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ABSTRACT

The detection and quantification of naturally occurring microbial cellular densities is an essential component of environmental systems monitoring. While there are a number of commonly utilized approaches for monitoring microbial abundance, capacitance-based biosensors represent a promising approach because of their low-cost and label-free detection of microbial cells, but are not as well characterized as more traditional methods. Here, we investigate the applicability of enhanced alternating current electrokinetics (ACEK) capacitive sensing as a new application for rapidly detecting and quantifying microbial cellular densities in cultured and environmentally sourced aquatic samples. ACEK capacitive sensor performance was evaluated using two distinct and dynamic systems – the Great Australian Bight and groundwater from the Oak Ridge Reservation in Oak Ridge, TN. Results demonstrate that ACEK capacitance-based sensing can accurately determine microbial cell counts throughout cellular concentrations typically encountered in naturally occurring microbial communities (10^3 – 10^6 cells/mL). A linear relationship was observed between cellular density and capacitance change correlations, allowing a simple linear curve fitting equation to be used for determining microbial abundances in unknown samples. This work provides a foundation for understanding the limits of capacitance-based sensing in natural environmental samples and supports future efforts focusing on evaluating the robustness ACEK capacitance-based within aquatic environments.

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1. Introduction

A biosensor consists of a biological recognition element such an enzyme, antibody, nucleic acid, or organism that is capable of outputting a signal to an interfaced transducer capable of monitoring and measuring that signal (Ahmed et al., 2014; Close et al., 2009). The small size, low power requirements, and flexibility of

biosensors has made them highly deployable for the identification of target cells and/or molecules, for monitoring changes in cell biomass, and for detecting bounded biomolecules. However, the majority of biosensors developed to date have been engineered specifically for estimating biomass and monitoring growth within batch reactors, or for the detection of specific pathogens (Ivnitski et al., 1999; Kim et al., 2009; Li et al., 2014; Matanguihan et al., 1994; Radke and Alocilja, 2004). Despite the advantages they can provide, substantially fewer biosensors have been developed for monitoring environmental samples due to difficulties in modulating sensor sensitivity relative to target abundance dynamics, the inherent interference resulting from exposure to the expanded environmental geochemical milieu relative to defined growth mediums, and the difficulties in differentiating target detection from total community abundance. Overcoming these hurdles would allow the application of biosensors for *in situ* environmental cellular detection as an enhanced technique for rapidly screening aquatic samples, particularly in environments where

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sample volumes may be limited. Such screenings would enable effective environmental sampling strategies by quickly estimating microbial abundance and providing a more cost-effective alternative to traditional sampling.

Particularly promising in this application are capacitance-based technologies that have been preliminarily demonstrated capable of estimating microbial cellular abundance in environmental samples, but have not been thoroughly investigated in this role. To determine bioparticle count, biosensors often require fluorescent labels for amplification of signals, long incubation times, and utilization of specialized instrumentation (e.g., optical microscopes) (Cui et al., 2013; Li et al., 2013, 2014). Recently, Cui et al. (2013) have recently developed an alternating current electrokinetic (ACEK) enhanced capacitive sensor that can rapidly detect and distinguish between bound biomolecules without the need for prior fluorescent labeling. Unlike conventional biosensors, the ACEK enhanced capacitive sensor employs dielectrophoresis (DEP) concentration with capacitive measurement simultaneously, thus providing enhanced detection sensitivity and selectivity by the microelectrodes.

Using this technology, target molecule detection is achieved by applying an appropriate AC signal to induce movement of the target biomolecules towards the sensor electrode, resulting in a change of the interfacial capacitance on the sensor surface that correlates with the biomolecule binding rate (Cui et al., 2013; Li et al., 2013, 2014). Overall, this label-free, one-step approach does not require extensive training and provides a “from sample to result” within minutes with a volume size of less than 10 μ L (Li et al., 2014). A schematic overview of ACEK capacitive sensor is provided in Supplemental Fig. S1.

In this study, we investigate the application of ACEK capacitive sensing technology as a rapid screening tool for the detection and quantification of microbial abundance in aquatic environments. Capacitive measurements for multiple sample types inclusive of bacterial and algal cells were tested to determine sensor sensitivity in response to different classes of microorganisms (e.g., Gram-positive, Gram-negative, algal) and variable target sizes. Sensor performance was evaluated using laboratory-sourced batch culture samples, and environmentally sourced groundwater and seawater samples representative of the most commonly encountered sample formats. For all tested conditions, microbial abundances were verified against direct Acridine orange-stained organismal counts to benchmark biosensor performance.

2. Material and methods

2.1. Organisms and culture conditions for laboratory-sourced batch culture experiments

Bacillus subtilis was obtained as a glycerol stock from the Center for Environmental Biotechnology at the University of Tennessee. Batch cultures of *B. subtilis* were prepared by adding the glycerol stock to 50 mL of Luria-Bertani broth (Difco) and growing overnight at 37 °C with continuous shaking. *Alkanivorax borkumensis* SK2 (DSMZ 11573) was obtained as a glycerol stock from the Hazen laboratory at the University of Tennessee. Batch cultures of *A. borkumensis* were prepared by adding the glycerol stock to 50 mL of Marine Broth (Difco) containing 50 mg of sodium pyruvate and growing overnight at room temperature with constant shaking. *Microcystis aeruginosa* (UTEX B2662) was obtained directly as a batch culture from the UTEX culture collection of algae and propagated in BG-11 freshwater media (Sigma Aldrich Co. LLC) pH adjusted to 8.4 with 1 M NaOH at 25 °C. During propagation, all *M. aeruginosa* cultures were shaken three times a day, maintained in a 12:12 h light-dark cycle using a controlled photobioreactor (UTEX,

Austin, TX), and diluted into fresh BG-11 media at a ratio of 1:5 every 7–10 days.

