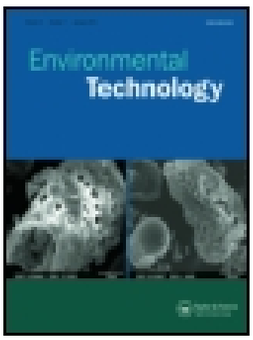

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Creation of a low cost, low light bioluminescence sensor for real time biological nitrate sensing in marine environments

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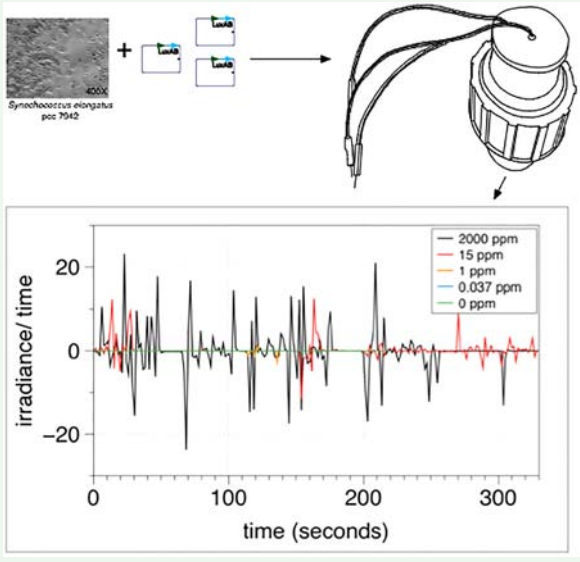
ABSTRACT

The concentration of nitrate (NO^{3-}) in Narragansett Bay has been shown to undergo considerable temporal and spatial variation. However, the dynamics of this flux has never been monitored on a fine-scale (<100 m, < 1 d) or in real-time. Whole-cell bio-reporters are promising candidates for low cost environmental sensing of bioavailable nutrients. Yet difficulties remain in creating sensors for long term deployment in the marine environment. This paper describes the creation and validation of a low-cost sensor using a self-bioluminescent strain of the cyanobacteria *Synechococcus elongatus pcc* 7942 for the direct measurement of bioavailable nitrate. Nitrate bioavailability was measured by monitoring light emission from a luxAB based promotor fusion to glnA using a light to frequency sensor and single board microcontroller. Sensor designs are presented in this manuscript with specific focus on storage, cell viability, and compatibility with the marine environment. Sensors were able to consistently assess nitrate standards as low as 1 ppm (16.3 µM). Using a wavelet denoising approach to reduce white noise and hardware noise, nitrate detection of standards as low as 0.037 ppm (0.65 µM) was achieved. Good sensitivity and low cost make these sensors ideal candidates for continuous monitoring of biological nitrates in estuarine systems.



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Biosensor; nitrate; cyanobacteria; microcontroller; low cost

Introduction

Nitrogen is a fundamental building block of living organisms and plays a critical role in the ecology of the marine system. Nitrate, the most oxidized form of nitrogen, provides the largest inorganic reservoir of this nutrient in the marine environment and is one of the nutrients that exerts the greatest control on eutrophication in these systems.

The temporal and spatial variation of nitrate concentrations in estuarine systems is large [1,2]. Often knowledge of estuarine systems is based on limited measurements from studies focused on a particular

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area or environmental condition. Current methods of tracking these variations are often inadequate when compared to the significant scale of perturbations found in these systems [3]. This hinders the creation of baseline data and the establishment of predictive models. A sensor network collecting real time data in an estuarine system could provide key insights into the fate and transports of biological nutrients. However, current methods of analytical modelling are often too complex and costly for environmental analysis. The size, power requirements and fragility of these systems limit their use to the confines of the traditional laboratory.

Microbial biosensors could provide a unique solution for nutrient analysis in the marine environment. Over the past decade, overall improvements in electronics, software, and components have led to the development of low cost, low energy data loggers, that can record and store data over long-term deployments. Marine microbes such as cyanobacteria are simple to cultivate, thrive under adverse conditions, and are easily adapted to modern recombinant DNA technologies suggesting that they are not only excellent indicators of the productivity of the marine environment, but excellent candidates for inexpensive and specific sensors [4,5]. However, difficulties remain in creating robust whole-cell sensors for real time measurement of bioavailable nutrients over long term deployments. Particular bottle points occur in maintaining the viability of the whole cell bioreporter and in reducing sensor costs while retaining sensor specificity and sensitivity [6–8].

