Medicine in Novel Technology and Devices 10 (2021) 100059

Contents lists available at ScienceDirect

Medicine in Novel Technology and Devices

journal homepage: www.journals.elsevier.com/medicine-in-novel-technology-and-devices/

Review Article

Cyanobacterial toxin biosensors for environmental monitoring and protection

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ARTICLE INFO

Keywords:
Cyanotoxin
Biosensor
Coefficient of variation
Harmful algal blooms
Microcystin
Limit of detection

ABSTRACT

Cyanobacterial toxins are primarily monitored using a variety of commercially available techniques, such as enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–mass spectrometry (LC-MS). Indeed, official protocols created and utilized by regulatory bodies have these methodologies ingrained into the technical documentation. However, biosensor technology has been advancing for decades and are positioned to replace many existing methods. The requirements of biosensors include, but are not limited to, real time diagnostics, ease of use (reducing requirements for trained personnel), lower costs, high sensitivity, robust platforms, and good reproducibility. In contrast, laboratory techniques like ELISA require immobile equipment and the use of certified standards for toxin quantification. The use of biosensors may reduce the overall burden of materials and associated costs of production. Given the regulatory limits for toxins in drinking water and recreation, a wide array of biosensor platforms (graphene-based, optical, immunological, etc.) reported in the literature have sufficient sensitivity to comply with these guidelines, however, currently no biosensor has been approved for use in the same manner or accepted as a suitable alternative. In many cases, biosensors have been compared in a limited capacity to established technologies such as the previously mentioned ELISA, LC-MS, and HPLC (High Performance Liquid Chromatography) and would serve as good tools to be used as proxy screening methods. Biosensors examined in this review are evaluated on four criterion: (1) feasibility for point-of-care (POC) use, (2) assay time (time from sample collection to receipt of data), (3) variation between measurements (reported coefficient of variation values), and (4) dynamic range and/or limit of detection (LOD) to obtain a measure of a given technique's suitability to be used for toxin quantification and detection.

1. Introduction

Harmful algal blooms (HABs), occur in numerous water systems (freshwater, brackish, and marine) worldwide and are a notable issue for nearly every continent and country. These HABs cause damage to natural ecosystems and fisheries, reduce or destroy agricultural yields, as well as compromise recreation and drinking water [1,2]. In the United States, the economic damage caused by HABs is estimated to be in the realm of $100 million annually [3]. Looking toward the future, most experts anticipate that climate change-induced environmental conditions will only exacerbate the frequency and severity of HABs in aquatic systems [4,5]. These HABs can produce a variety of harmful toxins called cyanotoxins, which generally include: microcystins, nodularins, saxitoxins, anatoxin-a, and cylindrospermopsins. Microcystins, nodularins, and cylindrospermopsins are known hepatotoxins, while saxitoxins and anatoxin-a are potentially potent neurotoxins, all of which can result in illness or death in both humans and wildlife [6–9]. Ingestion is usually the primary route of exposure, but cyanotoxins can also be aerosolized under certain conditions and have been found in the nasal passages of coastal residents [10–12]. Many of the areas impacted by the formation of HABs and the toxins they produce can be seen in Fig. 1. The microcystins are produced by *Microcystis*, a species of cyanobacteria most prevalent in freshwater bodies and generally responsible for toxin-related concerns. Other toxins, such as anatoxin-a and cylindrospermopsins are produced by *Anabaena* and a variety of other freshwater species of cyanobacteria [13,14]. Saxitoxins may also be produced by several species of marine dinoflagellates [15]. These 4–5 groups are generally the most discussed cyanobacterial toxins, but microcystins have up to 70 cogeners [16], making a thorough analysis and detection of toxins within a sample set potentially very involved.

Conventionally, methods such as enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–mass spectrometry (LC-MS)

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https://doi.org/10.1016/j.medntd.2021.100059
Received 18 September 2020; Received in revised form 14 December 2020; Accepted 3 January 2021
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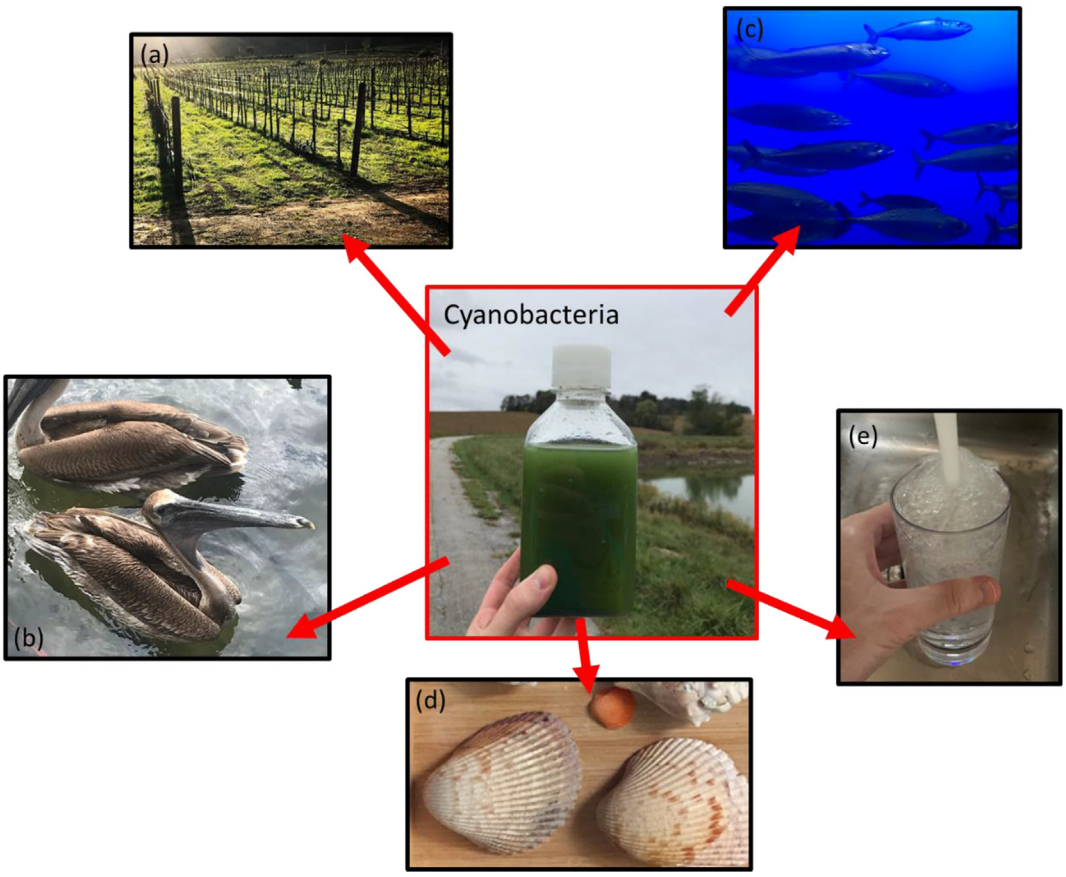