2.2. Preparation of laboratory-sourced batch culture samples

To prepare for biosensor exposure, the growth characteristics of *B. subtilis*, *A. borkumensis*, and *M. aeruginosa* batch cultures were monitored to over a 6–12 h period to generate a growth curve based on optical density and direct cell counts. Growth curves were generated using a Genesis 10S UV-visible Spectrophotometer (Thermo Fisher Scientific, Inc.) at optical densities (OD) of 600 nm (*B. subtilis* and *A. borkumensis*) or 680 nm (*M. aeruginosa*). Acridine orange direct counts (AODC) were performed as previously described (Francisco et al., 1973; Smith et al., 2015). Batch cultures for capacitive measurements were established by adding 1 mL of inoculum into 50 mL of medium and, using the characterized growth patterns for each sample, extracting samples during each culture's exponential growth phase (10^6 – 10^7 cells/mL). Each extracted sample was then serially diluted to densities of 10^6 , 10^5 , 10^4 , and 10^3 cells/mL in $1 \times$ phosphate buffered saline (PBS) solution and deionized water, and immediately analyzed for capacitance. Additionally, serial dilutions of *B. subtilis* and *M. aeruginosa* from cells extracted during stationary growth phase were similarly prepared. PBS and DI water were employed as blanks for each biosensor measurement, respectively.

2.3. Collection of environmental samples

Seawater samples were collected from the Great Australian Bight (GAB) in April 2013 aboard the RV Southern Surveyor using Niskin bottles mounted to a conductivity, temperature, and depth rosette. After sample recovery from depth, water was decanted into glass amber bottles and immediately stored at 4 °C until analysis. A map of the seawater sample collection sites is presented in Fig. 1A.

Groundwater samples were collected from four groundwater-monitoring wells located along the Bear Creek watershed in at the Oak Ridge Reservation (ORR) in Oak Ridge, Tennessee. Samples were collected between November 4, 2013 and December 4, 2013. For each well and sampling point, groundwater collection was performed as previously described in Smith et al. (2015). Briefly, each well was purged until *in situ* parameters (e.g., pH, conductivity, and oxidation-reduction) were stabilized to ensure the standing water line was fully removed. Next, 40 mL of groundwater was preserved in 4% formaldehyde (final concentration) and stored at 4 °C (Hazen et al., 2010; Smith et al., 2015). A map of the groundwater sample collection site and well locations is presented in Fig. 1B.

2.4. Preparation of environmentally-sourced samples

For both sample types (groundwater and seawater), subsamples of the collected water were aliquoted into three 2 mL tubes. To represent the full community, one of the aliquots was stored unmodified at 4 °C, and the second aliquot filtered through a 0.2 μ m pore size Millipore filter to represent the small-particle size fraction of the community. The third aliquot was centrifuged at $5000 \times g$ for 10 min and the supernatant was retained to represent the submicron-particle size fraction of the community. The cellular density of the whole water sample was determined using the AODC method (Francisco et al., 1973), and controls were prepared by filtering 5 mL of seawater through a 0.2 μ m membrane filter.

