

Nano-Biotechnology for early detection of harmful algal blooms in coastal waters

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Abstract

Harmful algal blooms (HABs) represent a significant and growing threat to marine ecosystems, human health, and coastal economies worldwide. Traditional monitoring and detection methods for HABs, such as microscopy and chromatography, are often timeconsuming, labor-intensive, and incapable of providing real-time data needed for early intervention. Nano-biotechnology offers a transformative approach to HAB detection by integrating nanomaterials with biological recognition elements to create highly sensitive, specific, and rapid biosensing systems. This paper explores recent advancements in nanobiotechnological strategies for early HAB detection, including the use of quantum dots, gold nanoparticles, and graphene-based nanocomposites functionalized with aptamers or antibodies targeting key algal toxins like saxitoxin and domoic acid. Literature indicates that such nano-biosensors can achieve low detection limits, high specificity, and potential for miniaturization and field deployment. However, challenges related to sensor stability in marine environments, biofouling, and scalability remain. We identify key limitations in current detection methods and propose a novel biosensing platform integrating cadmium-free quantum dots with aptamer-based recognition for fluorescence detection, deployed via autonomous monitoring systems. The proposed methodology aims to enable real-time, on-site detection of HABs with improved sensitivity and specificity,

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contributing to more effective monitoring, early warning systems, and mitigation strategies to protect coastal resources and communities.

Keywords: Nano-biotechnology, Harmful algal blooms, Biosensors, Early detection, Algal toxins, Quantum dots, and Environmental monitoring

Introduction

Harmful Algal Blooms (HABs) becoming more well-known for the damage they can inflict on water ecosystems, people, and the economy of coastal areas. Rapidly multiplying algae, or phytoplankton, can form algal blooms under favorable conditions (Qian et al., 2024). They can also generate harmful toxins that poison seafood, deteriorate water quality, and result in massive fish kills. Climate change, anthropogenic activities, and nutrient enrichment have significantly increased the occurrence and harm posed by HABs (Pabbi and Mittal, 2017; Shokhimardonov et al., 2024) Standard methods for identifying HABs include using a microscope, pigment, and chromatography techniques. These methods produce results but take an unusually long time to perform, which does not help prepare and respond to alerts (Kulkarni and Jain, 2023). Because of this, we deem them inefficient and obsolete. Early detection methods would allow us to manage and respond to **HABs** appropriately. Constructing methods that allow for sensitive, accurate, and easily transported monitoring techniques is necessary (Samantaray et al., 2018; Banerjee et al., 2021).

Nanobiotechnology offers novel solutions to the previously described challenges. Combining nanomaterials with biological recognition elements allows for greater sensitivity, specificity, and miniaturization in nanobiosensors. An electrochemical algal biosensor using silica-coated ZnO quantum dots demonstrated selective acephate detection, proving that nanomaterials can enhance the electrochemical signals in biosensing (Rani Saritha and Gunasundari, 2024). Also, functionalized CdTe quantum dots were used to fabricate fluorescence nanosensors to detect heavy metal ions in water, which shows the application of quantum dotbased biosensors for environmental monitoring (Chowdhury, 2021; Maguire et al., 2018).

Detection capabilities are further enhanced by development in microfluidic analytical systems. A new microfluidic sensor was developed that allows the simultaneous detection of three algal toxins, which proves high sensitivity and the ability to be used for multiplexed analysis (Malhotra and Mahadik, 2024). Also, a range of reviews has focused on electrochemical biosensors for algal toxins, where authors point out the use of nanomaterials for faster electron transfer and lowering the detection limit for remote measurements (Zhang *et al.*, 2018).

Biological components such as nanobodies have further increased the specificity of detection provided by biosensors. One study created biosensors based on nanobodies that selectively and stably detected harmful microalgae over long periods, which is a more suitable shim alternative to conventional antibody-based methods (Murugan et al., 2024). (Thomas and Iyer, 2024) is an example where. beyond designing artificial biosensors. intelligence, alongside deep learning, has been used to augment biosensor data. Predictive models based on physical, chemical, and biological parameters have been trained to forecast cyanobacterial blooms, which supplement these warning systems (Salem and Stolfo, 2010). In addition, automated monitoring of algal species in water samples is possible with computer vision-based image processing employing deep learning algorithms (Marrone et al., 2023).

