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ORIGINAL PAPER

Rapid detection of bacterial proliferation in food samples using microchannel impedance measurements at multiple frequencies

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Abstract We present a novel method for detecting viable bacteria in suspensions such as milk and apple juice. Underlying the technique is the fact that bacteria in aqueous suspensions can store a large amount of charge, and thus act like (non-ideal) capacitors. Thus increased numbers of bacteria due to proliferation increases the capacitance of the bulk of the suspension. However, this increase cannot be directly measured since the capacitance of the solid–liquid interface ("double layer") in effect "screens" the latter. We present a method (derived from an earlier one) that is able to discern such changes with high sensitivity and robustness. We also demonstrate its ability to monitor food quality/safety by detecting bacterial proliferation in "real world" liquid food samples like milk and apple juice. We are able to detect ~ 1, 10, 100, and 1000 CFU/mL of *E. coli* in milk in about 4.5, 3, 2, and 0.5 h, respectively. For the same initial loads, the corresponding times to detection (TTDs) for *Lactobacillus* in apple juice are approximately 8, 6, 4, and 1 h. These represent a greater than 4-fold reduction in TTD when compared to automated systems on the market such as RABIT, Bactometer etc. We can achieve such low TTDs for low initial loads since, due to the much greater effective charge holding capacity of bacterial cells (compared to surrounding media), we are able to detect a change in the overall bulk capacitance of the suspension as the bacterial numbers cross a threshold of around 500 CFU/mL.

Keywords Rapid detection · Impedance · Microfluidics · Food quality · Food safety · RABIT · Bactometer

Introduction

Some bacteria may survive procedures like pasteurization designed to eliminate them in various liquid food products like milk [1], juices [2], etc., or may be inadvertently introduced during further processing [3, 4]. These bacteria typically cause spoilage, leading to estimated economic losses of $1 billion each year [5]. The surviving bacteria, when pathogenic may even cause outbreaks of food borne illnesses among consumers who assumed that the product was risk-free since it had been pasteurized [6–8]. In USA alone, 76 million food borne illnesses occur per year leading to 5000 deaths [9] and are estimated to have an economic impact of $6.5–34.9 billion each year [10].

Detecting and quantifying bacteria that survive treatments such as pasteurization is an important step in assuring the food quality and safety, and in meeting standards set by appropriate governing bodies or trade organizations. For instance, the US Pasteurized Milk Ordinance requires "Grade A" pasteurized milk to have a total bacterial count of ≤20,000 CFU/mL and a coliform count of ≤10 CFU/mL [11]. As a consequence, those who produce/market the food have to perform microbiological tests to satisfy themselves, and the governing bodies, regarding the efficacy of their processes designed to keep the numbers of bacteria within the stipulated range. It is important to their

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economic operation that they do so with the least possible expenditure of resources (material and labor).

There are presently several ways to detect bacteria in liquid samples like milk and juice. They can be broadly classified into three broad classes: (a) traditional methods such as plate cultures and biochemical assays, (b) DNA and antibody based methods, often involving micro/nano particles and fluorescence, (c) other "automated" techniques that rely on monitoring the effects of bacterial metabolism on the medium. Of these, traditional methods are the most extensively used, and often serve as the standard to which other techniques are compared. However they are tedious, labor intensive and have very long times of detection ranging from overnight to weeks depending on the type of the organism and medium used. DNA and antibody based methods overcome many of the disadvantages of the traditional methods. They are rapid, require less reagents and labor, and are able to identify the species/strain of the bacteria present relatively easily. However, they are not able to distinguish between viable and dead bacteria, and hence their applicability in many situations (such as that described earlier) is limited. There have been several electrochemical biosensors that have been developed for various diagnostic purposes that involve use of DNA, nanoparticle based assays, but have problems associated with non-viable detection, and low sensitivity [12].

Hence, there has been a lot of interest in developing methods that are automated (to reduce labor) and miniaturized (to reduce material costs) for bacterial detection in samples. There are a large number of automated methods already commercialized. They include devices such as the Bactec^{TM} that detects the amount of radio-labeled carbon dioxide released, Coli-Check^{TM} swabs that use Bromocresol Purple as an indicator to measure the decrease in pH due to bacterial metabolism, and the Bactometer^{TM} (Bactomatic Ltd.), Malthus 2000^{TM} (Malthus Instruments Ltd.) and RABIT^{TM} (Don Whitley Scientific Ltd.) systems, that use electrical impedance. A summary of various automated methods already commercialized, and the times to detection (TTD) for these methods (for various mentioned initial loads) are given in Table 1.

The common underlying feature of these techniques, including those which use electrical impedance, is that they rely on bacterial metabolism to produce a discernable change in a material property of the medium (such as pH, optical density, amount of carbon dioxide dissolved, electrical conductivity). The amount of metabolite processed by an individual bacterium is extremely small. [Based on our knowledge that the specific oxygen consumption rate for *E. coli* is 20 mmol of oxygen per hour per gram (dry weight) of bacteria [13] and a typical bacterium has a dry weight $\sim 10^{-12}$ g [14], we estimate that one bacterium consumes only 2×10^{-14} mol of oxygen in 1 h]. Hence, there has to be a sufficiently large number of bacteria present (either *a* priori or arising due to proliferation from the smaller number initially present) before the signal generated (change in the material property of the suspension) can be effectively measured. If the bacterial count in the original suspension happens to be small (1000 CFU/mL or lower), one must wait for cells to proliferate to an appropriately high number (often $\sim 10^6$ CFU/mL or greater) before a discernable change in the physical properties of the medium (such as pH, O_2/CO_2 concentration, conductivity etc.) can be noticed. Thus, for low initial loads, current commercial automated systems take almost as long as the plate-cultures (overnight or longer) to provide the desired result.