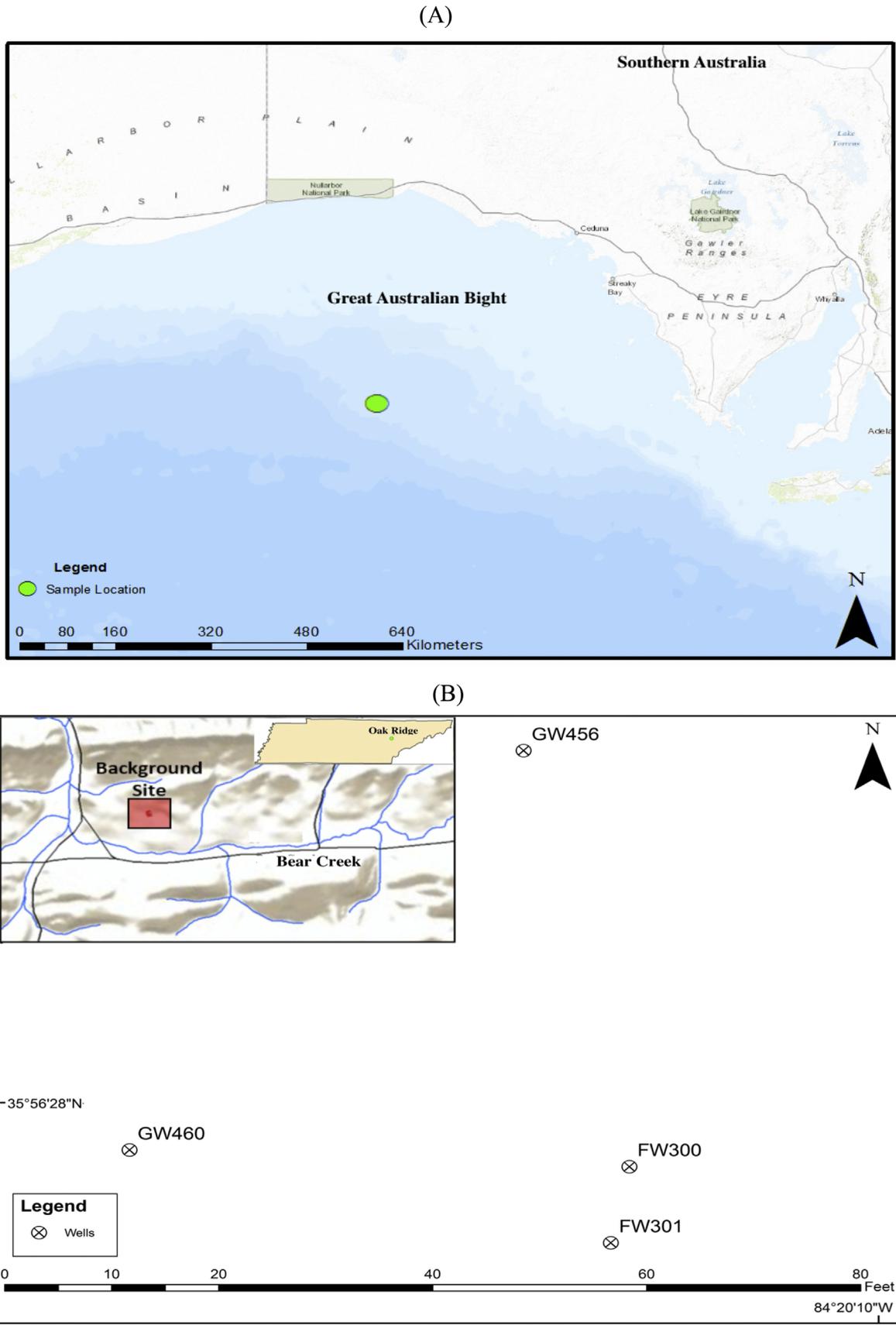


Fig. 1. Field collection sites for environmentally sourced samples. (A) Seawater samples were collected from the Great Australian Bight in 2013 across five sampling locations (green circle). (B) Groundwater samples were collected from groundwater monitoring wells located in the background area of the Oak Ridge Reservation in Oak Ridge, TN.

2.5. Correlation curve and blind study

To establish a correlation curve for quantification of cellular densities in environmentally-sourced samples, preserved groundwater from monitoring well GW-460 (Fig. 1B) was collected and stored at 4 °C. The cellular density of this sample was determined using the AODC method (Francisco et al., 1973). Samples for the correlation curve were prepared by adding 1 mL of groundwater into 9 mL of filtered groundwater and then serially diluted to densities of 10^6 , 10^5 , 10^4 , and 10^3 cells/mL. The diluent media or filtered groundwater was prepared by filtering preserved groundwater through a 0.2 μm membrane filter to remove all cells. Additionally, a 2 mL aliquot of filtered groundwater was collected to serve as a control. Each sample was analyzed and capacitive measurements were recorded. Using the capacitance rate change (dC/dt) and cell densities of each sample, both polynomial and linear fitting curves were established. The equation for linear fitting was calculated as $y = -2 \times 10^4 x - 1.41 \times 10^5$. The equation used polynomial fitting was calculated as $y = 571.07x^2 + 464.59x - 30000$. A mathematical comparison was performed to determine the most appropriate fitting curve for quantifying cell density from the blind study by comparing the linear and polynomial fitting curves using groundwater samples collected from the field site. Using this approach, the linear fit equation was determined to provide the best fit ($R^2=0.91$) for calculating cellular densities.

Using this fitting procedure, the sensor was then validated for estimation of microbial cell abundance through a blind study based on 10 groundwater samples collected from four different monitoring wells at the background area of the ORR field site. For each sample, the capacitance rate change was measured and the cellular density estimated using the linear fit equation. All sensor-based results were then compared to direct counts obtained using the AODC method.

2.6. Microelectrode sensor assembly

Silicon wafer-based capacitive biosensors, consisting of a pair of planar interdigitated microelectrodes, were used for capacitance measurements of microbial cells in cultured and naturally sourced samples. Microelectrodes consisted of 100 nm platinum over 5 nm chromium on oxidized (100 nm SiO_2) with equal width and gap spacing of 2 μm . As the determination of broad spectrum microbial density is desired, no specific probe was coated on the microelectrodes. Electrodes used in this study were fabricated using a standard lift-off process at the Center for Nanophase Material Sciences at Oak Ridge National Laboratory (Oak Ridge, TN) as previously described (Wu et al., 2005; Yuan et al., 2014).

Prior to each measurement, the sensors were sequentially cleaned with acetone, isopropyl alcohol, and de-ionized water to remove possible contamination, and a 2.5 mm diameter \times 0.9 mm depth silicone microchamber (Grace™ Bio-labs JTR24R) was sealed onto the wafer surrounding the electrodes to create a microchamber for sample loading. The bare electrodes were then visually inspected for contamination and functionality was verified with an impedance analyzer to confirm that the average capacitance variance for the bare electrode was less than 0.07%.