With all this in place, numerous issues still need to be solved before nanobiosensors can be practically employed to detect HABs. The primary hurdles include sensor fouling, marine environment stability, technology scaling up, and cost-effectiveness. In addition, integrating these biosensors into the autonomous platforms designed for realtime monitoring, including developing protocols for automated calibration, also needs to be addressed (Salem and Stolfo, 2010; Mehta and Dutta., 2024).

This paper aims to analyze new research the application on of nanobiotechnology for the early detection of harmful algal blooms, assess existing gaps, and outline a treatment plan for designing complementary a nano biosensor system. (Tao et al., 2024) Progresses in nanomaterials, biosensor design, and data processing enable the development of sensitive, specific, and real-time detection systems that can alleviate the ecological and economic consequences of HABs (Hui et al., 2019).

Methodology

A. Sensor Design and Fabrication

order to monitor freshwater In ecosystems susceptible to harmful algal blooms (HABs), a highly sensitive biosensor capable of detecting microcystin-LR (MC-LR)—a potent cyanobacterial toxin—was designed. The biosensor is based on a graphene fieldeffect transistor (GFET) configuration. The exceptional electrical conductivity, increased surface area, and impressive biocompatibility of graphene makes it suitable for biosensors. It is crucial for MC-LR detection owing to its ion exchange and aptamer functionalization transducing channel. The procedure for fabrication begins with the substrate preparation step. The silicon wafers together with the SIO2 300 nm thick coating underwent an organic and inorganic residue cleansing in the RCA standard cleaning step. This step is critical for device efficiency and the subsequent adhesion of graphene. After substrate preparation, monolayer graphene was synthesized by chemical vapor deposition (CVD) followed by transfer onto a silicon dioxide substrate via a wet transfer method using PMMA. This method mitigates the formation of post transfer defects while preserving the structural integrity of the graphene.

A photolithographic procedure was conducted to develop the pattern for the source and drain electrodes. The electrodes were structured using liftoff after a Cryl-Cr 5 nm/Au 50 nm deposition which was done using e-beam evaporation. Cr works well as an adhesion layer, and Au has one of the best electrical conductivity values of any metal. The designed geometry of the

electrodes maximally optimized the electric field distribution for the operation of the transistor. Aptamers for MC-LR were added to the graphene surface for the selective detection of MC-LR. Aptamers are short, single-stranded nucleic acids that bind tightly and specifically to their targets. Functionalization was achieved by applying PBASE to the graphene surface as it undergoes noncovalent π - π stacking with the graphene surface. The Nhydroxysuccinimide ester moiety of PBASE binds covalently to amineterminated aptamers. Thus, MC-LRspecific aptamers are fixed onto the graphene channel. Α **GFET** was incorporating assembled. the functionalized aptamers as its primary sensing element.

B. Sample Collection and Preparation

For the study, water samples were collected from coastal locations where known HABs frequently occur. These sites typically harbor significant cyanobacteria blooms, which are known to produce microcystins. Special care was taken during sampling to avoid any that could compromise disruption potential biological and toxin constituents.

In order to eliminate suspended particulate matter and debris, water samples were immediately filtered with 0.45 µm pore-size membranes. Algal cell concentration was facilitated by centrifuging the filtered water for 10 minutes 10,000 at rpm. The cyanobacterial cells were concentrated into a pellet which was then resuspended in a phosphate-buffered saline (PBS) solution in order to model physiological conditions and stabilize the aptamers for the subsequent detection steps. With these preparations, the biosensor could be tested under an environment simulating actual aquatic ecosystems.

C. Sensor Calibration and Testing

Sensitivity and specificity of the GFET biosensor were evaluated by calibrating it with standard solutions of MC-LR in PBS. A range of 0.1 to 10 µg/L concentration was selected because it corresponds to the toxic levels the World Health Organization (WHO) Guidelines for drinking water quality sets. The calibration steps included increasing the MC-LR concentration and recording the change of the GFET drain current (ID). It is anticipated that the current response will increase with an escalating amount of target toxins that attach to the spongelike graphene surface modified with aptamers.