In the recent past, there have been efforts to increase the ease of handling, cut costs, and most importantly, reduce TTD by using microfluidic systems to miniaturize the automated methods. Bashir and co workers have developed chip-based micro-devices in which pH and impedance are monitored in order to detect bacterial metabolism[15, 16], and various additional modifications like the use of interdigitated microelectrodes [17, 18], and arrays of microelectrode based biosensors [19, 20] have been tried in order to increase the sensitivity of measurements (with respect to conventional electrodes), and thus further decrease the TTD. While these efforts were successful (in the sense that

Table 1 Comparison of some of the commercially available automated systems for bacteria detection

Commercial name	Method employed	Initial load (CFU/mL)	Microorganisms	TTD (h)
RABIT (Don Whitley Scientific Ltd., Shipley, UK) [41, 42]	Change in solution conductance	1	Coliforms	16.1
Bactometer (Bio Merieux, Nuertingen, Germany) [43]	Impedance microbiology	$>10^5$	Mainly *E. coli*	4
Malthus systems (Malthus Instruments Ltd., Crawley, UK) [34]	Conductance change of the fluid	100	*C. Sporogenes*	15.5
BacTrac (Sylab)[36]	Impedance analyzer	100	*P. Aeruginosa*	30

their TTDs are lower than those of the commercially available devices), they continue to be limited by the amount of time it takes for bacterial metabolism to significantly alter the composition of the medium when bacterial loads are low. Bashir and co-workers attempted to overcome this drawback by concentrating the bacterial cells from dilute samples to a small volume by using dielectrophoresis (DEP) prior to culture, and then detecting changes in medium composition as before [21]. While the culture time needed for detection was reduced, one needs to take into account the time needed for concentration using DEP (an additional 2–3 h) as well to get effective TTDs. Again, while successful, the actual method of detection still relies on bacterial metabolism, with its inherent limitations (as discussed earlier).

In our previous work [22], we proposed a different approach to detect the presence of viable bacteria in a given suspension. We argued that since bacteria can store a lot of charge ($\sim 100\times$ more than an equal amount of water) [23], an increase in the number of bacteria through proliferation would lead to a measureable increase in the charge storing capacity (capacitance) of the medium, and hence the reactance (out of phase component of the impedance) between two metal electrodes in contact with the medium. Other previous researchers [24] had also had the same idea, but they had failed to detect any such change in reactance. We showed, using mathematical models, that this was because, given the range of AC signal frequencies they used (<1 MHz) and the geometry of the system, the electrochemical interface between the electrodes and the aqueous solution (the "double layer") effectively "screened" the bulk solutions. Our models also led us to propose that we could detect increased capacitance of the bulk medium due to bacterial proliferation by (a) changing the geometry of the system (using narrow and long, capillary-like geometries as depicted in Fig. 1) to decrease the inverse resistance capacitance time of the system, and (b) measuring the reactance of the system at multiple frequencies, and tracking the frequency at which a minima was recorded. We showed, using our proposed method and reactance (impedance) measurements up to 1 MHz, our ability to detect an initial load of $\sim$ 100 CFU/mL *E. coli* in Tryptic Soy Broth (TSB) in about 3 h.

In this paper, we report two advances. Firstly, we enhance the sensitivity of our electrical technique through the use of (a) high frequency impedance measurements up to 100 MHz (b) a refined electrical model for our system that is better able to describe the behavior of the bacterial particles at these high frequencies, and (c) more rigorous data analysis. Secondly, we demonstrate that the technique is suitable for monitoring food quality/safety by detecting bacterial proliferation in "real world" liquid food samples like milk and apple juice.

Materials and methods

Sample preparation and inoculation of bacteria into samples

Escherichia coli K12 (ATCC 23716), and *Lactobacillus acidophilus* (Nature's Life™ Apple-honey *Lactobacillus acidophilus* probiotic) were used in this study. In order to obtain load cultures, *E.coli K12* was incubated overnight at 37 °C in TSB (Bacto™, BD), *Lactobacillus acidophilus* was incubated at 30 °C for about 48 h in MRS Broth (Difco™, BD). These were then used, in appropriate dilutions, to seed the samples in which we monitored bacterial proliferation using our method. These samples included those of TSB into which we loaded *E. coli* (to compare our new technique to our previous work), and two representative liquids to study the ability of the method to detect bacteria in food samples, [2% reduced fat milk (Prairie Farms™) for *E. coli* and preservative free Apple juice (Florida's Natural™) for *Lactobacillus acidophilus*].

To facilitate growth of *lactobacilli* in the apple juice, its pH was adjusted to about 6 by adding potassium hydroxide (about 1 mL of 10 M KOH to 50 mL of Apple Juice). The media and the food samples were all autoclaved at 121 °C at 15 psi to ensure no presence of live bacteria in them. This ensures the right concentration of the bacteria in the sample when we artificially inoculate it with our bacteria of interest. The samples are allowed to cool down to room temperature before bacterial inoculation. The bacterial suspension after being incubated for specified time periods was initially assumed to contain approximately 10^9 CFU/mL bacteria. 1 mL of *E.coli K12* and 1 mL of *Lactobacillus acidophilus* were taken in separate eppendorf tubes and centrifuged for 8 min to settle the bacteria down as pellet. Then the supernatant was discarded and pellets were resuspended in equal volume of food samples in which they were to be detected. Then the suspension was serially diluted and inoculated into the liquid samples to have different initial concentration of bacteria in them and also simultaneously the samples were plated onto petriplates to get the actual initial concentration of the inoculated bacteria in the sample.