2.7. ACEK capacitive measurements

For each sample type (batch culture, seawater, or groundwater) and each community size fraction, triplicate capacitive measurements were recorded, with the previously defined filtered samples serving as controls. All ACEK capacitive measurements were obtained using a 4292A high precision impedance analyzer (Agilent). Data were transferred to a laptop for analysis via a local area network connection using Data Transfer V3.0 software (Agilent).

All measurements were performed by continuously recording the capacitance of the microelectrode sensor using a 100 kHz AC signal at no greater than 10 mV (environmentally-sourced samples) or 500 mV (cultures) for 30 s as these parameters have been shown as optimal for capturing the enrichment effect of biomolecules without inducing electrochemical electrode disturbance (Li et al., 2014; Li et al., 2013; Liu et al., 2011). A brief description of AC signal selection is provided in the Supplemental file. For each sample type, ACEK capacitive measurements were repeated three times, with a new electrode chip employed for each measurement.

2.8. Capacitance analysis

The width of each electrode and the gap between electrodes was determined to be 2 μm and the average capacitance was 68 nF. Microbial abundance was detected by measuring the electric signal representative of the rate of change of biosensor capacitance. As previously described (Cui et al., 2013), sensor impedance was directly measured by an impedance analyzer as $R_s + j/\omega C_s$, where R_s and C_s are the resistance and capacitance of the sensor when it is represented as a serial connection of a resistor and a capacitor. While usually a complex network of resistors and capacitors is needed to represent the impedance spectrum of an electrode biosensor, at the measurement frequency chosen in this work, C_s is predominately made up by the interfacial capacitance C_{int} . Therefore, C_s is used here to assess the change in C_{int} . It is established that C_{int} of an electrode biosensor consists of electric double layer (EDL) and the deposition of macromolecules on the electrode surface. The capacitance of EDL is determined by the ionic strength of background fluid. Since the background fluid remains unchanged during 30s' measurement, the change in C_{int} arises from the macromolecular deposition on the electrode surface. Normalized capacitance change was adopted here and calculated as $\text{Norm}(C_{int}, t) = (C_{int, t} - C_{int, 0}) / C_{int, 0}$, where $C_{int, t}$ and $C_{int, 0}$ are the measured interfacial capacitances at time t and time zero, respectively. This allowed analyte deposition onto the electrode to be represented using the percentage change in the measured capacitance, which was calculated using the slope of normalized capacitance change versus time (%/minute) as found by the least square linear fitting method.

3. Results and discussion

3.1. Biosensor validation using laboratory-sourced batch culture samples

Previous work has demonstrated that variances in microbial cell morphology and physiology can influence biosensor-based AC signals and negatively influence detection capabilities (Maskow et al., 2008; Yang, 2008). To determine the robustness of the ACEK capacitance-based biosensor against these phenotypic differences, it was validated against three known microbial cultures representative of the primary cellular physiologies found in naturally occurring aquatic communities. *B. subtilis*, a ubiquitous bacterium that can found in a number of habitats, including subsurface sediments and aquatic environments (Earl et al., 2008; Kunst et al., 1997) was chosen to represent Gram-positive bacteria. *A. borkumensis*, which is commonly found in coastal and oceanic waters (Golyshin et al., 2003; Schneiker et al., 2006), was chosen to represent Gram-negative bacteria. *M. aeruginosa*, a toxic, unicellular, colony-forming, freshwater cyanobacteria, was chosen due to its unique cellular physiology, which exhibits characteristics of both Gram-positive and Gram-negative microorganisms (Kaneko et al., 2007) and thus cannot be easily classified as one or the other (Hoiczky and Hansel, 2000; Kim et al., 1997).