To maintain novel whole cell bioreporter systems throughout the process of sensor storage and deployment a number of strategies have been developed within the literature, the most successful of these has been the creation of robust porous bio-composite materials that mimic natural biofilms [9]. The current gold standard of these types of coatings are waterborne latex coatings made from polydispersed acrylate and vinyl acetate copolymer mixtures with low VOC content [9–12]. While these biological coatings are suitable for use in a laboratory setting, their use within an environmental application is problematic due to their toxic constituents [13,14].

Graphene biomaterials have received a great deal of attention in both biomedical [15] and environmental sensing [16]. Graphene foam especially has been shown to be a unique, biocompatible, non-toxic material that provides microenvironments for cell growth and proliferation [17]. However, pure graphene foams are fragile, prone to breakage, and may not be suitable for long term use in a sensor environment.

Natural latex is a clear alternative coating that could be used on graphene biosensor surfaces. It has high cellular compatibility and low toxicity [18,19]. Thin films of natural latex are also highly transparent, and non-ammoniated latex is naturally hygroscopic [20,21]. In addition, graphene aerogels stabilized with natural rubber latex have shown increases in mechanical stability as well as increased adhesion to water, suggesting that these materials are highly compatible [22].

Creating a sensitive, specific and inexpensive method for bioluminescence monitoring in the marine environment is another key challenge. Microcontrollers such as the Arduino Uno have become controllers for a number of environmental sensors as they have a small form factor, have low power requirements and are low cost [23–25]. The addition of light sensors to microcontrollers such as the Arduino Uno have been used successfully to track naturally occurring bioluminescence in the marine environment in both the scientific literature and in the wider citizen scientist community [26,27]. The sensitivity and specificity of these sensors remains a limitation to their wider use. Sensors of natural bioluminescence have only proven useful for large scale sensing of multiple bacteria or for highly luminescent organisms such as plankton. This project proposes a wavelet denoising approach to improve sensor sensitivity. Wavelet denoising has proven beneficial for the removal of both high frequency white noise and hardware noise from microcontrollers [28,29].

The objective of this study was to combine these novel technological advances to develop and validate a low cost, whole cell biosensor device capable of the sensitive and specific detection of bioavailable nitrogen in the marine environment of Narragansett Bay.

Conceptual design

To design a robust, and economical sensor we created a sensor design where bioluminescent cyanobacteria with a reporter gene for nitrate detection were added to a replaceable cartridge element and then inserted into the main body of the sensor, which contained an inexpensive housing, a light to frequency sensor and a microprocessor for datalogging. Sensors, can then exposed to environmental conditions allowing the bacteria inside the capsule to come into contact with nitrate in environmental samples triggering luminescence of inserted lux gene. Light intensity (or irradiance) is then measured by the light-to-frequency sensor and is outputted as a square wave of the frequency that is linearly proportional to the light intensity. In this study we attempted to improve device detection limits by removing background noise using wavelet analysis. The results

of this study show that irradiance signals retrieved from this inexpensive device can be well correlated with nitrate standard.

Methods

Bio-reporter development

Synechococcus elongatus PCC *7942* was acquired from the American Type Culture Collection (*ATCC*, Manassas, VA). Bacterial cultures were grown in 70 mL of BG-11 media in a 2L photobioreator system (Grofizz LLS, Austin, Texas) at room temperature (22°C) and were bubbled with air. Cultures were exposed to a diurnal lighting cycle of 12 h and grown to a pellet wet weight of 0.5 mg. Genomic DNA was isolated according to the manufacturers protocol using a ZR Fungal/Bacterial DNA miniprep kit (Zymo Research, Irvine, California). Initial lysis steps used a Beadbug homogenizer (Benchmark Scientific, Edison, NJ) at 4000 rpm for a series of three 20s pulses.

Reporter plasmids for biological nitrate were constructed according to Gillor et al. [30] Briefly, the highly transcribed glnA promotor region was amplified from *Synechococcus elongatus pcc 7942 (ATCC*, Manassas, VA) using the following primers (*Getal fwd 1 (5′- GAT TAA GCG GCC GCT CCC GAG TG-3′) and GetalRev (5′- CAT TAA GGA TCC AGG CCT GAG CGA C-3′)*). The resulting PCR project was gel purified and cloned into pAM1414 plasmid using restriction enzyme based cloning and ligation. Gel purified DNA was digested with NotI-HF and BamHI-HF and ligated into the multiple cloning site of pAM1414 using T4 DNA ligase. Fragment insertion into pAM1414 was confirmed with colony PCR and diagnostic digests. Digested fragments were analysed using an Agilent Bioanalyzer (Agilent, Santa Clara, California) for length. (Supplementary Figure S1). Constructs were transformed into *Synechococcus elongatus pcc 7942* using a standard procedure for natural transformation. Transformants were selected from BG-11 plates with spectinomycin (Teknova, Hollister, California)

Design of bioreporter sensor

Sensor design

A prototype sensor was designed to quantify bio-reporter luminescence in marine systems and can be seen in Figure 1.