Fig. 1. Web of areas impacted by HABs and the cyanobacteria they produce. These include but are not limited to: (a) agriculture, (b) marine life, (c) fisheries, (d) shellfish/restaurants, and (e) drinking water. Images courtesy of Laura Bertani and Marissa Ganzfried.

are the most common techniques for detecting and quantifying cyanotoxins. Two techniques, ELISA and LC-MS, are employed by the United States Environmental Protection Agency (USEPA) for this purpose. "Method 546" and "Method 544" are the official methodologies that describe protocols for the detection of total microcystins and nodularins in water samples for ELISA and LC-MS respectively [17,18]. LC-MS is used when the investigator needs higher sensitivity and the ability to differentiate cogeners within a toxin group. However, LC-MS is more expensive and involved than ELISA, which is more time efficient and economical by comparison. ELISA is most often employed as it allows for total toxin quantification and gives sufficient information to give water use advisories to the general public. That said, drawbacks for ELISA still include the requirement for a laboratory setting (and thus sample transportation) and a limit of detection (LOD) of approximately 0.1 μg/L for microcystins/nodularins, which limits diagnostic capabilities when toxin concentrations are low [19]. In short, notable improvements to a cyanotoxin diagnostic technique would be in these two areas, improved assay time and improved LOD. For the purposes of this review, we will be primarily focusing on the detection of cyanobacterial toxins in water samples, as that is the most common area of interest for their quantification and detection. Research techniques will be reviewed with respect to four criteria: (1) feasibility for point-of-care (POC) use, (2) assay time (time from sample collection to receipt of data), (3) variation between measurements (reported coefficient of variation/relative standard deviation (CV/RSD) values), and (4) dynamic range and/or LOD. Often, biosensor research platforms for the detection of a wide array of analytes only show that the device successfully responds to a chosen analyte, without exploring the methodology and considerations required to correlate device signal output to real analyte concentrations.

2. Microcystin detection

Of the cyanobacterial toxins, perhaps none attract more attention than the microcystins group (Fig. 2a [20]), and specifically microcystin-LR (MC-LR), the most toxic of the cogeners. Microcystins are the most numerous of all the cyanobacterial toxins and the most relevant to societal interests. When designing any technique for the detection of microcystins (and nodularins as seen in Fig. 2b), a common approach is to exploit the ADDA side chain. Structurally, microcystins are cyclic peptides composed of seven amino acids [21]. Of these, the ADDA moiety is highly conserved among microcystin cogeners and stable in a wide array of environments [22], making it a good target for detection. One common approach is the use of an optical sensing platform. In 1999, *Marquette* et al. first demonstrated a sensor based on chemiluminescence for the detection of okadaic acid, a toxin similar to MC-LR, with an LOD of 2 μg/kg within mussel homogenate [23]. The authors reported a stable response with a CV value of 11.7% across 34 measurements with a variation of 7% and 12.6% for negative controls and "contaminated signals" respectively. Sensor membranes showed no degradation of detection capabilities after storage of up to one month (assuming proper storage conditions) and post-sample processing, assay results were available in approximately 20 min time. While the authors underscored the ability of this technique to detect toxin in crude mussel homogenate (i.e no toxin extraction required), the technique is not label free and would require additional efforts to translate into a POC-style system.

More recently, a portable "Leopard Array Biosensor" (the commercial version of the Naval Research Laboratory Array Biosensor shown in Fig. 3a) demonstrated the parallel detection (six samples) of microcystins in freshwater in approximately 60 min with an LOD of approximately 16 ng/L (reported dynamic range of 0.06–1.5 μg/L) [24–26]. The device