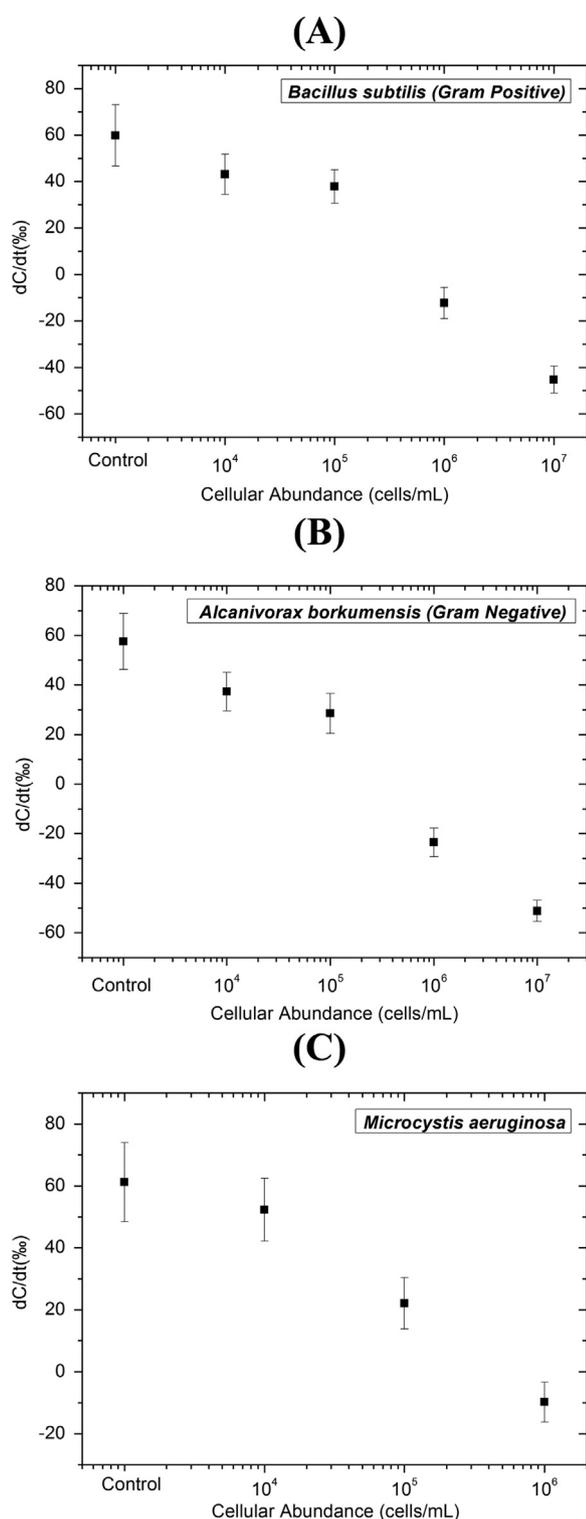


Fig. 2. Capacitance changing rate as function of concentration level of (A) *Bacillus subtilis*, (B) *Alcanivorax borkumensis*, and (C) *Microcystis aeruginosa*.

For biosensor validation, the dC/dt elicited upon exposure to pure cultures of *B. subtilis*, *A. borkumensis*, and *M. aeruginosa* were measured following re-suspension of each microbial culture in PBS. The ACEK capacitance-based biosensor was capable of detecting cells from each culture at concentrations ranging from 10⁴ to 10⁷ cells/mL, independent of their unique physiological characteristics. The observed differences in dC/dt between cultures were not statistically differentiable, indicating that biosensor is

capable of detecting all three representative cultures. For *B. subtilis* and *A. borkumensis*, the capacitance change rate values ranged from 59.8‰ to –51.2‰ and 57.5‰ to –45.3‰, respectively (Fig. 2A–B). Similarly, the capacitance change rate for *M. aeruginosa*, was within one standard deviation of *A. borkumensis* and *B. subtilis*, ranging 61.2‰ to –9.8‰ (Fig. 2C). The observed negative dC/dt values were determined to be due to the difference in permittivity between the bacterial cells and the medium, as negative capacitance change rate values were observed only upon exposure to higher cell concentrations.

These results suggest that the major physiological differences between Gram-positive, Gram-negative, and cyanobacteria do not negatively impact ability of the ACEK capacitance-based biosensor to obtain and report capacitance measurements. Furthermore, the consistency of these measurements across four orders of magnitude in cellular concentration demonstrates that ACEK enhanced sensing technology can function reliably at the scales commonly encountered for naturally occurring microbial communities.

3.2. Validation of biosensor robustness against target growth phase

Physiological and morphological changes that occur during an organism's natural growth can influence biosensor detection and lead to bias reporting of target population concentrations (Mukamolova et al., 1998; Zhu et al., 2010). In particular, variances in cell size, permeability of cell membranes, and intracellular composition can alter a cell's electrical polarizability, thus interfering with the biosensor's ability to accurately separate and detect biomolecules (Zhu et al., 2010). These variances are particularly applicable to environmental samples, or to un-preserved laboratory-derived samples, which contain target population members from across the full growth phase spectrum. Therefore, to validate that capacitive measurements of the ACEK capacitance-based biosensor were not biased by differences in growth phase, the capacitance of cells growing at exponential and stationary growth phase were measured. Exposure of the biosensor to pure cultures of *A. borkumensis* and *B. subtilis* (Fig. 3) did not result in any observed changes to sensor output at either exponential or stationary growth phases. This suggests that the time point at which the sample is measured does not create a bias or interfere with target detection.

3.3. Evaluation of biosensor performance using environmentally-sourced seawater samples

The detection of microorganisms and the quantification of their cell densities by capacitive biosensors are reliant upon the accurate measurement of change in the DEP at the electrode surface (Li et al., 2013, 2014). Due to the sensitivity of the sensor to changes in the DEP force, it is important to identify if factors such as physiological and growth differences will interfere with the AC signal and result in biased measurements (Ahmed et al., 2014; Varshney and Li, 2009). In environmental samples, monitoring of biological inhibitors is often difficult due to the number of physiological and morphological differences observed within a microbial community at a single time-point. Differences such as cell size, cellular structure, and permeability of the cellular membrane have all been shown to affect changes in the DEP force (Ahmed et al., 2014; Matanguihan et al., 1994; Zhu et al., 2010). To determine the ability of the ACEK capacitance-based biosensor to detect cells from naturally occurring microbial communities harboring these complicating factors, the capacitance of bacterial cells from the GAB were measured. The GAB is located in the Western region of Australia's southern continental margin and provides a natural habitat for a number of ubiquitous marine heterotrophic bacteria (Seymour et al., 2012), including *A. borkumensis*. Due to observed

