. 2. The \( f \) shift is reported in Hz whereas the \( D \) shift is plotted in terms of 10\(^{-6}\) dissipation units (step A). When glutaraldehyde is injected into the flow cell, substantial \( f (\sim-30 \text{Hz}) \) and \( D (11.6 \text{DU}) \) shifts are observed (step B). The large frequency and dissipation shifts observed during this step are most likely due to the difference in viscosity between the 2% glutaraldehyde solution and the PBS solution (Kurosawa et al., 1998). This is supported by the results obtained following a PBS rinse (step C) where the frequency and dissipation shifts return closer to the original baseline values (A). Following antibody binding (D), any unbound antibody is removed from the flow cell by rinsing with PBS (step E). BSA is injected for 20 min to block any unreacted sites on the crystal surface (step F), then left to sit for 60 min in the flow cell before rinsing with PBS (step G) to remove any unbound BSA. After its preparation, the biointerface is used for detection of the target bacteria (step H). When \( E. coli \) \( O157:H7 \) enters the flow chamber and binds to the immobilized antibodies, a decrease in the resonance
frequency (Fig. 2a) and a corresponding increase in the dissipation signal (Fig. 2b) of the functionalized sensor crystal are observed due to specific binding of bacteria to immobilized antibody.

3.2. QCM-D sensor response as a function of cell concentration

After preparation of the functionalized crystal, the sensor baseline was obtained in PBS as shown in the representative data presented in Fig. 3 (step A). Phase B in Fig. 3 shows a portion of the bacterial attachment step. Only the first 10 min of bacteria injection are shown in this plot to highlight the differences in the measured frequency and dissipation shifts when suspensions of varying cell concentration are injected. The data presented in Fig. 3 show that the observed f and D shifts for E. coli are more significant at a higher cell concentration.

In traditional QCM-based bacterial biosensors (Kim et al., 2004; Mao et al., 2006; Park and Kim, 1998; Park et al., 2000; Su and Li, 2004, 2005; Vaughan et al., 2003; Vaughan et al., 2001), the frequency shift is selected as the key sensor response signal. To compare the sensor response obtained when solutions of different cell concentration were injected in the flow module, the f and D shifts recorded after 60 min of bacterial injection were used as a basis of comparison. Fig. 4 shows the negative f and absolute D shifts after 60 min of E. coli O157:H7 injection as a function of injected cell concentration (note: the negative of the f shift is used to allow presentation in a log scale plot). As seen in Fig. 4a, the negative f shift after 60 min of cell injection increases linearly (in log–log plot with $R^2 = 0.93$) from 0.21 Hz at 10⁶ cells/mL to 42 Hz when the cell concentration reaches 10⁸ cells/mL. A slight decrease or leveling out in the negative f shift is observed as the cell concentration is further increased to 10⁹ cells/mL. Below a cell concentration of 10⁶ cells/mL, the f shift could not be distinguished from the negative control.

The f shifts recorded following injection of the target bacteria into the prepared sensor module are not comparable to those reported in previous studies using QCM technology. In fact, the observed f shifts after 60 min were typically lower than previously reported findings. Park et al. (2000) report a f shift of −900 Hz after 100 min of injection of Salmonella at 4.75 × 10⁸ cells/mL. Fung and Wong (2001) report f shifts of −35 Hz after 50 min of injection of 1.7 × 10⁴ cells/mL of S. paratyphi A. (Vaughan et al., 2001), on the other hand, observed a positive f shift during injection of bacteria. Su and Li (2004) report a frequency shift of about −4 Hz after 60 min of injection of 10⁸ cells/mL, while in the current study the f shift observed during injection of cells at the same concentration was approximately −40 Hz after 60 min. These observed differences in the measured f shifts reported in the literature could be attributed to differences in the fundamental resonance frequency of the crystals utilized, the configuration of the crystal holding module, and the biorecognition element implemented. For instance, in the current study, we used a 5 MHz crystal, whereas Su and Li (2004) used a 8 MHz crystal and Fung and Wong (2001) used a 10 MHz crystal.

Although it has not directly been demonstrated for the biosensing of microorganisms, a crystal with a higher fundamental resonance frequency will generally exhibit greater changes in f for the same amount of adsorbed mass. Moreover, a higher-density packing of biorecognition elements will also lead to improved detection and hence, greater f shifts.