Sensors were designed with low cost, easily sourced materials, and were optimized for easy construction and low power requirements. A PVC socket union (Ace Hardware, Warwick, RI) was chosen as the outer housing of the sensor cartridge. This PVC socket union was modified with two silicon O-rings (Dano Inc., Irving, TX) which were sealed to the PVC using marine safe silicone sealant. A 0.25 inch × 0.125 inch silicone washer was added to bushing of the PVC socket union to better accommodate bio-reporter capsules. A 0.1875 inch × 0.125 inch washer was sealed on the top outlet of the socket union to both restrict light within the chamber and protect electronics. A light to frequency converter (TSL-237LS, Mouser Electronics, Mansfield,

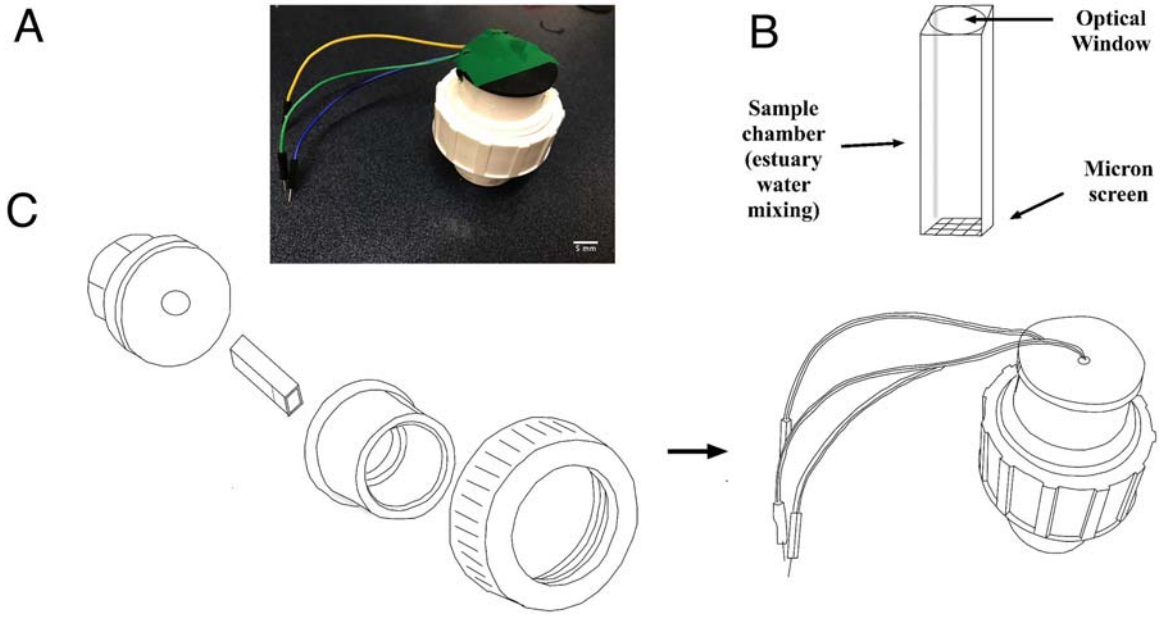

Figure 1. (A) Image of Prototype nitrate bio-reporter sensor (B) Diagram of nitrate bio-reporter sensor cartridges (C) Diagram of bioreporter sensor cartridge with included depiction of sensor housing and cartridge assembly.

Texas) was added to the upper sensor chamber and affixed with the sensor diode perpendicular to the sensor capsule. Sensor wires were threaded through the 0.25 mm O ring and secured to the inside. TSL-237LS sensors were attached to a 5 V Arduino pro mini (Keyestudio, Shenzhen, China) with an associated RTC DS3231 real time clock and a 5 V micro SD board. All capsules were made light-tight by the addition of a layer of marine safe silicone seal.

Bioreporter capsule and encapsulation techniques

Inner bio-reporter cartridges were created from Costar SpinX spin columns (Corning, New York). Graphene foam (Graphene supermarket, New York) was cut into 7 mm pieces and inserted into bottom of capsule. Cell culture or encapsulated cell materials were added on top of graphene in circular pattern with 200 µL pipet. All prototype sensors also had an optical window created from a transparent plastic film (Microseal 'B' PCR Plate Sealing Film, Bio-Rad, Hercules, California). To create cell- latex biocomposite material 200 µL of a 1–3 d old cyanobacterial culture was pelleted at 2500 × g. 20 uL of natural rubber centrifuged latex was then added to the wet cell pellet and agitated with gentle mixing.