Experimental design

Four sets of 9 mL of each of the liquid samples (TSB, milk or apple juice) were taken in the incubating tubes. Each tube was inoculated with the bacteria to be detected such that the final concentrations of the bacteria in the tubes were approximately 1, 10, 100 and 1000 CFU/mL respectively. The tubes were then allowed to incubate for a time period of 8 h for 1, 10, 100 CFU/mL concentrations and 5 h for 1000 CFU/mL concentration. At regular time intervals

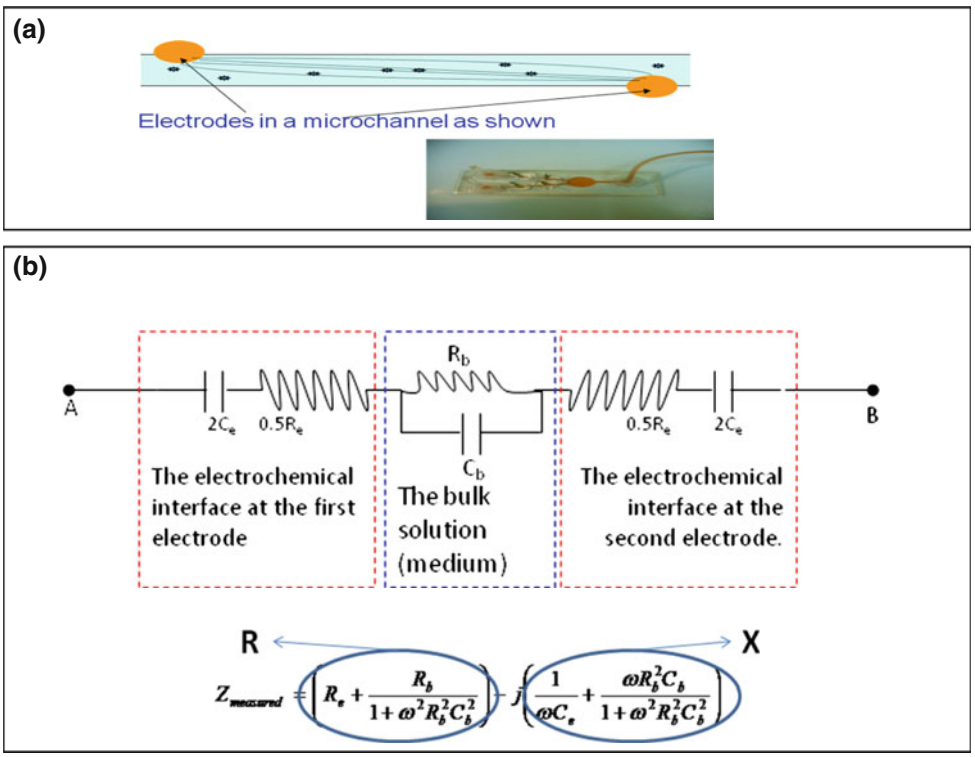

Fig. 1 **a** Schematic representation of the microchannel with electrodes on either end loaded with suspension harboring bacteria (actual image in *inset*). The spatial confinement of the electrical lines of force ensure sensitivity of electrical measurements to the bacteria present. **b** Equivalent circuit representation of the channel with suspension of bacteria and electrodes. R_e Resistance at the electrode-suspension interface, C_e Capacitance at the electrode-suspension interface, R_b bulk resistance, C_b bulk capacitance

(30 min for 1000 CFU/mL and 1 h for 1, 10, 100 CFU/mL) small volume (~250 µL) of the sample was taken out, injected into the cassettes for purpose of taking impedance measurements.

The microfluidic cassette (as shown in Fig. 1a inset) consists of a reservoir and two arms of 4 cm in length each with 800 µm depth and 1 mm in width. Gold coated crimp-pin electrodes are located 1 cm apart and on the opposite side of the channels, as shown in Fig. 1a used to make electrical measurements. The microfluidic cassettes used for the measurement were fabricated using liquid phase photo-polymerization of a commercially available UV curable polymer (Loctite 363™), a process that has been described elsewhere [22, 25] in detail. The gold electrodes on the cassette are connected to an agilent 4294A impedance analyzer (Agilent technologies, CA, USA) via 16047E connector that allows measurements of R and X across electrodes.

Along with taking the impedance measurements at every time interval, 100 µL of the sample was taken, diluted appropriately and plated onto petri-dishes to give actual concentration of bacteria at that hour in the sample. The whole process was repeated independently at least 3 times for each targeted initial load of the system (1, 10, 100 or 1000 CFU/mL) and for all liquids (TSB, milk and apple Juice). The cassettes were sterilized in an autoclave at 121 °C before use. After each of the experiment, the electrical connectors were replaced; cassettes were washed thoroughly with soap, bleach, alcohol and water, and then autoclaved.

Electrical measurement technique

When the cassettes are loaded with an aliquot from the sample being investigated, the suspension fills the microchannels along which electrodes are positioned as shown in Fig. 1a. An Agilent 4294A Impedance Analyzer is used to measure the electrical impedance between the electrodes at multiple (>500) frequencies between 1 kHz to 100 MHz. The instrument measures the magnitude and phase of the AC current that flows through the suspension upon the application of a sinusoidal AC voltage of 500 mV (peak-to-peak), and calculates the Impedance (resistance and reactance) from the measurements. At this low voltage, the cells do not get lysed [26] and hence the reading that is obtained gives the reading for the total viable cells in the channel and avoids interference by dead cells and giving false negative results. Since the current is not in-phase with the applied sinusoidal voltage, the Impedance, which can be considered as the AC analog of the DC resistance, has both an in-phase component called the resistance (R), and an out-of-phase component called the reactance (X). It is typically represented as a complex number and as shown in Eq. 1

$$Z = \mathrm{R} + j\,\mathrm{X} \tag{1}$$

where $j = \sqrt{-1}$

Alternatively, the impedance can also be represented completely by its magnitude (|Z|) and its phase angle θ. The magnitude and phase angle, respectively, of the impedance, are related to the resistance and reactance by the equations.

$$Z = \sqrt{(R^2 + X^2)} \tag{2a}$$

$$\theta = Tan^{-1}\left(\frac{X}{R}\right) \tag{2b}$$

The impedance analyzer measures impedance by measuring the resistance (R) and reactance (X) for each sample, over the frequency range of 1 kHz to 100 MHz and hence generates the data set containing the values of R and X at multiple (>500) frequencies.