The working range of the current biosensor based on the measured f shift of the functionalized crystal is comparable to some of the working ranges previously reported for flow-through QCM biosensors (Kim et al., 2004; Park and Kim, 1998; Park et al., 2000; Su and Li, 2005; Vaughan et al., 2003; Vaughan et al., 2001). Park et al. (2000) report a working range of 3.2 × 10⁶ to 4.8 × 10⁶ CFU/mL, while Wong et al. (2002) present a biosensor for S. paratyphi A that can be used between 10⁸ and 5 × 10⁸ cells/mL. Fung and Wong (2001) report a lower working range of 10⁸–10⁹ cells/mL, while Su and Li (2004) have sensed heat-killed cells from a concentration as low as 10⁴ cells/mL. Mao et al. (2006) used a nanoparticle amplification method to detect extracted DNA from cells at a concentration as low as 2.67 × 10⁷ up to 2.67 × 10⁸ CFU/mL. As described above, the lower detection limits achieved in certain studies may be linked to the greater fundamental resonance frequency of the QCM crystals utilized. To better understand the advantages of using higher frequency QCM crystals, further studies are required utilizing QCM crystals of different fundamental resonance frequencies in the same experiments.
would be an indicator of the number of viscoelastic cells bound to be a better response signal than the frequency since the dissipation of the crystal’s oscillation (Rodahl et al., 1995) and could thus times underestimate the actual mass of cells attached to the surface. Thus, the simultaneous measurements of the attached layer of cells. Thus, the simultaneous measurements increase log–log linearly ($R^2 = 1.0$) from a cell concentration of $3 \times 10^5$ up to $10^8$ cells/mL where the sensor response reaches a plateau. The log–log linear working range of the biosensor is larger and more stable for the $D$ shift (Fig. 4b) than for the $f$ shift response (Fig. 4a). Moreover, the $D$ shift after 60 min can be better distinguished from the acceptable instrument drift than for the $f$ shifts at low cell concentrations; specifically, the lower detection limit for the $D$ shift is $3 \times 10^5$, whereas for the $f$ shift, it is $10^6$ cells/mL. From the comparison shown in Fig. 4, the dissipation shift after 60 min is shown to be a better choice as a sensor response signal in this study than the frequency shift which is more commonly used.

Because cells are very viscoelastic, the frequency response is not well described by the Sauerbrey equation. The Sauerbrey equation (Sauerbrey, 1959) can be used to directly relate adhered mass to changes in the crystal resonance frequency only for rigid adhered films, where the $D$ shift is much lower than the $f$ shift. In the present study (Fig. 3), the measured $D$ shifts are significantly greater than the measured $f$ shifts, hence highlighting the viscoelastic nature of the attached layer of cells. Thus, the simultaneous measurements of $f$ and $D$ presented here highlight the fact that the $f$ shift can sometimes underestimate the actual mass cells attached to the surface. The dissipation signal, on the other hand, is a measure of the damping of the crystal’s oscillation (Rodahl et al., 1995) and could thus be a better response signal than the frequency since the dissipation would be an indicator of the number of viscoelastic cells bound to the surface.

To confirm that the recorded biosensor response was due to specific binding of cells to the immobilized antibodies rather than an experimental artifact, a control experiment was performed where sensor crystals were prepared without the addition of antibody. These “no antibody” crystals were then exposed to a suspension of $10^8$ cells/mL $E. coli$ O157:H7. The results of this control experiment are plotted in Fig. 3 as dashed lines. When no antibody is added to the crystal surface, the $f$ and $D$ shifts are equivalent to the instrument drift, thereby confirming that the $f$ and $D$ shifts observed when using the full antibody immobilization protocol result from the binding of target bacteria with the antibody.

To further verify whether the QCM-D measurements corresponded to cell attachment events, after each experiment, the sensor crystals were viewed by optical microscopy (IX-71, Olympus). Fig. 5a shows a representative optical microscopy image of a crystal prepared with both antibody and BSA while Fig. 5b is an image of a crystal prepared without antibody. Inspection of Fig. 5a reveals that the crystal surface is covered in a monolayer of cells when the crystal is functionalized with antibody, whereas very few cells are attached to the crystal surface when no antibody is present (Fig. 5b). Together, the data presented in Figs. 3 and 5 show that the observed $f$ and $D$ shifts are a result of binding of the target bacteria to the layer of immobilized antibodies.