Programing design

Frequency oscillation from the TSL-237LF sensor was measured by the ATmega328P microcontroller using a modified version of Gammon's Arduino timer and counter code that uses the input capture unit to time an interval [31]. The main code of the sensor recorded time from the DS3231 real time clock, count, frequency, irradiance at the sensor face and the first order difference of the irradiance at the sensor face. Irradiance at the sensor face was calculated from frequency at the output pin using the following equation from the data-sheet [32] where E_e is the incident irradiance in µW/cm^2, f_o is the output frequency, f_d is the output frequency for E_e=0 (dark condition) and R_e is the device responsivity for a given wavelength of light given in kHz/(µW/cm^2).

$$E_e = (f_o - f_d)/R_e$$

Both f_d and R_e were specified according to the data sheet [32] and were set at 0.1 Hz and 2.3 kHz/(µW/cm2) respectively. The first order difference of the irradiance at the sensor face was also calculated and recorded by the Arduino. All data streams were then processed using a maximal overlap discrete wavelet transform (MODWT). Thresholding for wavelet denoising was accomplished using the universal threshold prescribed by Donoho and Johnstone [33] and hard thresholding. The selection of the wavelet base for this analysis was a daubechies' extremal phase wavelet of phase 2, or a haar wavelet due to its improved utility in estimation of transient signals [34]. All wavelets in this analysis were analysed using the wmtsa library in R. [35].

Sensor evaluation

Cell culture and evaluation of viability

Viable cell counts were performed using a neutral red staining procedure and a 4-chip disposable hemocytometer (Bulldog Bio, Portsmouth, NH). Briefly, cells were removed from culture by centrifugation at 2500 × g at 1, 3 and 5 days and re-suspended in PBS. Cells were then stained with a solution of 0.33% neutral red for a period of 5 min. Cells were imaged with an inverted microscope and the percentage of viable cells was determined. Viable cell counts were also performed after cell encapsulation and entrainment. Cells were removed from entrainment materials after 0, 24, and 48 h by centrifugation at 2500 × g with sterile PBS, stained with a 0.33% neutral red solution, and were finally imaged with an inverted microscope.

Nitrate sensor evaluation

Collection of estuarine water samples

Surface water was assayed from two collection sites along the Narraganset bay Estuary in March 2018 and August of 2019. Surface water samples were taken in polypropylene bottles and samples were filtered with a 0.22 micron syringe filter before analysis. Temperature was recorded at time of collection and at time of sampling.

Spectrophotometric determination of nitrate

Nitrate concentration of standards and filtered samples was determined by UV absorption at 220 nm using a UV-Vis spectrometer. UV absorption at 275 nm was also determined to correct for organic matter absorption. A calibration curve was prepared in the range of 0–11 mg/L by diluting a 150 mg/L nitrate standard with millipure water.

Quantitative assessment of sensor

A quantitative assessment of the nitrate bio-reporter *Synechococcus elongatus* PCC 7942 and sensor cartridge was performed in a range of nitrate standards (0–150 mg/L) and in an estuarine sample from Narraganset bay. Testing procedures consisted of the following steps. At the beginning of each test a solution of 50 mM decanal dissolved in ethanol/water (50% v/v) was added to the top left of the optical window of the sensor cartridge. Sensor cartridges

were then sealed within the sampling chamber. Tests were initialized from a serial monitor connected to the Arduino and ran for a period of 30 min. All samples were done in triplicate ($N = 3$).

Results

Cell viability assessment of encapsulation protocol

A neutral red viability analysis of samples after a period of 24 h at room temperature can be seen in Figure 2. Gains in viability were seen in samples with graphene nanomaterials and latex encapsulation. Samples patterned onto graphene foam alone retained about 42% of their original viability. With the addition of latex, 68% of cells remained viable.

Nitrate

UV-Vis spectrophotometric analysis of nitrate standards produced a nitrate standard curve with the following equation, $y = 0.012177 + 0.0016164x$ ($R^2 = 0.99$) (Figure 3). Figure 3 also shows a spectrophotometric analysis of seawater samples (sterile filtered) with an absorbance of 0.0205 ± 0.0046 for the April 2018 sample and an absorbance of 0.012 ± 0.0058 for the August 2019 sample.