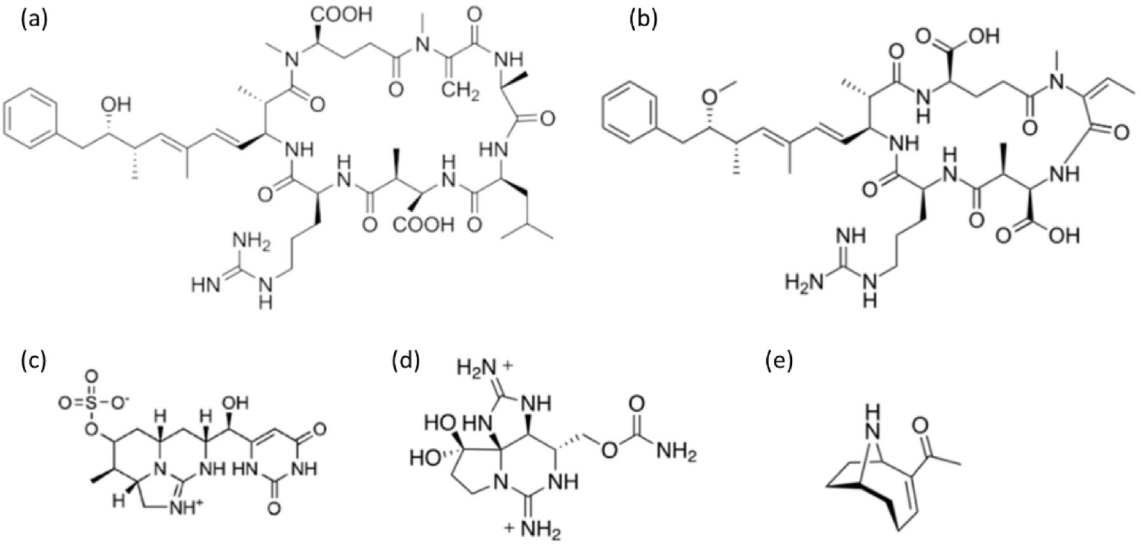

Fig. 2. Cyanotoxin chemical structures: (a) microcystin, (b) nodularin, (c) cylindrospermopsin, (d) saxitoxin, and (e) anatoxin-a [20].

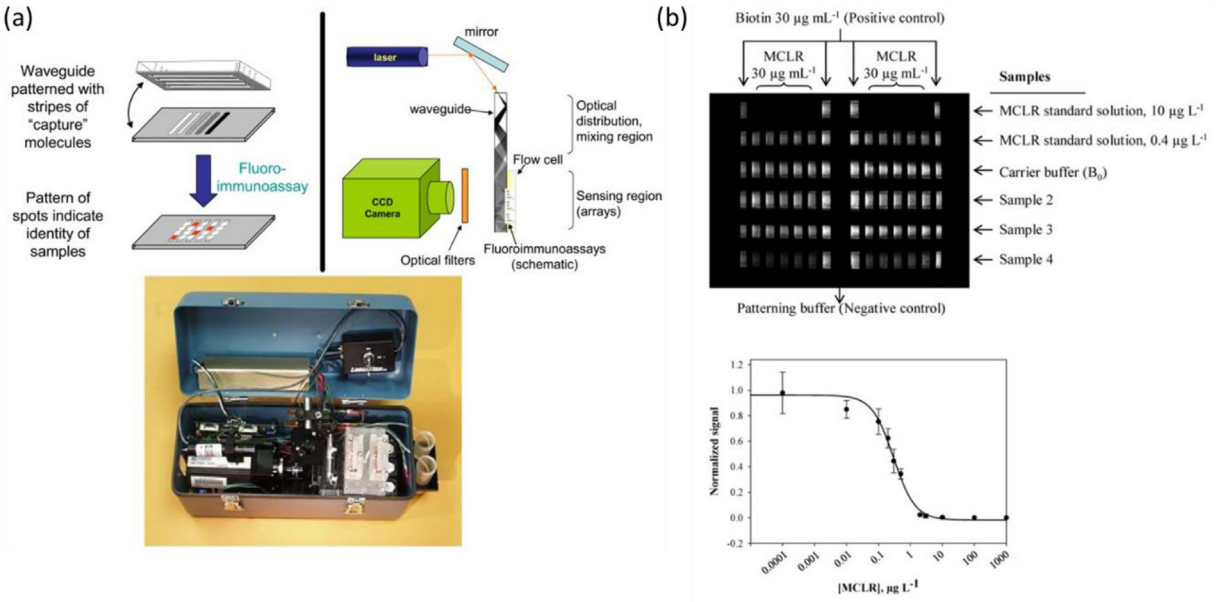

Fig. 3. (a) Naval Research Laboratory Array Biosensor Prototype with detection schema. Optical configuration with separated patterning leading to the formation of a fluorescent spot array on the waveguide surface [24]. (b) MC-LR detection biochip image utilizing this style of detection device (upper), and the corresponding dynamic range (or "calibration") curve for MC-LR detection. (lower) [26].

works by utilizing a competitive fluoroimmunoassay performed within a multi-channel system across a glass slide. Fluorescence intensity signals (Cy5) indicative of binding were extracted from charge-coupled device (CCD) images taken by the camera. A sample biochip MC-LR testing image is shown in Fig. 3b with the corresponding calibration/dynamic range curve. This curve takes on the form of a four parameter logistic regression model (also known as the Hill equation) to map device output to analyte signal [27]. This model is of the form:

$$y = d + \frac{a-d}{1+\left(\frac{x}{c}\right)^b}$$

Here, a is the minimum value, d is the maximum value, c is the point of inflexion (middle of the sigmoid) or sometimes called the IC_{50} (50% inhibitory concentration) value, and b is referred to as either the "Hill coefficient" or the "Hill's slope" of the curve. This value indicates whether or not the receptor-analyte binding is positively cooperative ($b > 1$) or negatively cooperative ($b < 1$).