### 3.3. Improved detection of target bacteria using initial dissipation slope

Careful inspection of the data presented in Fig. 3 reveals that the initial slope in the $f$ and $D$ signals during injection of $E. coli$ O157:H7 is readily distinguishable among the different concentrations. This observation suggests that the initial slopes of the $f$ and $D$ may be valid response signals for detection of $E. coli$ O157:H7. The initial slopes of the $f$ and $D$ were calculated from the QCM-D measure-
ments for the different cell concentrations and are presented in
Fig. 6.

In Fig. 6a, the negative of the initial slope in the f shift is plotted as a func-
tion of the injected cell concentration on a log–log scale. The
data obtained during detection of \textit{E. coli} O157:H7 are plotted with
open circles where the f slope is shown to increase in a fairly log–log
linear fashion as a function of cell concentration. The minimum
detection limit is $10^6$ cells/mL and the measurements can be carried
out up to a maximum of $10^9$ cells/mL ($R^2 = 0.95$). Comparison of
Fig. 6a with Fig. 4a demonstrates that the linear working range for
the biosensor response is larger when using the initial slope of the
f shift rather than the absolute f shift observed after 60 min, which
saturates at a concentration of $10^8$ cells/mL. The initial f slope for
injected cell concentrations lower than $10^6$ cells/mL could not be
distinguished from the recognized instrument drift, thus these data
are not included in Fig. 6a.

Fig. 6b shows the calculated initial slope in the energy dissi-
pation response (i.e., $D_{\text{slope}}$) following injection of \textit{E. coli} O157:H7
(open circles). The data show a log–log linear increase in $D_{\text{slope}}$
over a wide range of injected cell concentrations ($R^2 = 0.99$), from
a concentration of $3 \times 10^5$ cells/mL to a maximum detection limit
of $10^9$ cells/mL. A comparison of the results shown in Fig. 6b with
those presented in Fig. 3b reveals that the sensor response range
based on $D_{\text{slope}}$ is wider and more linear (log–log) than the response
based on the D shift after 60 min. The biosensor performance based
on the measurement of $D_{\text{slope}}$ is also significantly better than that
obtained with either the f shift after 60 min or the initial slope in
the f response, thus indicating that the $D_{\text{slope}}$ is the best choice as a
sensor response signal for \textit{E. coli} O157:H7 detection in this system.

An important advantage of the $D_{\text{slope}}$ as a biosensor response is
the fact that it provides for rapid detection. The $D_{\text{slope}}$ is calculated
from the energy dissipation measurements during the first 5 min of
bacteria injection into the flow chamber. Using this approach, there
is no need to wait for a stable plateau to be reached in the QCM-D
response (i.e., the frequency or dissipation shifts). Hence, bacteria
detection can be confirmed very rapidly with this approach, using
a very small sample volume (as little as 250 µL).

3.4. Effect of BSA layer

BSA is widely used as a blocking agent to block unreacted sites
on sensor surfaces in biosensor applications (Fung and Wong, 2001;
Su and Li, 2004, 2005; Wong et al., 2002). However, the potential
effects of BSA in cell detection using QCM technology have not
been reported. To verify the potential influence of BSA in the
sensor response, additional QCM-D experiments were performed
where the sensors were prepared without the addition of the BSA
layer after antibody adsorption. The results of these experiments
are presented in Fig. 6 as open squares. As shown in Fig. 6b, the
initial $D_{\text{slope}}$ values are indistinguishable when the sensor crystals
are prepared with or without BSA and exposed to \textit{E. coli} O157:H7.
This comparison confirms that the addition of BSA to the system
does not introduce any experimental artifacts in the detection pro-
cess and that the observed cell attachment can be considered to be
specific.