Sensor data

Results of sensor data collection from a set of Potassium nitrate (KNO_3^{2-}) standards can be seen in Figure 4(a). To better visualize changes in light intensity across sensor trials the first order derivative of the raw sensor data was taken. Standard deviations of raw sensor data were assessed for both blank samples and nitrate standards (Supplementary Figure S2). Results showed that standard deviations of sensor data increased with increasing concentrations NO_2^{3-}. For standards with NO_2^{3-} that were greater or equal to 1 ppm, the standard deviation of sensor data was substantially greater than that of the blank.

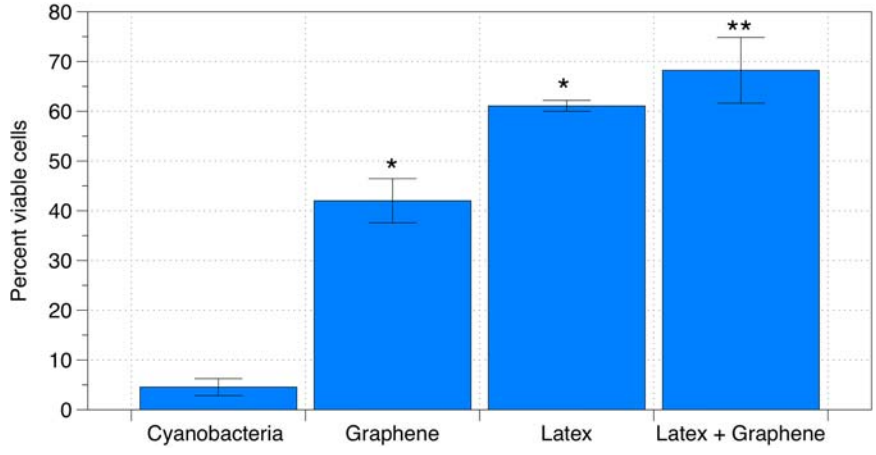

Figure 2. 0.33% neutral red viability analysis of encapsulated *Synechococcus elongatus* pcc 7942 bioreporter. Bioreporter was encapsulated and then air dried at room temperature (22°C) for a period of 24 h. Data = mean ± SEM; $N = 3$; *$p < 0.05$, **$p < 0.01$.

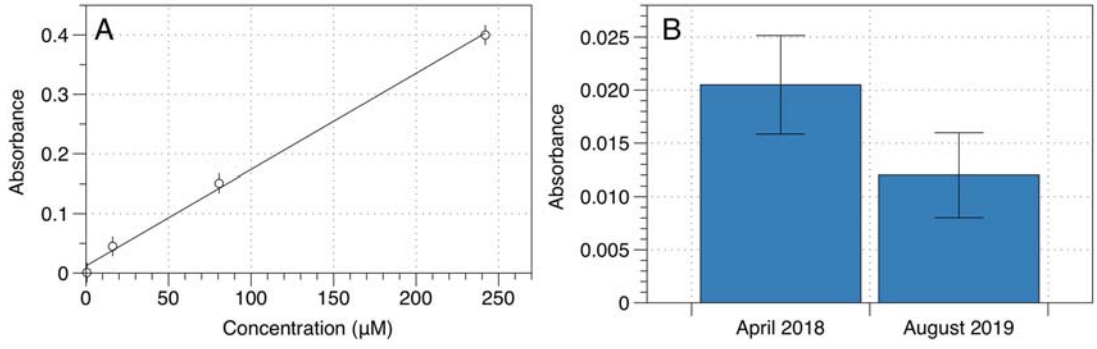

Figure 3. Spectrophotometric analysis of nitrate standards and estuarine samples. (A) Calibration curve of concentration vs. absorbance in potassium nitrate standard solutions. (B) UV-Vis spectrophotometric analysis of estuarine water samples from Narrragansett Bay.

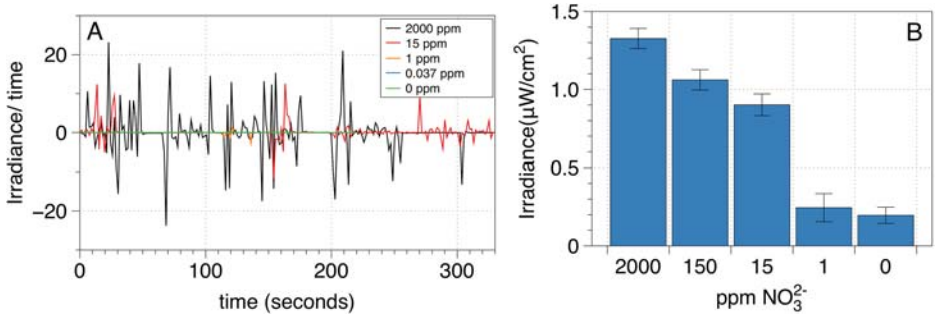

Figure 4. Irradiance vs. nitrate concentration in ppM of a series of potassium nitrate standards evaluated in sensor. (A) Output of sensor capsule in difference in irradiance over time (B) Maximum irradiance at sensor face in μW/cm^2 versus parts per million nitrate of a potassium nitrate standard.