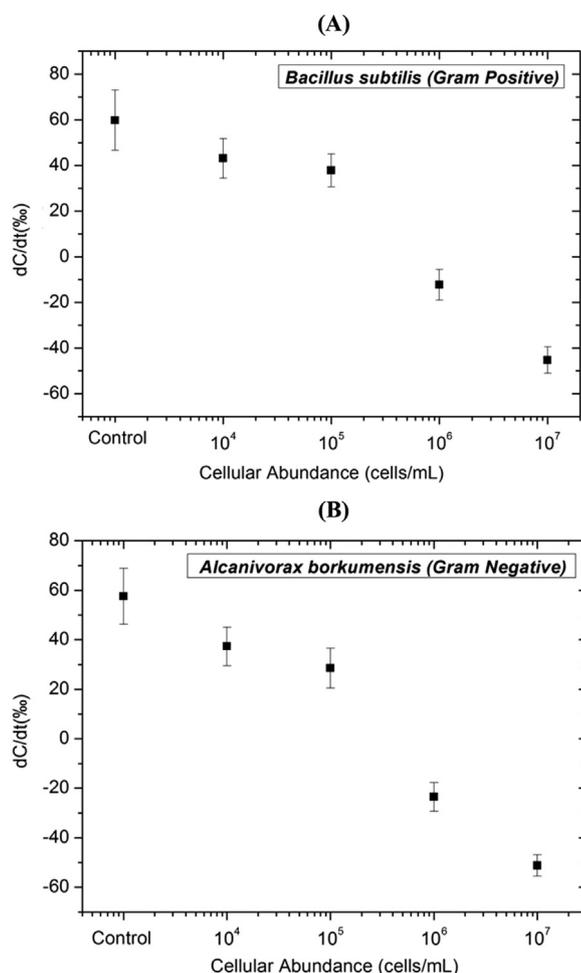


Fig. 3. Capacitance changing rate as function of concentration level of (A) *Alcanivorax borkumensis* and (B) *Bacillus subtilis*.

high salinity near the basin head and the presence of boundary currents, the GAB is an active and highly conductive environment (Godfrey and Ridgway, 1985; Smith et al., 2015) that is ideal for assessing the biosensor's ability to detect naturally occurring microbial cells.

To validate that the ACEK biosensor can measure naturally occurring microbial cells despite physiological differences, the dC/dt of GAB samples size-fractionated into unfiltered and filtered ($< 0.2 \mu\text{m}$) water, and the supernatant collected from centrifuged water were assayed. Using this approach, the whole water sample is representative of all microbial cells and physiologies present in the sample at the time of collection, the filtered size-fraction is representative of ultra-small bioparticles such as viruses, and the supernatant is free of bacterial cells.

For all three size-fractions, the average dC/dt values ranged from -3.78% to -11.96% (standard deviation = 2.66 – 1.87) with the highest value measured in the supernatant sample (Fig. 4). Within the supernatant, bacterial cells and most biomolecules are removed during centrifugation, as are most, if not all, biological inhibitors. Since this sample has the highest level of purity in regards to the absence of microbial cells, it can be considered a control environmental sample. In contrast, the lowest dC/dt value was measured in the whole water sample ($-11.96 \pm 1.87\%$). While the negative dC/dt values observed in the centrifuged and filtered samples suggest that a non-negligible amount of molecular coating may exist on the electrode surface, the negative values measured by the biosensor in the whole water samples are more likely due to differences in permittivity between microbial cells and the

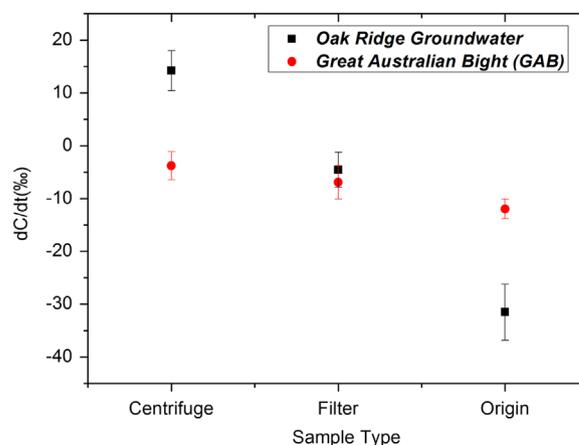


Fig. 4. Capacitance response for whole water, filtered, and centrifuged Great Australian Bight and Oak Ridge Reservation groundwater samples.

ionic concentration of the seawater. This is due to the greater cell density of (10^4 cells/mL) within these samples and their increased likelihood to contain higher amounts of biomolecules and cell products.

The observed variations in dC/dt for the three size fractions were statistically different (ANOVA, p -value = 0.02), indicating that the biosensor is capable of distinguishing between the seawater sample types. This suggests that the high conductivity of the GAB samples does not interfere with target detection. Rather, even under these environmental conditions the sensor is capable of differentiating between a sample containing only nano-sized biological particles and a sample containing all potential biological inhibitors. Combined with its ability to detect cells at low (10^4 cells/mL) concentration levels, these results indicate that capacitance-based sensing can be used as a biosensor in marine environments.