Circuit model and data analysis

In our previous work [22], we had proposed that a system consisting of an aqueous solution in a microfluidic channel with electrodes on either end (Fig. 1a), can be represented by an equivalent electrical circuit as shown in Fig. 1b. As seen, this takes into account the existence of electrochemical "double layers" on the surfaces of charged metal electrodes. Hence, the net measured impedance ($Z_{measured}$) is, as shown by the accompanying equation, affected by not only by the presence of conductive and capacitive (charge-storing) elements in the bulk, but also by such elements present at the electrode-solution interface. In fact, the interface capacitance is typically more than 1000 times greater than the bulk capacitance, as a result of which previous investigators [24] were not successful in measuring any changes in the latter, although it was expected that since bacteria can store charge [23], their proliferation would lead to an increase in bulk capacitance. In our previous work, we showed how the use of micro-channel geometry serves to concentrate the electrical field and raise the effective bulk resistance of the suspension, thereby allowing us to pick up signatures of increased bulk capacitance. Specifically, the signature that we looked at was a shift in the plot of measured reactance (X) vs. frequency (ω) in the range of 1 kHz to 1 MHz.

Since the effects of the electrochemical double layer are less pronounced at high frequencies (as can be seen from the equation accompanying Fig. 1b), we had initially assumed that obtaining readings at higher frequencies (up to 100 MHz) would enable us to obtain more sensitive readings using the same metric (shift in X vs. ω curves). However, our experiments (results not shown) were unsatisfactory. Due to the increased contribution to the measured reactance from the bulk resistance at these higher frequencies, even extremely small changes in the value of this quantity (that we attribute to thermal fluctuations in our lab) caused shifts in the X vs. ω curve. Thus our efforts to increase the sensitivity of our old measurement technique led to false positives. We hence need a way to distinguish the true positives (shift due to an increase in bulk capacitance) from false positives (shift due to other reasons such as an increase in the bulk resistance).

To overcome this hurdle, we chose to adopt a different procedure, viz. to evaluate changes in all individual components of the circuit. To do so, we take the values of resistance (R) and reactance (X) measured by the impedance analyzer and fit that to our equivalent circuit model using a commercial circuit analysis software (Z view). This software when given the circuit model and data set is be able to estimate each of the individual parameters (R_e, C_e, R_b and C_b) of the circuit. Using this technique, it was further seen that while the circuit model used earlier Fig. 1b works well at low frequencies, it does not hold very well at higher frequencies. Replacing the bulk capacitance (C_b) with a Constant Phase Element (CPE) in the circuit (yielding the circuit shown in Fig. 2a provided a much better fit to the data obtained – as shown in the Fig. 2b.

The CPE is a non-intuitive circuit element that replaces a capacitor in a circuit when the there is some type of non-homogeneity in the system, delaying or impeding the movement of charge carriers [27]. In more mathematical terms, the impedance of a CPE is given by the equation

$$Z = 0 - j\left(\frac{1}{(wQ)^n}\right) \tag{3}$$

As shown in Eq. 3, the impedance of the CPE is defined by two values: the magnitude component CPE-T (Q) that is measured in farads and the phase component CPE-P (n) that is measured in radians. If CPE-P (n) equals 1 then the equation is identical to that of a capacitor. Since it intuitively seems likely that bacteria while able to store charge, may not behave like ideal capacitors, compensating its non-ideality with CPE element seems appropriate. We could identify need for using a CPE for our data analysis, as the arc of the Cole Plot for our impedance data was a depressed semicircle or an arc of the circle rather than a perfect semicircle as would be the case if the bacteria behaved like ideal capacitors [28].

The value of the CPE-P (n) is not a constant but is different for different samples. When doing the analysis using the Z view software, initially the data is loaded to the software and the circuit to which it had to be fit is constructed. Then, each of the circuit parameters (R_e, C_e, R_b, CPE-T, and CPE-P) are provided with initial guess values and the system numerically optimizes the values to obtain the best fit for the system as a whole over the range of frequencies examined. Convergence is often not guaranteed, and sometimes the system converges to a solution that is "obviously" wrong as seen from the visual fit of the