Figure 4(b) shows a graph of the maximum irradiance at the sensor face for each nitrate standard calculated from the raw data. To better evaluate irradiance data with respect to the analytical noise of the instrument, the limit of the blank and limit of detection were calculated for the sensor using the raw data. Replicates of cyanobacterial samples without decane were evaluated within the sample chamber to yield the limit of the Blank (LOB) at a value of 0.024 ± 0.018 μW/cm^2·s. Nitrate peaks above the level of the analytical noise of the instrument (limit of detection) were seen in nitrate standards as low as 1 ppM (or approximately 16.13 μM).

Finally, a wavelet denoising of sensor signal with a MODWT methodology was used to improve the analytical detection of the sensor. This methodology eliminated high frequency white and grey noise from sensor output reducing the limit of the blank for sensor 1 and giving a final limit of detection for low concentration nitrate standards at 0.037 ppm (0.65 μM) of 0.00112± 0.0003 μW/cm^2·s. As seen in Figure 5, Nitrate standards tested at 0.037 ppm (0.65 μM) had detectable peaks at the limit of detection.

Analysis of inter-sensor variation among 3 TSL-237LS light to frequency sensors showed that sensor deviation between 3 sensors to be less than 0.02 μW/cm^2. The average percentage coefficient of variation for the three sensors was also calculated and was found to be 8.94%.

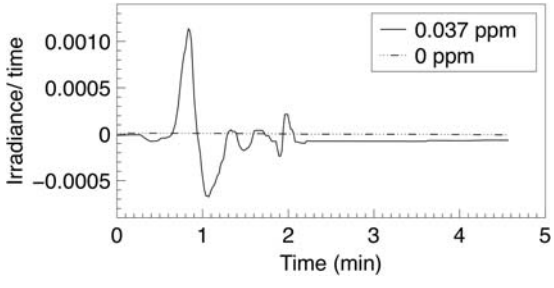

Figure 5. Irradiance vs. nitrate concentration in ppM of a series of potassium nitrate standards evaluated in sensor. Output of sensor capsule in difference in irradiance over time.

Discussion

This study designed and validated a novel optical sensor for nitrate detection in the marine environment. This design is low cost, low complexity and requires easily obtainable parts. Biosensors provide a sensitive means of biological analysis within the laboratory environment. However, remote applications of these sensors remain limited due to factors like cost, longevity, and bacterial viability.

Results of this investigation suggest that the optical biosensor is highly effective at sensing bacterial luminescence in response to nitrate levels. Evaluation of the standard deviation of sensor data show that standard deviations for NO_2^{3-} standards increase with concentration and are substantially greater than that of the blank, which suggests that these sensors are measuring the changes in irradiance at the sensor face due to light produced from the cyanobacterial biosensor.

Tests of three TSL-237LS light to frequency sensors showed inter-sensor variation among these sensors was low for this sensor. The average CV of these sensors was shown to be <10%. Low CV has been associated with high reproducibility and CV of less than 10% have been shown in previous studies to be well suited for use in low cost applications [36,37].

Most importantly, validation of the bioreporter sensor showed this sensor not only matched the sensitivities of other cyanobacterial bioreporters within the literature [30,38,39], but by using a wavelet denoising methodology, this sensor was able to differentiate signal at nitrate levels found within the estuarine environment of Narraganset Bay [40].

Results of this study also showed that graphene nanomaterials present a novel addition to current biocomposite dehydration methodologies. Latex-graphene

Table 1. Literature review of current nitrate whole cell bioreporters [30,38,39,41].