As might be expected, the error gets larger as MC-LR concentration is reduced. Here, the sensor surface is capable of being regenerated using a 50 mM NaOH solution and re-used up to ~15 times without appreciable signal loss [26]. This technique would qualify as a "fully characterized technology" suitable for comparison to established commercial techniques like ELISA. A graphene-based sensor using the rapid fluorescence quenching of a microcystin-DNA-fluorescein reports an LOD of 0.14 μg/L [28]. This LOD is based on a 3σ (standard deviation) method after evaluating variation in blank samples. However, as other reviewers have noted [29], based on the dynamic range plots and fittings shown in Fig. 4, this seems like an overly optimistic assessment and is not an improvement over the sensitivity offered by ELISA. Assay time is reported to be < 35 min, though the corresponding dynamic range curve (calibration curve) is created using fluorescence signal measurements at t = 20 min.

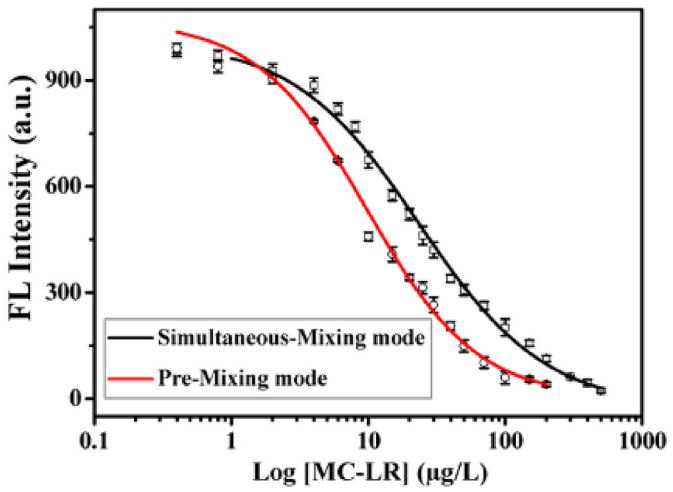

Fig. 4. Standard calibration curve for fluorescence intensity signal at 20 min from immunosensors using both "simultaneous-mixing mode" and "pre-mixing mode" approaches [28].

Other approaches with LODs similar to, or slightly better than, ELISA (roughly 0.01–0.1 μg/L) include Gold nanoparticles used to quench the photoluminescence of graphene oxide resulting in an LOD of 0.5 μg/L for microcystin detection [30], a fiber-optics-based biosensor demonstrating highly reproducible detection of microcystins with an LOD of 0.03 μg/L [31], and an automated online biosensing system (AOBS), seen in Fig. 5a, for the detection of microcystin-LR reported an LOD of 0.09 μg/L. This approach is similar to total internal reflection fluorescence systems and also utilizes a competitive binding assay, where fluorescently labelled antibodies are pre-mixed with the sample then the remaining antibodies are allowed to react with the device surface (Fig. 5b). This results in a reduction of the peak fluorescence signal allowing for quantification of the toxin (Fig. 5c). This AOBS system's most remarkable feature, however, was not the LOD, but the breadth of measurements performed, which were performed continuously every 6 h, with a single calibration run per day over the course of several months [32,33]. This type of setup (online integrated, potential for constant surveillance, etc.) is ideal for a variety of applications and likely an optimal incarnation of an environmental monitoring apparatus, though given the LOD, may not provide warning as early as desired. Additionally, this methodology was compared with high-performance liquid chromatograph (HPLC) for validation purposes. The good agreement between this technique and HPLC lends a higher degree of confidence in the validity of this approach for others interested in alternate cyanotoxin detection techniques.

Immunologically modified sensors display the best LOD among the technologies investigated. To begin, a biosensor based on silver particles was reported with an LOD of 7 pg/L for MC-LR and a linear range between 10 pg/L and 1 μg/L, where the device could be regenerated up to 43 times [34]. However, this LOD was unable to be verified by HPLC, as only high toxin concentrations were compared (mg/L range). A graphene-based, electrochemical sensor utilizing a PtRu alloy for signal amplification reported a 10 ng/L LOD [34], but the provided calibration curve and sensor measurements do not make this value abundantly clear. The lowest reported LOD found in this review is demonstrated by an electrochemical immunosensor based on a graphene-gold nanocomposite conducting polymer/gold nanoparticle/ionic liquid composite film with a remarkable LOD of 37 fg/L for MC-LR [36]. Differential pulse voltammetry (DPV) was used for sensor measurements where the peak current (I_p) decreases with increasing microcystin binding events due to the insulative nature of the bioconjugate blocking electron transfer. The phenomenon can be observed in Fig. 6a where curves a, b, and c correspond to 0, 0.8, and 2 pg/L MC-LR concentrations respectively. These devices are further tested with a wide range of MC-LR concentrations (0.1 pg/L – 8 pg/L denoted as 'a' though 'l' in Fig. 6b), where the change in peak current (ΔI_p) varies linearly with MC-LR (inset of Fig. 6b). Based on a signal-to-noise ratio of approximately 3, a theoretical LOD of ~37 fg/L is determined [36]. This sensor was compared with LC-MS for verification of accuracy using pg/L MC-LR concentration solutions showing good agreement. Other reports have also shown highly sensitive approaches such as an electro-chemiluminescent immunosensor based on CdS quantum dots for the detection of MC-LR, with a proposed LOD of 2.8 ng/L [37] and an Fe_3O_4 nanocomposite technique reporting an LOD of 4 ng/L [38]. As can be seen, these approaches have a wide range of merits, ranging from very impressive to few appreciable benefits over commercial techniques with only some validated against established technologies to an appreciable degree. Across all the methods presented, there is no technique that allows for highly sensitive POC detection with low CV/RSD in a short time period, though some may possess that potential. This combination of beneficial attributes is likely necessary to supplant ELISA as a "gold standard" technology for the detection of microcystins. EPA acceptance and validation of any technique would also be mandatory to instill confidence in researchers and investigators to switch over to new technologies. Theoretically speaking, biosensor techniques based on amperometric approaches should demonstrate superior sensitivity compared to optical methods as the signal is based on the modulation of charge instead of quantization of a fluorescence signal via computer algorithm. Indeed, the top performing devices compare in this review use current as the detection signal. However, the consistency (precision) of the technique when comparing optical and current-based approaches may be similar. All microcystin detection techniques discussed in this section are summarized with respect to the four criterion in Table 1.