3.4. Biosensor performance using environmentally-sourced groundwater samples

One of the challenges of utilizing capacitance-based sensors for environmental monitoring is the ability of a sensor to detect biological targets across various habitats. While the ACEK biosensor is capable of detecting microbial cells in marine water samples, it is unclear how the sensor will respond to a more dynamic system that is repeatedly exposed to seasonal stress factors and biases, such as groundwater. Therefore, to validate the versatility and applicability of the sensor to groundwater environments, dC/dt values of size-fractionated groundwater samples from monitoring wells located in the background area of the ORR were measured. In this environment, the groundwater flow system is embedded in fractured shale and carbonate rocks, providing pathways for the introduction and transport of potential electrode inhibitors such as chemical contaminants, particulates, dissolved organic matter, dissolved salts, and colloids to the monitoring wells (Moline and Schreiber, 1996; Solomon et al., 1992). This site is also subject to seasonal and temporal events such as rainfall, snowfall, and temperature fluctuations, all of which can potentially impact its hydrological, geochemical, and groundwater microbial community structure (Solomon et al., 1992).

To prepare for dC/dt measurement, the groundwater samples were processed similarly to seawater samples and aliquoted into unfiltered, filtered ($< 0.2 \mu\text{m}$), and centrifugal supernatant sub-samples for analysis. The average dC/dt values for the three size-fractions ranged from 14.22% to -31.50% (standard deviation = 3.8 – 5.3 ; see Fig. 4). Consistent with the previous

results, the highest dC/dt values were recorded in the supernatant from centrifuged samples, where microbial cells are absent. The lowest dC/dt values were measured in whole water samples, which had a microbial cellular abundance of 10^5 cells/mL. This further supports our findings that dC/dt values decrease with increasing numbers of bioparticles, such as microbial cells.

A greater difference in capacitance response was observed between sample types in the groundwater samples relative to their seawater counterparts, with all three groundwater size-fractions displaying statistically significant (ANOVA, p -value=0.00003) dC/dt differences. Similar to the seawater samples, a negative average dC/dt value was detected for these samples. Given that the groundwater in this study is representative of a dynamic, low salinity freshwater system, it is likely that the presence of minerals and ions in both the groundwater and its surrounding subsurface sediment contributed to the conductivity of the medium. Under this hypothesis, the thicker electric double layer within the groundwater samples relative to the seawater samples surrounds the surface deposition, resulting in more pronounced decreases in capacitance within these samples, while the presence of dissolved compounds contributes to the formation of permittivity gradients between the microbial cells and their conductive medium.

Since the capacitance of these samples is directly related to the conductivity and permittivity of the groundwater medium, the presence of dissolved compounds would therefore cause the observed negative average difference in capacitance response. Fortunately, despite the presence of the unknown inhibitors, geochemical variation, and surface deposition that lead to this negative change in dC/dt , the sensor was able to reliably detect cells within the highly dynamic groundwater system.

3.5. Estimation of cellular density using ACEK enhanced sensing technology

Based on the results from the batch culture and size-fractionated environmental experiments, the capacitance-based sensor is capable of differentiating between samples consisting of varying microbial abundances despite differences in cell physiology, growth stage, and media type. Therefore, we theorized that it would be possible to determine cell population densities of *in situ* environmental samples with unknown inhibitory compound profiles by correlating their capacitance values with the observed change in dC/dt . To determine if this was the case, a blind study was conducted using groundwater from the ORR field site.

A single correlation curve was established from groundwater well GW-460, which is located in close proximity to the wells for the blind study (Fig. 1B). This site was chosen because previous characterization by Smith et al. (2015) indicated that cellular abundance at the site remained relatively consistent between 10^4 and 10^5 cells/mL regardless of the well being sampled. Cell abundance from this reference well ranged from 5.5×10^2 to 10^5 cells/mL, with average $\Delta dC/dt$ measurements ranging from -3.57% to -30.80% (1.59–3.88 standard deviation) and decreasing with increased cell abundance (Fig. 5A).

The average dC/dt values for the blind test wells ranged from -11.03% to -28.74% for preserved groundwater samples. Measured cellular densities varied from 3.34×10^4 to 2.08×10^5 cells/mL using the AODC method and 7.97×10^4 – 4.34×10^5 cells/mL using the ACEK capacitive sensor (Table 1). Sensor-derived cell density estimates correlated well with AODC measurements ($R^2=0.91$) for all observed samples with the exception of sample 10 (Fig. 5(B)). It was determined relative difference observed in this sample was due to the sample being either out of the sensor's linear range of 7.97×10^4 – 4.34×10^5 cells/mL, or that there was error associated with actual measurement of the sample. Alternatively, it is