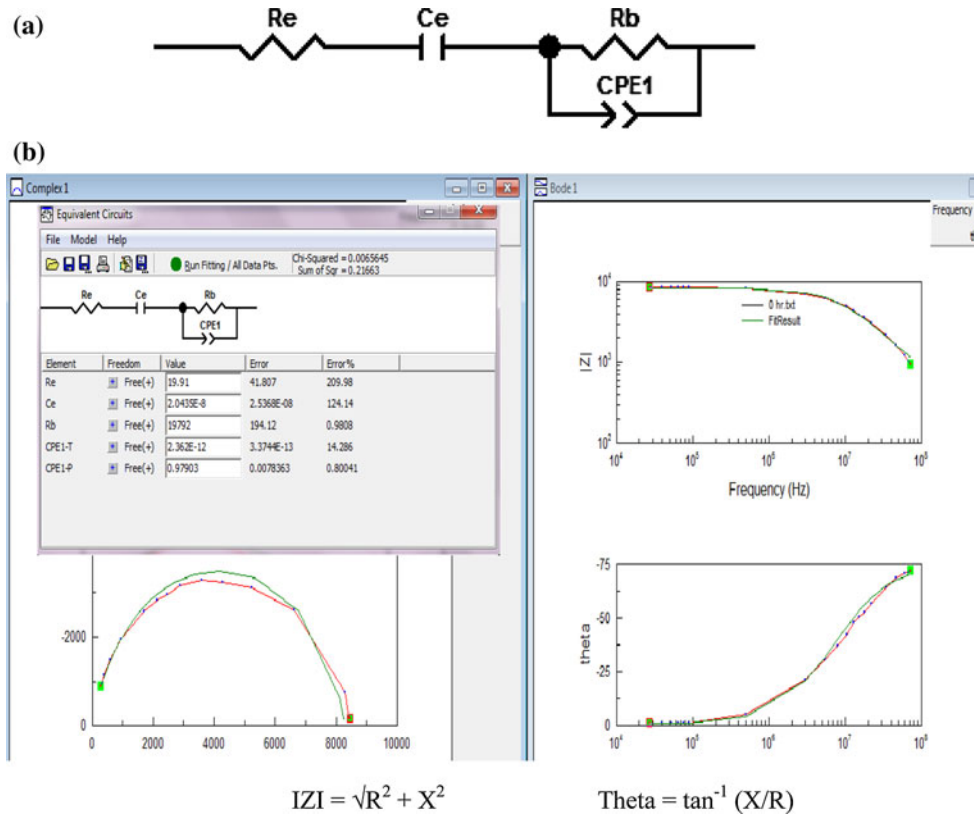

Fig. 2 **(a)** Equivalent circuit diagram as used in the Z view software with a Constant Phase Element used to represent the charge-holding behavior of the bulk suspension (bacteria), instead of an ideal capacitor (as in Fig. 1) **b** Results obtained using Z-view®, when the data from Impedance Analyzer (*represented by line with dots*) was fitted to the circuit model with the parameters on the right (*represented by line without dots*)

computed circuit model impedance curves (green lines in Fig. 2b) to the actual data (red lines in Fig. 2b). Since the charge-holding behavior of individual bacteria cells presumably do not change with an increase in their number, in some (but not all) cases, the value of the CPE-P is fixed to the value obtained earlier for the same sample to help the software reach an apparently correct numerical solution. In the other cases, it is seen that the software itself arrives at a set of values for the circuit parameters in which the value of the CPE-P is extremely similar (if not identical) to that obtained previously for the same sample. It may be noted, though, that the values of CPE-P that we obtain appear to be a function of the bacterium studied and the medium used: with values of approximately 0.96, 0.97, and 0.986 for *E.coli* in TSB, *E. coli* in Milk, and *L. acidophilus* in Apple Juice respectively. We do not have, at present, have any basis for predicting a-priori what the value of the CPE-P will be for a particular system. Presumably, they may even differ among different samples of the same product (e.g. different batches of milk, depending on the protein and fat content).

Establishing TTDs for individual samples

The CPE-T value generated by the software provides a measure of the charge-storing capability of the suspension being investigated. Over a period of time, this quantity is expected to increase with increase in the number of bacteria, and one can conclusively state that there are viable bacteria in the sample when one observes this quantity (CPE-T) to increase significantly. The time needed to make this observation (significant increase in the value of CPE-T) is the TTD for our system. As illustrated in Fig. 3, the confidence interval (error bar) of the CPE-T value obtained from the 1 h impedance readings overlaps with that of the corresponding 0 h (initial) value. In contrast, the confidence interval of the CPE-T value obtained at the 2 h impedance measurement does not overlap with that of the initial reading. Thus it is possible to state conclusively that the charge holding capacity of the suspension increased (presumably due to proliferation of bacteria) after 2 h, but not after 1 h. Hence our TTD for this case is 2 h. It may be noted that the values of CPE-T closely track the plate counts (red squares in

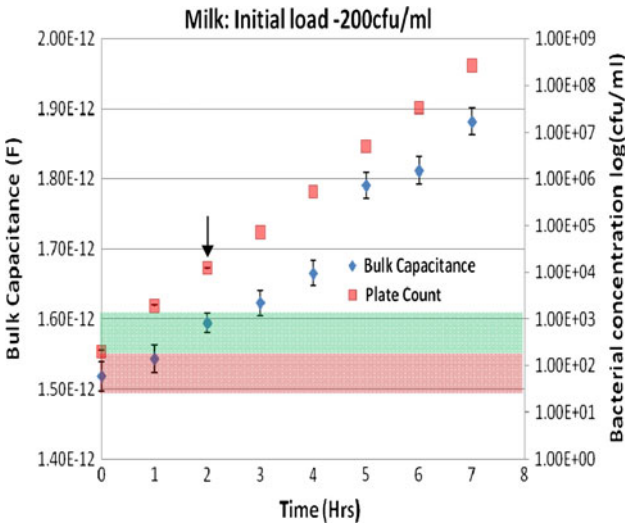

Fig. 3 Plot showing the increase in the bulk capacitance (*diamonds*) with actual increase in the concentration of the bacteria (*squares*) in the suspension. The plot also indicates the time to detection (*shown by the arrow*), For this sample, the error bar of CPE-T value of 2 hours does not overlap with the error bar of zero-hour reading and hence 2 hours is considered as the time to detection