3. Saxitoxin detection

Following microcystins, saxitoxin (Fig. 2d) is perhaps the second most relevant of the cyanotoxins and the best known "paralytic shellfish toxin." This moniker is derived from the ingestion of contaminated

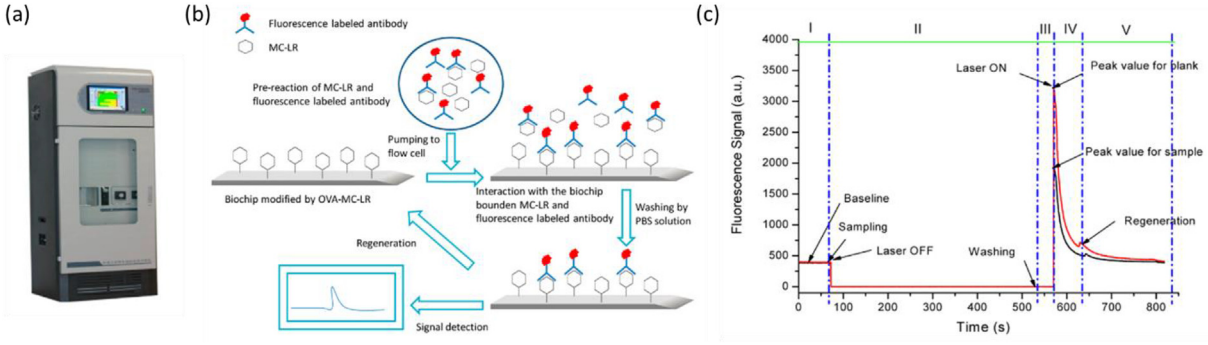

Fig. 5. (a) Automated online biosensing system (AOBS) for the detection of microcystin-LR with (b) sensing paradigm and (c) example fluorescent signal measurement of a sample versus a blank measurement [31].

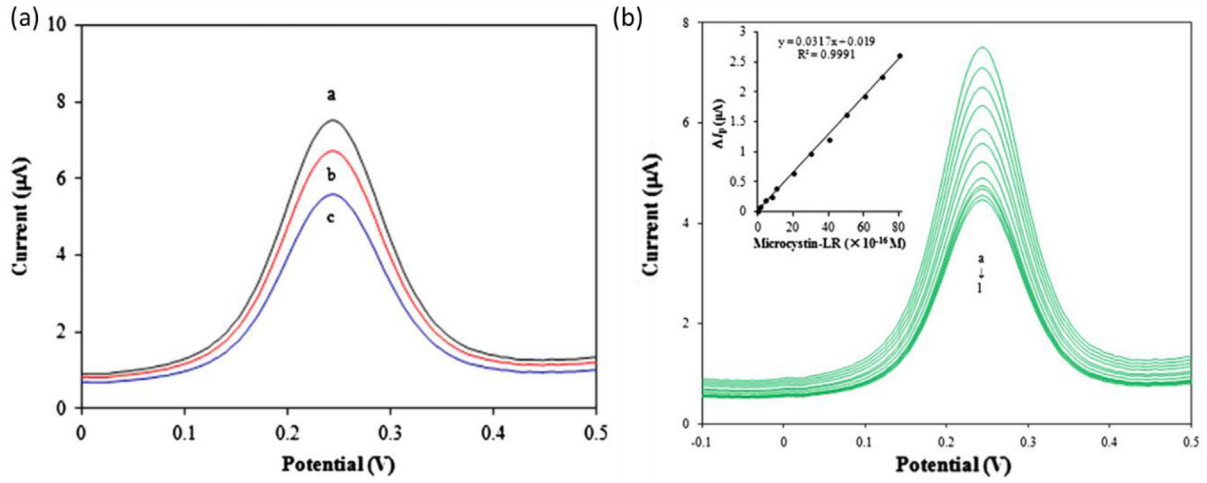

Fig. 6. (a) DPV sensor values of the sensor measured in a PBS solution laden with 0, 0.8, and 2 pg/L MC-LR concentrations denoted as 'a', 'b', and 'c' respectively. (b) DPV sensor values for 0.1 pg/L – 8 pg/L MC-LR concentrations denoted as 'a' though 'l' with Inset: calibration fitting of microcystin-LR concentration vs. peak current change (ΔIp) [36].

Table 1
Summary of microcystin detection techniques.

Technique Description	POC Compatible	Total Detection Time	Reported LOD	CV/RSD Values (%)	Reference
Chemiluminescence for Okadaic Acid	Potentially	20 min (assay time)	2 μg/kg	11.7%	[23]
Leopard Array Biosensor	Yes	60 min	16 ng/L	4–9%	[26]
Rapid Fluorescence Quenching Graphene Sensor	N/A	<35 min	0.14 μg/L	~8%	[28]
Graphene oxide - fluorescence resonance	N/A	N/A	0.5 μg/L	N/A	[30]
Portable optical immunosensor	Yes	<20 min	0.03 μg/L	<5%	[31]
AOBS	Yes	5 min	0.09 μg/L	<8%	[32]
Ag Particle Biosensor	Potentially	~25 min	7 pg/L	<4%	[34]
PtRu Immunosensor	Potentially	~2 h	9.63 ng/L	5.6%	[35]
Graphene-gold nanocomposite	Potentially	~15 min	37 fg/L	1.2%	[36]
CdS quantum dots	N/A	1.5 h	2.8 ng/L	6.45%	[37]
Fe_3O_4 nanocomposite	N/A	1 h	4 ng/L	5% (estimated)	[38]