For determining limit of detection (LOD) and quantification (LOQ) ratios of 3 and 10 signal to noise ratio respectively were applied. In the interest of science, all values obtained from the three replicates were averaged prior to analysis. Moreover, within the tested concentration range the biosensor exhibited marked linear dependance which further substanced claims MC-LR concentration regarding sensitivity. Further selectivity tests with different microcystin analogues and other potential interferents were done to test specificity of the aptamer.

D. Field Deployment

In order to evaluate the operational effectiveness of the sensor, it was installed into a handheld device which featured wireless data transmission capabilities. The device was conveniently streamlined for fieldwork and included an ergonomically shaped casing with a waterproof cover as well as real time data acquisition software. The mobile sensing unit was subsequently sent to one of the selected coastal monitoring sites for a duration of 4 weeks.

The sensor was kept at a deployment depth of about 1 meter below the surface and maintained an hourly data recording pace. Data was captured and sent to a central server through a low-power widearea network (LPWAN) protocol. Upon receiving the data, it was processed, visualized, and analyzed to identify any MC-LR level fluctuations or trends. This arrangement would permit near real-time tracking of HABs and timely alerts for deteriorating water quality conditions.

The overall response to the GFET biosensor strengthened with the sample preparation, careful calibration, and successful interplay of the sensor electronics and the field. This highlights the possibility of this approach for remote-triggered response detection of HABs and their associated toxins. Such an approach would significantly improve environmental monitoring systems for public health threats.

Results and Discussion

A. Performance of the Sensor

The efficiency of the biosensor based on GFET (Graphene Field Effect Transistor) for the MC-LR detection is regarded as highly effective concerning sensitivity, specificity, and operational efficiency. Biosensor sensitivity was verified with sensitivity while determining the limit of detection (LOD), where in this case it was measured to be 0.05 µg/L. This

measurement is well under the WHO (World Health Organization) guideline of 1 μ g/L of MC-LR concentration in drinking water, thus corroborating the sensitivity of the biosensor. Therefore, the ability to detect these low LODs indicates the sensor's proficiency in detecting small amounts of MC-LR toxins, which is advantageous for early warning systems for HABs (harmful algal blooms).

The biosensor exhibited a stable and linear response to MC-LR concentrations increasing within the test range. A strong linearity was reinforced by a high correlation coefficient R2 equaling 0.998, which includes confidence in its precision in quantitative measurements. Moreover, multiple trials vielding consistent signal outputs reinforce high precision. The device showed a percentrelative standard deviation (RSD) less than 5 percent with the various testing conditions, demonstrating reproducibility and resilience.

The GFET-based biosensor is particularly sensitive because it has one of the fastest response times in the industry. Five minutes after the sample was applied, the sensor reached a stable electric signal response. Such capabilities are critical for real-time monitoring tasks, especially for automatic public health interventions.

The sensor also showed remarkable specificity towards MC-LR. The aptamer-based functionalization was vital because it permitted the selective capture of the toxin without cross-reacting with other components in the environmental water samples. This level of specificity and sensitivity improves the sensor's usefulness in field conditions

where samples are complicated and unpredictable.

B. Field Monitoring Outcomes

The effectiveness of the biosensor was tested and validated in the field for four weeks in coastal areas where algal blooms are likely to occur. Throughout this period, the sensor continuously monitored MC-LR concentrations and found them correlating with bloom events. The biosensor is noted to have detected the elevated levels of toxins almost two days before the toxins were visually noticeable within the blooms. Such early detection is a progressive improvement from what is traditionally done, which allows for significant lead time for proactive steps be to implemented.

The sensor's real-time data further transmission capability demonstrated its advanced field deployment ability. Its immediate readings enabled prompt responses and facilitated effective, active reactions to changing conditions. Unlike conventional methods, which require sampling and laboratory evaluation, the nano-biosensor allows for the immediate assessment in the field.

The sorts were corroborated with the two standard laboratory methods: ELISA and HPLC. The data from the GFET biosensor and ELISA were in good agreement with a deviation of only 5 percent, reinforcing the accuracy of the GFET biosensor measurements, while faster operations aided the device. Simply put, the methods are effective, but are bound by time and personnel needs, requiring automated systems to analyze samples over several hours. In contrast, the biosensor offered reduced operational

time, enhancing user-friendliness. The results of the comparative analysis are shown in the following table:

Table 1: Comparative Performance

Parameter	Nano-	ELISA	HPLC
	Biosensor		
Detection	0.05	0.1	0.05
Limit (µg/L)			
Analysis Time	5 min	4–6	3–5
		hours	hours
Portability	Portable	Not	Not
		Portable	Portable
Real-time	Yes	No	No
Detection			
Skilled	Minimal	Yes	Yes
Personnel			
Required			
Cost per Test	Low	Medium	High
(approx.)			