3.5. Verifying biosensor selectivity

The objective of this study was to develop a biosensor for detec-
tion of \textit{E. coli} O157:H7 and other indicator \textit{E. coli}. Thus, it is of interest
to verify the selectivity of the biosensor to the organisms of inter-
est. Biosensor selectivity was evaluated by using a Gram-positive

![Fig. 5. 10× magnification light-microscopy images of (a) sensor prepared with antibody and BSA, and (b) sensor prepared without antibody after exposure to 120 min injection of \textit{E. coli} O157:H7 and subsequent rinses with PBS and DI and drying with UHP N2. The negative of the microscope images is presented for improved clarity (i.e., bacteria are shown as black spots on a white background).](image-url)*
the response in the initial negative bacterium, Bacillus, and not in a log–log linear fashion. Below an injected concentration of 10^5 E. coli K12 cells/mL, the initial f slope and instrument drift could not be discriminated, while the initial Dslope could not be distinguished from the acceptable instrument drift below a cell concentration of 10^6 cells/mL. Both the initial f and D slopes are lower for E. coli K12 than for E. coli O157:H7 (Fig. 6), providing evidence that the developed biosensor is more selective to E. coli O157:H7 than to E. coli K12. It is interesting to note that when a PBS rinse was performed after the 120 min bacteria injection, an increase in the f shift and a corresponding decrease in D were observed for E. coli K12, indicating a loss of mass (i.e., a release of cells) at the crystal surface (data not shown). In contrast, when PBS was used to rinse the crystal surface following injection of E. coli O157:H7, the f and D measurements are not disturbed (i.e., follow the same trend or reach a stable value), confirming the weaker binding of E. coli K12 to the functionalized sensor surface in comparison to E. coli O157:H7. Nevertheless, the results shown in Fig. 6 indicate that the biosensor can be used for detection of both organisms over a broad range of cell concentrations.

Because the selected antibody is specific to E. coli, the biosensor is not expected to exhibit a response when exposed to other bacterial species. Results of experiments conducted with Bacillus are presented in Fig. 6 as an open cross. As can be seen in Fig. 6b, when Bacillus is injected, the initial Dslope is the lowest observed at an injected cell concentration of 10^5 cells/mL. The initial f slope is not shown on Fig. 6a since it was determined to be positive, +0.2 Hz/min. While a positive f shift was never observed for the E. coli, the positive f shift observed with Bacillus may be indicative of very weak association of this organism with the functionalized crystal. Moreover, this positive slope may be a useful signal to discriminate between specific and non-specific binding. Specifically, the ratio f/D will be negative for binding of the target bacterium, but may be positive for a non-target organism. Optical microscopy imaging of the crystal surfaces after each experiment shows that the bacterial surface coverage is much lower for the case of Bacillus when compared to E. coli O157:H7 (images not shown). This further supports the hypothesis that Bacillus binds weakly or non-specifically to the sensor surface and is readily washed off the crystal.

4. Conclusions

A flow-through QCM-D-based biosensor for detection of E. coli O157:H7 was developed via immobilization of polyclonal antibodies using a cysteamine SAM. The sensor preparation steps were characterized using QCM-D, with the exception of the formation of the cysteamine SAM, which was done ex-situ. Four QCM-D sensor responses were evaluated: the f and D shifts after 60 min of bacteria injection, and the initial f and D slopes after bacteria injection. The initial Dslope yielded the best sensitivity, 3 × 10^6 cells/mL, and the largest linear working range, 3 × 10^5 to 10^9 cells/mL. The linear working range was found to be comparable to most QCM-based bacteria biosensors and satisfactory selectivity was also demonstrated. This study reports, for the first time, the application of the initial Dslope as a useful sensor response in QCM-D technology. The Dslope provides an important advantage of rapid on-line bacteria detection, in as little as a few minutes. Results of this study suggest that the slope in the D shift can be a useful signal to monitor in a broad range of QCM-D applications. Current research in our laboratory is exploring the application of this technique for detection of a wider range of microbial contaminants, including Cryptosporidium parvum. Experiments are also underway to determine the long-term stability of the prepared sensor crystals.

Acknowledgements

This research was supported by NSERC (Discovery Grant and PG-S awarded to C.P.), FQRNT (Team Grant), the FQRNT Centre for Biorecognition and Biosensors (CBB), the Canada Research Chairs Program and the Canada Foundation for Innovation. The authors acknowledge S. Omanovic (McGill), J. Fatissin (McGill), P. Bjoreen (Q-Sense), and D. Thid (Q-Sense) for helpful discussions and S. Grunewald (McGill) for providing ATCC 700927.

References