shellfish resulting in "paralytic shellfish poisoning" (PSP). Symptoms include tingling of the mouth and extremities, which may progress to loss of muscle control and difficulty breathing [39]. If the ingested dose is sufficient, death may result due to extended paralysis of the respiratory system and there is no known antidote, so clinical treatment is restricted to supportive care [40]. The commercial or recreational harvesting of mussels, clams, oysters, and scallops may be detrimentally impacted due to the potential toxins present in tissue commonly sold as food items [41]. Globally, there are approximately 2000 PSP cases per year with a 15% mortality rate [42]. As may be apparent, the presence of saxitoxin in water systems or seafood items is of paramount concern from both a public health and economic perspective. While not as well studied as biosensors for microcystins (or MC-LR) detection, biosensors have been used for the detection of saxitoxin in a research setting. For reference, the ELISA LOD for saxitoxins is reported to be ~0.02 μg/L in aqueous samples [43,44].

Some reported biosensors show a similar or higher LOD compared to ELISA for the detection of saxitoxin. For example, one sensor based on interferometry reports a "low" LOD of 0.5 μg/L [45]. This is suitable for limits set by the USEPA, but not appreciably more sensitive than ELISA. However, the technique improves detection in other areas by offering a label-free and real-time sensing platform. For some applications, this may be a superior approach and also avoids the requirement of a mouse assay and can be regenerated. However, it is unfortunately not verified against commercial techniques. Another approach uses surface plasmon resonance to create a biosensor capable of triplicate measurements [46]. This technique is also label free and purports results in roughly 20 min (total), but a "fast assay" may also be used to reduce the time to around 5 min with little loss in sensitivity. While no LOD or CV/RSD values are explicitly given by the authors, they appear to be around 1 μg/L and <3%. The authors claim that the LOD could be "shifted" by varying the percentage of antibody in the mix solution, but this claim remains uninvestigated. One of the more sensitive devices reported was a colorimetric biosensor based on aptamer functionalized Au nanoparticles (Fig. 7) [47], which a reported LOD of 3 pg/L for saxitoxin in seawater. This technique utilizes an aptamer that reacts specifically with saxitoxin in solution, which results a0067gregation of Au nanoparticles subsequently changing the color of the Au NP solution. This color change is then detected and given an absorbance value. While the authors do compare the LOD with other techniques, they do not verify their technology against established methods or comment on is POC compatibility or CV/RSD. From their data, one might estimate that the CV would hover somewhere in the range of 4–6%. A similar approach is reported by *Zhong* et al. but emphasizes the portability of the sensor over the LOD [48]. The authors therein also utilize a colorimetric biosensor based on Au nanoparticles, but with a significantly higher LOD (~1 μg/L) and shorter detection time (~2 min). Additionally, this technique was based on a modified ELISA platform and is thus verified using a conventional ELISA approach, lending more credence to the results. From the perspective of precision, this approach had a wide range of CV values (4.6–23.2%) depending on the type of shellfish tissue analyzed. Another highly sensitive approach used a magnetic electrochemical immunosensor with a palladium-doped graphitic carbon nitride-based methodology [49]. This technique uses magnetic beads functionalized with saxitoxin-specific antibodies and palladium-doped graphitic carbon nitride peroxidase nanoparticles which catalyze tetramethylbenzidine for signal generation. While highly sensitive, this technique can require substantial sample preparation (e.g. toxin extraction from tissue) and requires labelling for

Fig. 7. (a) The detection methodology of the colorimetric saxitoxin sensor based on gold nanoparticles, (b) the structure of saxitoxin and utilized aptamer, and (c) the UV–vis spectra of gold nanoparticles under various conditions [46].

successful operation. However, risk of false positives would be very low with this approach. Comparisons with LC-MS yielded good agreements and CV values were less than 5%. Two additional techniques, aptamer-based Surface-Enhanced Raman Scattering (SERS) [51] and an amperometric sensor using a gold electrode modified with carbon nanotubes [51] are reported for saxitoxin detection. The SERS approach has the benefit of using an established technique (Raman) for detection and thus, some level of credibility is inherent. However, the LOD is not appreciably better than other techniques in the literature and it is not verified against LC-MS, HPLC, etc. The authors do note the technique may be compatible with on-site detection. Total detection time is not entirely clear, but measurements are completed within a matter of minutes. In this instance, the amperometric sensor uses an aptamer-based approach, but in conjunction with methylene blue as an electrochemical indicator probe [51]. Differential pulse voltammetry is used to elucidate device signal where saxitoxin binds to the aptamer to form 'rigid' complexes. The quasi-rigid structure of folded aptamers prevents the exposure of the bases, which restricts electron transfer of the methylene blue released from sensor surface. This results in a decrease in oxidation current. Overall, we can see many techniques have an array of pros and cons, but while a few reports have an improved LOD in conjunction with shorter detection times, many methodologies show similar sensitivity to ELISA. This is likely due to the improved sensitivity of the ELISA assay to saxitoxin and also the more limited interest and prevalence compared with microcystins. However, biosensors still show as much promise for the detection of saxtitoxin as they do for microcystins and nodularins with respect to high sensitivity and precision. Interestingly, the best performing device reviewed for saxitoxin is based on a fluorescence technique. While the LOD for this technique isn't as low as that of the top performing MC-LR sensor, it is still an improvement over the sensitivity of ELISA and has been shown to be portable. It is possible then that a hypothetical amperometric sensor could potentially achieve even higher sensitivity due to the reason previously discussed in addition to saxitoxin being potentially more heavily charged, making it easier to detect. However, such as technique has yet to be shown in the literature at this time. All saxitoxin detection techniques discussed in this section are summarized in Table 2.