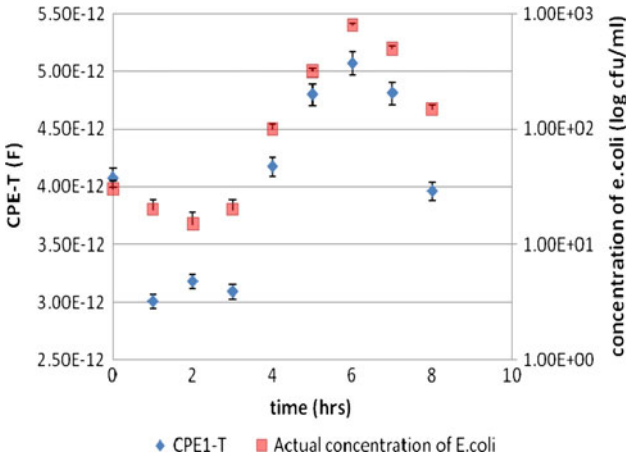

Fig. 4 Plot of CPE-T values (*diamonds*) and actual concentration of bacteria in the sample (*squares*) at various points in time for a system consisting of *E coli* suspended in tryptic soy broth (TSB). The data show the ability of our method to closely track the true bacterial count

Fig. 3) of bacteria in the system at that particular time (obtained from aliquots harvested at that time). Also, we had taken a reading at 1.5 h, it is possible we could have detected a significant change in CPE-T earlier, but since we did not do so, we can only report a TTD of 2 h.

Results

Ability of the calculated CPE-T value to track true bacterial counts

The validity of our method i.e. using the value of CPE-T (obtained as described earlier), as an indicator of bacterial load in the system, is illustrated in Fig. 4. As seen in this figure, the CPE-T values closely track the actual bacterial numbers present (as obtained using plate counts) irrespective of whether the bacterial numbers hold steady (as occurs in the lag and saturation/stationary phase), rise, or decline. The decrease in CPE-T values as bacteria die off seems to indicate that dead bacteria are not as capable as live ones of storing charge. We speculate that this is due to the active transport ion pumps no longer being functional for these dead bioparticles [29].

TTD as a function of initial bacterial loads

Three types of samples (TSB, Milk and apple juice) were inoculated with 4 different initial bacterial loads (targeted to be 1, 10, 100, 1000 CFU/mL), impedance measurements were taken at specific intervals (half hour or one hour), and the impedance data were analyzed using Z view software to obtain the CPE-T values as described in the previous section. These values were used to obtain TTDs using the criteria explained using Fig. 3. Some more of such typical plots of CPE-T vs. time which gives the TTDs for each sample are shown in Fig. 5, with the arrows indicating the TTD for that sample with respective initial bacterial load.

In a few cases, mostly for *L. acidophilus* in apple juice, a significant lag phase is observed. During this period, bacteria do not grow, and sometimes even die as indicated by the plate count data (e.g. red squares of Fig. 4). In such cases, a better estimate of the capabilities of our system is obtained by subtracting the lag phase time (2 h in our example) when we calculate our TTD for the given initial load in the given system. For example, in the case shown in Fig. 4, although the significant increase in CPE-T from the initial value is detected only at the 4th h, the TTD of the system is taken to be 2 h since for the first two hours, the bacteria in the suspension were in the lag phase.

Each experiment with the targeted initial load of bacteria in a specific sample is repeated three times to ascertain the reproducibility of the method. A more accurate estimate of the true value of the initial loads could only be obtained the next day, once plate counts were obtained. Hence, we have twelve points each for TSB, milk and apple juice (some of these points overlap very closely, and are hence not distinguishable). These points are used to calculate a line of bet fit using linear regression, and these lines are also shown in Fig. 6. The equations for these lines provide the best estimate of the time that our system will take to detect a given load of a particular type of bacterium in a particular substrate.