References	Strain	Gene	Reporter	Sensitivity
Mbeunkui et al. [39]	Synechococcus sp. PCC 6803	PnbIA	LuxAB	4–100 µM
Gillor et al. [30]	Synechococcus sp. PCC 6803	glnA	LuxAB	1 mM to 1µM
Ivanikova et al. [38]	Synechococcus sp. PCC 6803	nirA	LuxAB	1µM^{++}
Prest et al. [41]	E coli PK27	narG	LuxCDABE	0.3 ppm (4.84 µM)
Jensen et al. [42]	Pseudomonas fluorescens DF57	Tn5	LuxAB	10–90 µM

Note: $^{++}$Sensor measurement taken in BG-11 media with nitrate.

sensor materials retained 68% viability over a period of 24 h drying. At much shorter times, viability remained higher, about 80-90% after only 4 h. Our results showed similar viability to synthetic biofilms within the literature finding 80-90% viability after 4 h of drying as compared to 95% viability after 1 h in synthetic biofilms [9]. (Supplementary Figure S3) Additionally, the materials used within this novel sensor are non-toxic unlike VOCs and safe for aquatic life (Table 1).

Additional opportunities exist to integrate and optimize bioreporter sensors for future remote systems applications. Not only should long term studies of bioreporter expression in marine environments be assessed but particular attention should be paid to the interaction of other nutrients and chemicals upon the functioning these sensors. Additionally, the calibration of biosensor capsules within the marine environment needs to be investigated further to better anticipate the challenges of long, term remote sensing.

Conclusion

This study showed that an inexpensive cartridge based, bioreporter sensor could both maintain cell viability and perform sensitive detection of biological nitrate in marine samples. This suggests that inexpensive microcontroller based, bioreporter sensors may be a useful and accurate means of measuring biological nitrate in the marine environment and should be considered for sensing applications.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Cloern JE, Jassby AD, Schraga TS, et al. Ecosystem variability along the estuarine salinity gradient: examples from long-term study of San Francisco Bay. Limnol Oceanogr. 2017;62:S272–S291.
[2] Gruber N, Sarmiento JL. Large-scale biogeochemical-physical interactions in elemental cycles. The sea. 2002;12:337–399.
[3] Wild-Allen K, Rayner M. Continuous nutrient observations capture fine-scale estuarine variability simulated by a 3D biogeochemical model. Mar Chem. 2014;167:135–149.
[4] Rogers K. Recent advances in biosensor techniques for environmental monitoring. Anal Chim Acta. 2006;568:222–231.
[5] Webster DP, TerAvest MA, Doud DF, et al. An arsenic-specific biosensor with genetically engineered shewanella oneidensis in a bioelectrochemical system. Biosens Bioelectron. 2014;62:320–324.
[6] Bilal M, Iqbal HM. Microbial-derived biosensors for monitoring environmental contaminants: recent advances and future outlook. Process Saf Environ Prot. 2019;47:777–780.
[7] Bjerketorp J, Håkansson S, Belkin S, et al. Advances in preservation methods: keeping biosensor microorganisms alive and active. Curr Opin Biotechnol. 2006;17:43–49.
[8] Lei Y, Chen W, Mulchandani A. Microbial biosensors. Anal Chim Acta. 2006;568:200–210.
[9] Swope KL, Flickinger MC. The use of confocal scanning laser microscopy and other tools to characterize escherichia coli in a high-cell-density synthetic biofilm. Biotechnol Bioeng. 1996;52:340–356.
[10] Buitelaar R, Bucke C, Tramper J, et al. Immobilized cells: basics and applications. Amsterdam: Elsevier; 1996.
[11] Leonard A, Dandoy P, Danloy E, et al. Whole-cell based hybrid materials for Green energy production, environmental remediation and smart cell-therapy. Chem Soc Rev. 2011;40:860–885.
[12] Lyngberg O, Ng C, Thiagarajan V, et al. Engineering the microstructure and permeability of thin multilayer latex biocatalytic coatings containing E. coli. Biotechnol Prog. 2001;17:1169–1179.
[13] Kotzias D, Sparta C. VOCs and water pollution, in: Chemistry and analysis of volatile organic compounds in the environment. Springer. 1993: 175–201.
[14] Van Faassen A, Borm P. Composition and health hazards of water-based construction paints: results from a survey in the Netherlands. Environ Health Perspect. 1991;92:147–154.
[15] Yang Y, Asiri AM, Tang Z, et al. Graphene based materials for biomedical applications. Mater Today. 2013;16:365–373.
[16] Perreault F, De Faria AF, Elimelech M. Environmental applications of graphene-based nanomaterials. Chem Soc Rev. 2015;44:5861–5896.