Table 2
Summary of saxitoxin detection techniques.

Technique Description	POC Compatible	Total Detection Time	Reported LOD	CV/RSD Values (%)	Reference
Interferometry-based sensor	Potentially	~7 min	0.5 μg/L	6.5%	[45]
Surface plasmon resonance	Potentially	20 min	~1 μg/L (estimated)	<3%	[46]
Colorimetric biosensor based on Au nanoparticles	Yes	30 min	3 pg/L	N/A	[47]
Colorimetric biosensor based on Au nanoparticles	Yes	~2 min	0.4 μg/L	4.6–23.2%	[48]
Magnetic electrochemical immunosensor	N/A	75 min	1.2 ng/L	<5%	[49]
Surface-Enhanced Raman Scattering	Yes	~2–3 min	3.5 μg/L	6.6%	[50]
Amperometric carbon nanotube sensor	Yes	30 min	114 ng/L	8.1%	[51]

4. Anatoxin-a and cylindrospermopsin detection

While anatoxin-a (Fig. 2e) and cylindrospermopsin (Fig. 2c) are often included in the "cyanotoxin umbrella" description, they are generally toxins of lesser interest when compared to microcystins and saxitoxin. One reason for this is the short half-life of anatoxin-a. While anatoxin-a is a very potent neurotoxin, its half-life is only 1–2 h under natural conditions, which makes it far less likely to cause complications in people or wildlife, as it typically photochemically breaks down before it can do much damage [52]. That said, it was originally discovered by P. R. Gorham after it sickened and killed several herds of cattle in the 1960s [53]. Cylindrospermopsin is produced by the cyanobacteria *Cylindrospermopsis raciborskii* and is predominately found in tropical water systems but has been observed in temperate waters as well [54]. This toxin was first discovered in 1979 when 128 people were hospitalized in Queensland, Australia with symptoms ranging from abdominal pain and vomiting to kidney failure [55]. This illness was originally called the "Palm Island Mystery Disease," but those afflicted were all found to have commonly used water from a nearby dam and it was later discovered that a copper sulfate treatment used to disperse an algal bloom there resulted in the release of the toxin into the water [56]. Cylindrospermopsin was later found in water bodies worldwide, including the United States, Europe, South America, and New Zealand [57].

Biosensors for these two toxins have also been described in the literature. For anatoxin-a, one approach is exploiting the fact that anaotoxin-a is an organophosphate, which irreversibly inhibits acetylcholinesterase (AChE) [58]. However, this process is naturally non-specific, so extra steps must be taken to ensure selectivity when using this approach. In the first report, a set of four mutant AChE variants are used for the detection of anatoxin-a [59]. Interestingly, two are selected to be sensitive to anatoxin-a and two are sensitive to pesticides. As AChE is inhibited by any organophosphate and carbamate insecticides, two mutants found to be most sensitive to anatoxin-a could be used in tandem with two mutants that are sensitive to insecticides, but resistant to anatoxin-a, allowing the device to discriminate between both signals by comparing the relative rates of inhibition. Relative inhibition was measured amperometrically and determined using: RI = dI_0-dI_1)/dI_0, where RI is relative inhibition and I_0 and I_1 are the recorded current initiated by the enzymatic reaction and the current after inhibition respectively [59]. Using this approach, approximately 0.5 nM (82.6 ng/L) sensitivity was achieved in approximately 10 min, but no CV/RSD values are given or discussed, nor is any standardized technique used for verification. A similar approach is used to create a "disposable" acetylcholinesterase-based biosensor for anatoxin-a detection in natural water samples [60]. Here again, the inhibition of AChE is used as the detection methodology, but only *Electrophorus electricus* AChE was used rather than a set. In this case, oxime reactivation was used to show selectivity between the anatoxin-a target and pesticide contaminants. This reactivation was only possible with insecticide (specifically paraoxon) inhibition [61]. Here CV/RSD values are again not discussed, but graphical error bars suggest that these values may be large. The LOD for this approach is also higher and reported to be approximately 1 μg/L [60]. Lastly, an aptamer-based technique uses a self-assembled monolayer (SAM) of disulfide-derivatized aptamer formed on a gold electrode, where DNA aptamers were selected using a systematic evolution of ligand by exponential enrichment (SELEX) approach [62]. Anatoxin-a capture via immobilized aptamer is measured via electrochemical impedance spectroscopy (EIS) and binding events result in a decrease in electron transfer resistance. For this sensor, the optimal detection time is found to be ~60 min with a reported LOD of 82.6 ng/L. This approach has the advantage of being label-free and has good precision with CV values ranging between 1 and 6%.