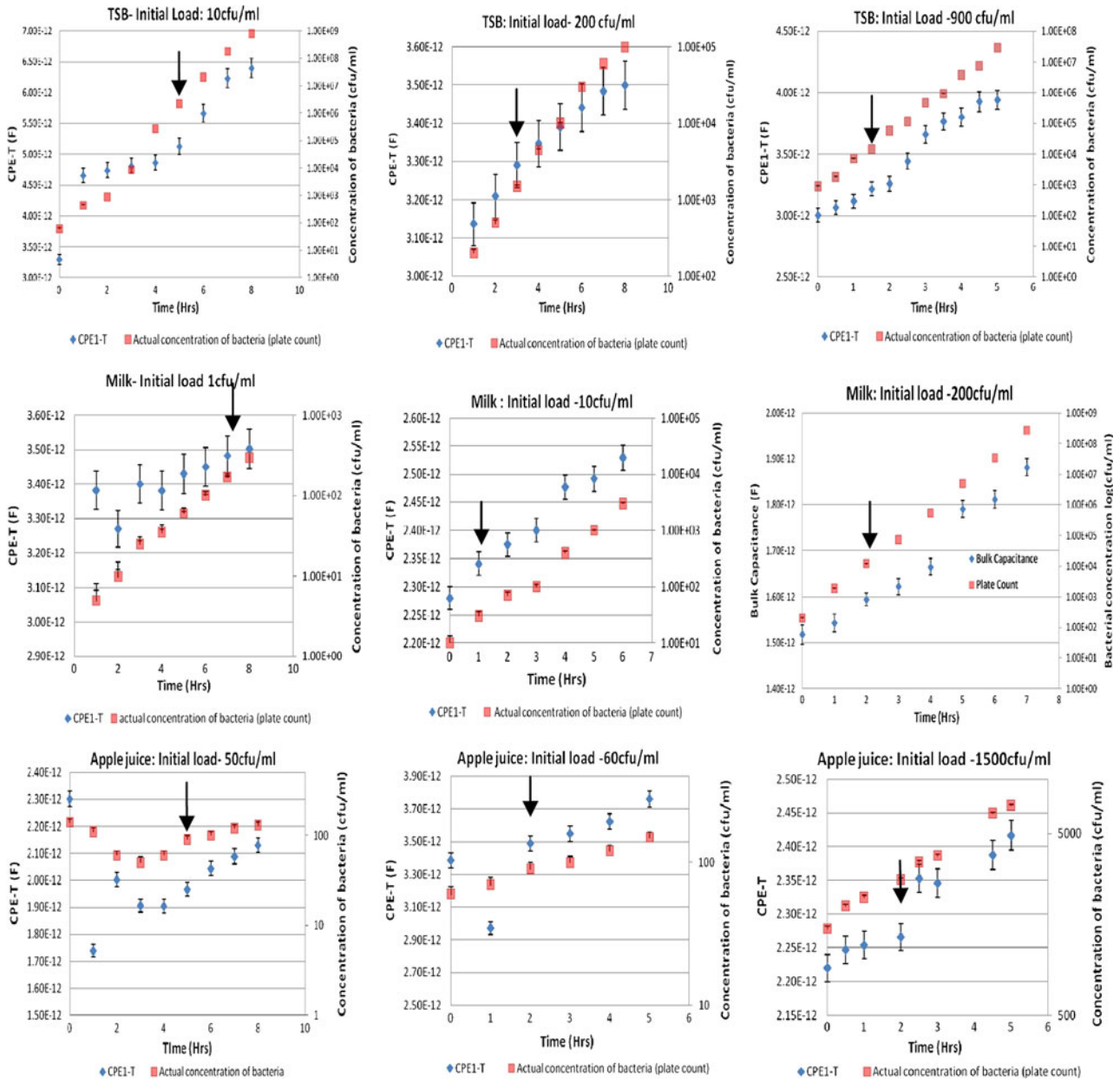

Fig. 5 CPE-T vs. time plot for some representative samples with different initial bacterial loads. The TTD for each is indicated by an *arrow*

Discussion

As seen in Fig. 6, there is an inverse relationship between the (log) initial load of bacteria in a sample, and the TTD of proliferating bacteria using our method. In this it is similar to methods that rely on detecting the effects of bacterial metabolism such as Bactometer, RABIT etc. This is expected since the presence of more bacteria (our method) leads increased metabolite consumption/generation. Also, as in the case of RABIT, Bactometer etc., one can generate a calibration plot for a particular type of suspension, and the TTD can be used to estimate the initial load of the system. The scatter that we observe seems qualitatively comparable to the data used to generate calibration curves for RABIT etc. [30]. The scatter arises due to multiple reasons. To that we believe contribute the most in our case are uncertainties in the estimates of the initial loads, and differences in metabolic state of members within and between populations seeded. In other words, although we plot our TTDs against a "known" initial load based on plate counts, this "known" value itself is subject to some degree of uncertainty typically of the order of the square

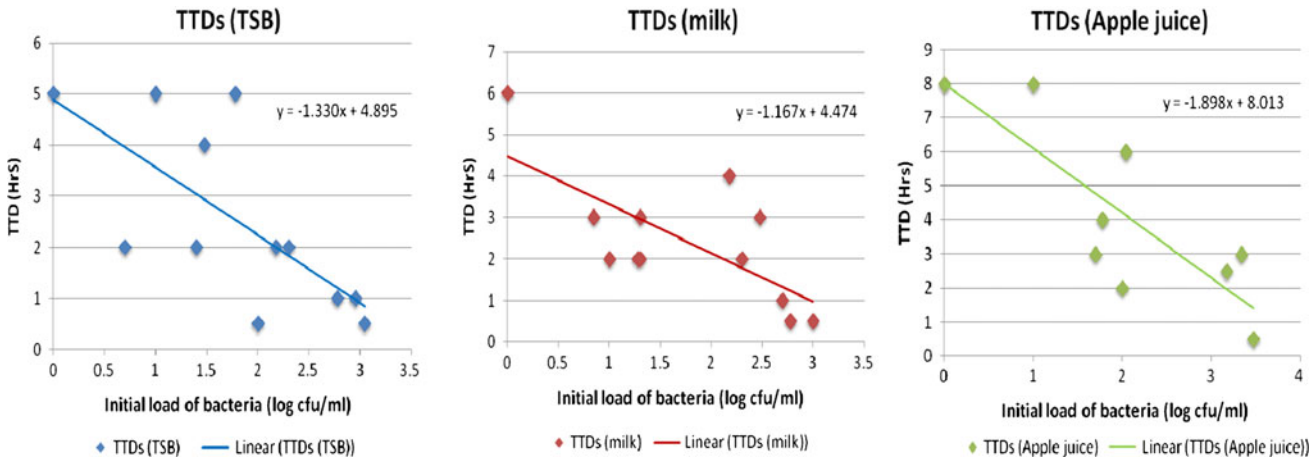

Fig. 6 Consolidated plots showing the variation of the TTD as a function of the initial bacterial load for multiple experiments with *E. coli* in TSB (*left*), *E. coli* in milk (*center*), and *Lactobacillus* in apple juice (*right*)

root of the true number of particles present (Poisson distribution) [31]. Thus, if the suspension being incubated had 100 CFU/mL of bacteria (true value), we expect that a 100 µL sample we introduce into our cassette, or use for plating, will have 10 CFUs. However, there is also a 33% chance that the sample we actually isolate will have either less than 7 $(10 - \sqrt{10})$, or greater than 13 $(10 + \sqrt{10})$ bacteria. In addition, a certain fraction of the bacterial cells that constitute the inoculum may remain in the lag phase slightly longer than others. This may not be readily captured by the plate counts taken to determine initial load (since in plates, they get adequate time to grow). Since we are operating at low concentrations (low numbers of bioparticles), these sampling uncertainties are liable to introduce a greater relative error. Despite these sources of error, our TTD data still show a clear trend in the manner expected (inverse with respect to log initial load).