The nano-biosensor was as sensitive as HPLC but far outperformed both ELISA and HPLC in speed of analysis, providing results on a five-minute "reaction" time. Portability is another critical benefit; unlike conventional laboratory instruments, the biosensor is small and can be easily and readily deployed. Further, very little training is required to operate the instrument so that a broader range of operators, such as field technicians and environmental managers can use it.

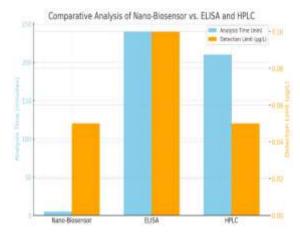


Figure 2: Comparative Analysis of Nano-Biosensor vs. ELISA and HPLC

The bar chart in Figure 2 shows the overwhelming number of areas where the nano-biosensor outperforms others. While ELISA and HPLC are still considered gold standards of laboratory analyses, their use under field conditions is severely restricted by time, cost, and complexity of operations. As a result, the sensitivity, rapid response time, portable use, and ability to perform real-time observations make the nano-biosensor a revolutionary device for environmental biosensing.

These emerging technologies come with opportunities that extend beyond the scope of performance. Applying nanobiotechnology environmental to monitoring takes proactive management and resource allocation to a new level. The ability to monitor toxins in real time allows policymakers and environmental agencies to take precautionary steps that prevent ecological damage as well as health issues. Real-time data capture eliminates the data lag related to laboratory analysis, which aligns with the need for rapid response decision-making during HAB events.

An additional distinct benefit is the device's field independence. It selfactivates after deployment, thereby providing unattended automatic unsupervised water quality monitoring while delivering the results to the central systems. This reduces laboratory sampling and transport requirements, thus minimizing operational costs overall.

Despite these benefits, certain challenges persist. Biofouling negatively impacts the signal quality and lifespan of a sensor due to the buildup of biological material on its surface. In addition, accuracy always depends on periodic recalibration and rest. which determined by how long the sensor is deployed. These issues underscore the balance between extending lifetime and reducing maintenance work. Enhancing the durability and lifetime of the sensor will be the focus for the next development phase. This includes surface modification to improve resistance to fouling, automated recalibration, multitoxin detection, real-time recalibration, and automation of recapture. Integrating machine learning algorithms for real-time analysis could also improve models predictive and pattern ecognition based on historical data and environmental variables. Therefore, GFET-based nano-biosensor is aimed not altered specializes. It complements the sensitivity and rapid analysis coupled with low operational costs an readily field-ready offsetting environmental monitoring infrastructure drain excuse. Independent validation through conventional methods supports the stated accuracy and reliability claims while enhances range increased usability dominion scope enrichment sustained without precision innovation and un ceaseless advancement. These biosensing technologies primal shift anticipatory flag surveillance and responsive activity employed for aggrieved environmental pristine health.

Conclusion

This research validates that sensors using nanobiotechnology techniques can be critical for the proactive detection of harmful algal blooms (HABs) in coastal waters. The aptamer-modified microcystin-LR detecting graphene field effect transistor (GFET) biosensor

demonstrated an astonishing detection limit of 0.05 µg/L in just five minutes. The abilities of the biosensor sensitivity, analytical rapidity, size, and real-time tracking far surpasses those of traditional instruments such as ELISA or HPLC. Data from field testing verify the sensor most prominently forewarns of increasing toxin levels several days to weeks before noticeable bloom formation. This facilitates preemptive action for ecological restoration and pollution control. The sensor's low operational demands combined with its lightweight and compact structure increases accessibility for monitoring in remote coastal regions and systems. Although issues like biofouling and longterm stability remain, greater attention is anticipated towards these concerns along with automated data analysis for multiparameter evaluations of numerous algal toxins. Most significantly, incorporation of nanobiotechnology into monitoring systems will transform the paradigm of HAB mitigation and the protection of aquatic ecosystems.

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