For cylindrospermopsins, two aptamer-based sensors are described. The first approach is essentially the same as the last anatoxin-a sensor and is reported from the same research group, with the main difference here being aptamers selected for cylindrospermopsin capture instead of anatoxin-a (Fig. 8a) [61]. A detection range of approximately 41.5 ng/L – 33.2 μg/L is reported with CV values ranging from 2 to 10% [63]. 100 min was determined to be the optimum binding time for these devices. The second approach also uses EIS for the device signal but utilizes a thionine–graphene nanocomposite platform [64]. As shown in Fig. 8b, here the receptor aptamer is immobilized onto the thionine-graphene nanocomposite surface via a glutaraldehyde cross-linker. While overall curve fitting is good ($R^2 = 0.997$), this approach gives a higher LOD value of 117 μg/L. Similarly, the optimal binding time for cylindrospermopsin is determined to be 120 min with CV values ranging from 1.4 to 8.5%. This material platform also shows the capability for surface regeneration and long-term stability over a period of one month. For reference, the LOD for cylindrospermopsin detection via ELISA is approximately 50 ng/L [65]. The breadth of studies for both toxins are clearly more limited compared to microcystins and saxitoxin and the maximum sensitivity demonstrated for both toxins is on the order of 10 ng/L. In order for biosensors to make headway in this area, more studies will be needed and more interests in these cyanotoxins will be required. That said, there is no reason why these toxins could not be detected with the same high sensitivity and good precision as demonstrated for microcystins and saxitoxin. All Anatoxin-a and cylindrospermopsin detection techniques discussed in this section are summarized in Table 3.

5. Conclusions and outlook

In summary, there are a vast array of biosensor platforms well suited to the detection of cyanotoxins. They offer novel methods that can detect toxins on-site in real time and with high sensitivity. Additionally, they can produce CV/RSD values and dynamic range curves that adhere to the USEPA guidelines of a CV < 15% and an $R^2 > 0.98$. The ability to detect

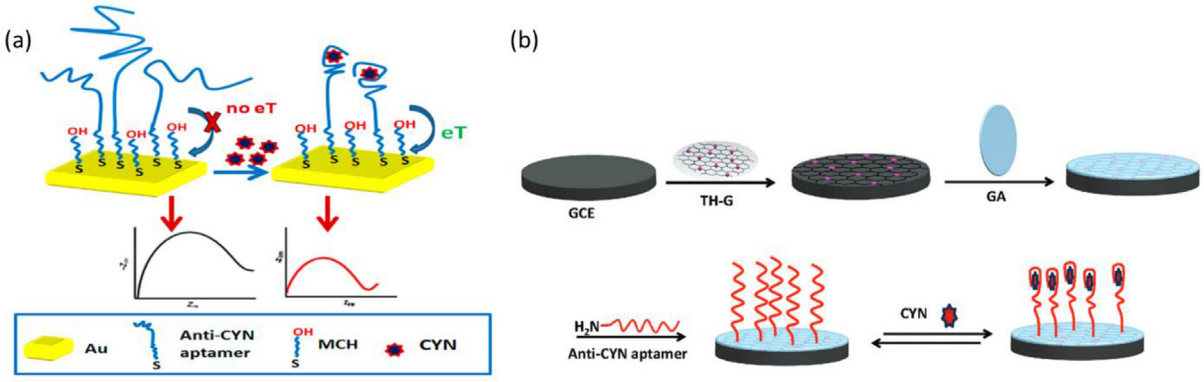

Fig. 8. (a) Aptasensor scheme for cylindrospermopsin detection [62] and (b) thionine–graphene nanocomposite approach for aptamer-based cylindrospermopsin capture and detection [63].

Table 3
Summary of anatoxin-a and cylindrospermopsin detection techniques.

Technique/Toxin	POC Compatible	Total Detection Time	Reported LOD	CV/RSD Values (%)	Reference
Engineered acetylcholinesterase biosensor	Yes	~10 min	82.6 ng/L	N/A	[59]
Acetylcholinesterase-based electrode biosensor	Yes	20 min	1 μg/L	N/A	[60]
Aptamer on Au surface	Yes	60 min	82.6 ng/L	6%	[62]
Aptamer on Au surface	Yes	100 min	41.5 ng/L	2–10%	[63]
Aptamer on thionine-graphene nanocomposite	Yes	120 min	117 μg/L	1.4–8.5%	[64]

toxins on-site and in real time make life easier for technicians and allow for near immediate sampling of natural water systems, so that water use advisories can be more rapidly implemented and information can be more easily disseminated to the public. The improved sensitivity of some biosensors opens up new possibilities such as the earlier detection of algal blooms, so that they might be recognized and dealt with before they become a larger and more costly problem. Additionally, the detection of cyanotoxins at very low concentrations allow for better clinical diagnostics to be able to determine if a patient has been exposed to cyanotoxins, even at very low dosages. This provides a wider window of opportunity as the toxin is filtered out by the body. Except for a select few, most biosensor technologies for the detection of cyanotoxins have been isolated to the research area, but given all their benefits discussed in this review, the future seems extremely bright in this area. Indeed, biosensors have been introduced into surveillance research and it would be not be farfetched to believe that in the future, some of these techniques will surpass ELISA and other accepted laboratory techniques as the new standards for cyanotoxin detection and quantification. While new technologies will slowly trickle into commercial and agency usage, the major hurdles biosensors will have to clear will mainly be establishing regulations and protocols for their usage so they can be used by non-technical staff. Additionally, many reports show excellent results that would typically rival ELISA and other commercial techniques, but often comparisons with mature technologies are not shown. This must be addressed before biosensors are employed for widespread use. A complete Hill's model will be necessary to evaluate device precision and sensitivity range as it has been done for ELISA and other typical laboratory techniques. As an "end goal", it would be ideal to have an array setup with the use of multiple sensors in parallel as a platform to detect and quantify all types of cyanotoxins using a single sample.

Author contribution to study

Paul Bertani: Original draft preparation, Conceptualization, Research, Editing Wu Lu: Editing, Supervision.

Funding source

This work was supported by the Ohio Sea Grant [Grant Number: F2F-000001]; Ohio Department of Higher Education [Grant Numbers: GRT00050966, GRT00053491, GR119066]; National Science Foundation [Grant Number: ECCS1809570].

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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