Another characteristic of our method is that the TTD is a function of the doubling time of the proliferating bacteria. The faster that a given bacterium doubles, the shorter is our TTD. In the present study, we worked with two different bacteria: *E. coli K-12* that has a doubling time of 27 min at 37 °C [32], and *Lactobacillus acidophilus* that has a doubling time of 50–60 min at 30 °C [33]. Thus the doubling time of *lactobacillus* is about 2 times that of *E. coli*. The TTDs for *lactobacillus* are also correspondingly longer (8 h for 1 CFU/mL and 4.5 h at 100 CFU/mL vs. 4.5 and 2 h, respectively, for *E. coli* at the same initial loads). For initial loads of 1,000 CFU/mL or higher, we are able to detect proliferation in half an hour (the shortest time interval we used) for *E. coli* (and in one case, for *lactobacillus* as well). Thus, at these relatively higher loads, we are able to detect the bacteria within one cycle of division. For lower initial bacterial loads, at the points in time where significant changes in CPE-T values are detected, their concentration in the sample (as estimated from the plate counts) is typically between 200–1000 CFU/mL. As a rough ballpark estimate, we can hence claim that we are able to catch the bacteria in the act of doubling their numbers when there are about 500 of them present per mL of suspension. Since the volume of liquid between our electrodes is $\sim$ 20 µL, this implies that we are actually observing about 10 of these bacteria (reproducing to form 20) at that time.

As shown in Figs. 7a, b, our TTDs compare very well with automated techniques already on the market and other automated techniques in development. Fig. 7a gives the comparison of our detection method with that of the commercial automated systems- RABIT^{TM} [30], Malthus 2000^{TM} [34], Bactometer^{TM} [35], BacTrac^{TM} [36] currently in market. Fig. 7b gives the comparison between our system and some of the systems currently under development. Virtually all of these systems under development continue to rely on detecting the effects of bacterial metabolism on the medium properties, such as changes in pH [15], conductivity [15, 37], oxygen concentration [38], for detection. Some employ features and capabilities available through microfluidic systems, such as microinterdigitated electrodes [18, 39] or preconcentration using dielectrophoresis [21, 40] to try and reduce the overall TTDs. While they achieve low TTDs (3–9 h) for very high initial concentrations of bacteria ($\sim$10,000 CFU/mL), they continue to have high TTDs (10–14 h) at low initial concentration of bacteria (1 CFU/mL). Thus, for any given initial load, our system is able to detect bacteria at least 3–4$\times$ faster than the competition.

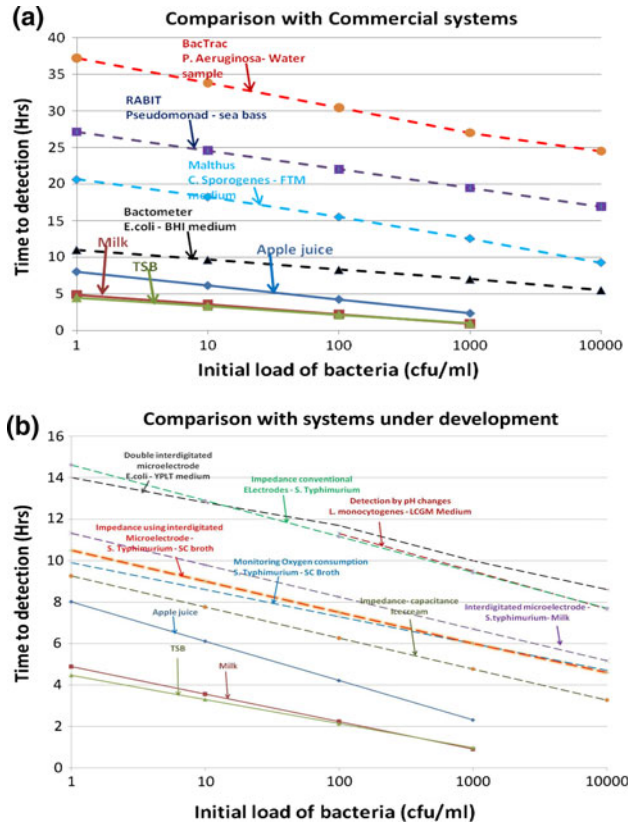

Fig. 7 Comparing TTDs obtained using our system (*solid lines*) to **(a)** those of the commercial systems already on the market, and **(b)** other, especially microfluidic, systems under development (*dashed lines*)

Conclusion

The method presented here represents a different approach to detecting viable bacteria than taken by virtually all other automated systems, including those currently under development. Instead of relying on monitoring the effects of bacterial metabolism, it monitors the charge carrying capacity of the suspension (which increases with increasing numbers of suspended particles capable of storing charge), and thus directly tracks bacterial numbers. We have been able to use this method to detect low loads of bacteria in milk and apple juice much more quickly than current automated methods. This coupled with the fact that handling requirements are very similar to those used for current methods and that the reported method appears to be robust to minor fluctuations in temperature, encourages us to believe that the latter can be widely adopted for monitoring food quality and safety. Further, it can in the future also be applied to other areas where rapid detection of low bacterial loads is important, such as for environmental and clinical applications.

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