[17] Li N, Zhang Q, Gao S, et al. Three-dimensional graphene foam as a biocompatible and conductive scaffold for neural stem cells. Sci Rep. 2013;3:1604.
[18] Balabanian CA, Coutinho-Netto J, Lamano-Carvalho TL, et al. Biocompatibility of natural latex implanted into dental alveolus of rats. J Oral Sci. 2006;48:201–205.
[19] de Barros NR, dos Santos RS, Miranda MCR, et al. Natural latex-glycerol dressing to reduce nipple pain and healing the skin in breastfeeding women. Skin Res Technol. 2019;25:461–468.
[20] Boggs C, Blake J. The absorption of water by rubber. Industrial & Engineering Chemistry. 1926;18:224–232.
[21] Ernst B, Johannes J. (1938). Process for concentrating rubber latex and similar vegetable juices.
[22] Zhang X, Yang G, Zong L, et al. Tough, ultralight, and water-adhesive graphene/natural rubber latex hybrid aerogel with sandwichlike cell wall and biomimetic rose-petal-like surface. ACS Appl Mater Interfaces. 2019;12:1378–1386.
[23] Beddows PA, Mallon EK. Cave pearl data logger: A flexible arduino-based logging platform for long-term monitoring in harsh environments. Sensors. 2018;18:530.
[24] Hund SV, Johnson MS, Keddie T. Developing a hydrologic monitoring network in data-scarce regions using open-source Arduino dataloggers. Agricultural & Environmental Letters. 2016;1:160011.
[25] Wickert AD, Sandell CT, Schulz B, et al. Open-source arduino-derived data loggers designed for field research. Hydrol. Earth Syst. Sci. Discuss 2018, in review. doi:10.5194/hess-2018-591.
[26] Le Tortorec AH, Hakanen P, Kremp A, et al. Stimulated bioluminescence as an early indicator of bloom development of the toxic dinoflagellate alexandrium ostenfeldii. J Plankton Res. 2014;36:412–423.
[27] Patrik. 2017. Highly Sensitive Arduino Light Sensor. Bioluminescence community project. Available from: https://www.instructables.com/Highly-sensitive-Arduino-light-sensor/
[28] Li T, Sunami Y, Zhang S. Perceptual surgical knife with wavelet denoising. Micromachines (Basel). 2018;9:79.
[29] Venkatnarayan RH, Shahzad M. Gesture recognition using ambient light. Proceedings of the ACM on Interactive, Mobile, Wearable and Ubiquitous Technologies. 2018;2:1–28.
[30] Gillor O, Harush A, Hadas O, et al. A Synechococcus PglnA:: luxAB fusion for estimation of nitrogen bioavailability to freshwater cyanobacteria. Appl. Environ. Microbiol. 2003;69:1465–1474.
[31] Gammon N. (2013). Frequency timer using input capture unit.
[32] TAOS. (2008). TSL237 high sensitivity light to frequency converter (Datasheet No. TAOS052J).
[33] Donoho DL. De-noising by soft-thresholding. IEEE Trans Inf Theory. 1995;41:613–627.
[34] Ma X, Zhou C, Kemp I. Automated wavelet selection and thresholding for PD detection. IEEE Electr Insul Mag. 2002;18:37–45.
[35] Percival DB, Walden AT. Wavelet methods for time series analysis. New York: Cambridge university press; 2000.
[36] Sousan S, Koehler K, Hallett L, et al. Evaluation of consumer monitors to measure particulate matter. J Aerosol Sci. 2017;107:123–133.
[37] Sousan S, Koehler K, Hallett L, et al. Evaluation of the alphasense optical particle counter (OPC-N2) and the grimm portable aerosol spectrometer (PAS-1.108). Aerosol Sci Technol. 2016;50:1352–1365.
[38] Ivanikova NV, McKay RML, Bullerjahn GS. Construction and characterization of a cyanobacterial bioreporter capable of assessing nitrate assimilatory capacity in freshwaters. Limnology and Oceanography: Methods. 2005;3:86–93.
[39] Mbeunkui F, Richaud C, Etienne A-L, et al. Bioavailable nitrate detection in water by an immobilized luminescent cyanobacterial reporter strain. Appl Microbiol Biotechnol. 2002;60:306–312.
[40] Pilson ME. Annual cycles of nutrients and chlorophyll in Narragansett Bay, Rhode island. J Mar Res. 1985;43:849–873.
[41] Prest AG, Winson MK, Hammond JR, et al. The construction and application of a lux-based nitrate biosensor. Lett Appl Microbiol. 1997 May;24(5):355–360. doi:10.1046/j.1472-765x.1997.00064.x. PMID: 9172442.
[42] Jensen LE, Kragelund L, Nybroe O. Expression of a nitrogen regulated lux gene fusion in Pseudomonas fluorescens DF57 studied in pure culture and in soil. FEMS Microbiol Ecol. 1998;25:23–32.