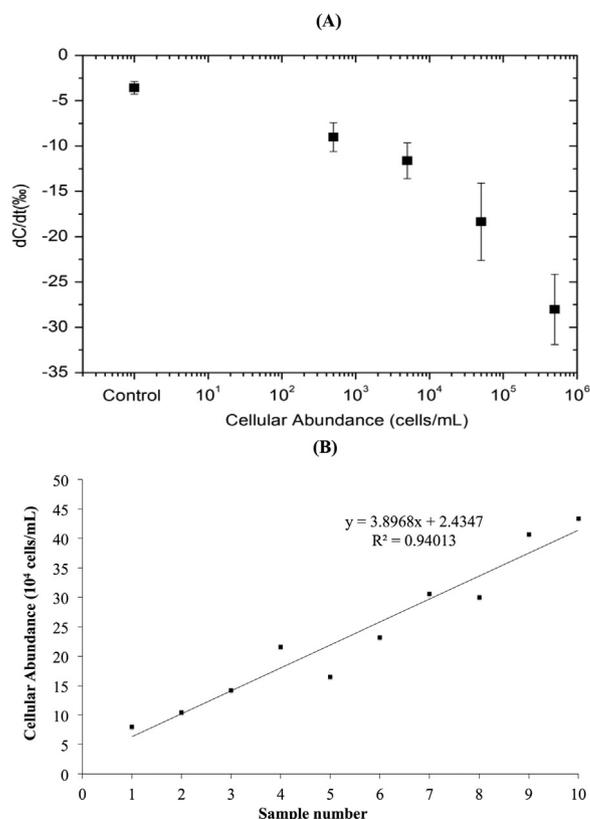


Fig. 5. Reference curve and linear fit for blind study samples. (A) Capacitance response and linear fit from GW-460 sample located at the Oak Ridge Reservation. (B) Correlation of cellular abundance for groundwater samples determined using the linear fit equation. Data points represent individual samples. For each sample, the total number of bacteria was counted in 10 randomly selected fields with an average of 27–142.75 cells (standard deviation=2.46–30.74) per field of view.

possible that this outlying sample may have resulted from the presence of an inhibitor that biased the electrode reading, as it is known that the individual geochemistry of each well can vary depending on its specific depth (Moline and Schreiber, 1996; Solomon et al., 1992). If this were to be the case, however, it would be expected that dilution of the sample prior to measurement with the ACEK capacitive sensor followed by back calculation of the original sample concentration would result in alleviation of the error and, based on the results of this study, the differences in conductivity or ion concentration in groundwater collected from shallow and deep wells does not impact results.

These results validate the use of only one reference curve for this study, however it is important to note that multiple reference curves may still be required when comparing samples from sites with large differences in geochemistry. In addition, to further constrain the limits of detection of the sensor and determine allowable differences in geochemistry prior to multi curve-based analysis, further testing across aquatic habitats will be necessary.

4. Conclusions

The results of this study indicate that the ACEK enhanced capacitance-based sensor is capable of detecting and estimating microorganism population sizes in batch cultures, as well as within environmentally sourced seawater and groundwater systems. Validation tests with cultured microorganisms reveal that the sensor is capable of reliably detecting microbial cells across varying physiological and environmental states. In particular, the detection of microbial cells and the ability to differentiate between

Table 1
Capacitance change rate values, AODC measurements, and cell counts based the linear fit equation for the blind study groundwater samples. S. D.=standard deviation.

Sample number	Well ID	Date sampled	dC/dt (‰) for blind samples					Cell abundance		
			Run 1	Run 2	Run 3	Average	S. D.	AODC (cells/mL)	Linear fit (cells/mL)	
1	GW-456	11/05/13	-15.72	-9.77	-7.60	-11.03	3.43	4.34×10^4	7.97×10^4	
2	GW-456	11/11/13	-16.28	-11.20	-9.45	-12.31	2.90	4.70×10^4	1.05×10^5	
3	GW-456	11/12/13	-16.76	-15.86	-9.77	-14.13	3.10	6.06×10^4	1.42×10^5	
4	FW-301	11/12/13	-22.74	-16.32	-14.43	-17.83	3.56	7.12×10^4	2.16×10^5	
5	FW-300	11/13/13	-21.00	-14.32	-10.64	-15.32	4.29	9.95×10^4	1.65×10^5	
6	GW-460	11/11/13	-23.70	-19.74	-12.57	-18.67	4.61	1.32×10^5	2.32×10^5	
7	FW-301	12/04/13	-26.56	-21.87	-18.68	-22.37	3.24	1.69×10^5	3.06×10^5	
8	FW-300	09/24/13	-27.84	-23.96	-14.35	0.2205	5.67	1.69×10^5	3.00×10^5	
9	FW-300	11/06/13	-31.92	-28.79	-21.40	-37.37	4.411	2.08×10^5	4.07×10^5	
10	GW-456	11/19/13	-34.90	-25.13	-26.19	-28.74	4.38	3.34×10^4	4.34×10^5	

sample types in challenging and dynamic environments, such as the GAB or ORR site, demonstrates that ACEK capacitance-based technology can function reliably in commonly encountered natural systems. This includes systems containing naturally low densities or significant amounts of potential inhibitors such as metals, colloids, or high salinity that are difficult to measure using traditional systems.

In addition to its high-resolution differentiation of varying microbial cell densities under standardized conditions, results from this study indicate that ACEK capacitance-based sensing can be used *in situ* for estimating microbial population densities in environmental samples. However, while a linear reference curve was sufficient for the samples assayed in this work, further testing will be necessary to understand the limits of the technology and to apply this information towards further development.

Overall, ACEK capacitance-based sensing was found to represent an accurate, robust, and rapid-screening tool for detecting and estimating the density of microbial communities in aquatic environments. It requires low (μ l) amounts of sample volume relative to other methods such as AODC, and can be used to monitor changes in microbial community abundance *in situ*. Although the primary scope of our work was *in situ* cellular detection, ACEK sensing can potentially be applied towards characterizing conductivity gradients in aquatic samples. While ACEK capacitance-based sensors are not widely used for environmentally sourced samples, these data suggest that they can provide a simplistic means for assessing groundwater quality and community response to environmental perturbations, and that with additional development they could serve as improved *in situ* capacitive sensing devices for biomonitoring.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2016.05.098>.

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