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Review

Recent trends in development of biosensors for detection of microcystin

Shweta Singh [a], Ankita Srivastava [a], Hee-Mock Oh [b], Chi-Yong Ahn [b], Gang-Guk Choi [b], Ravi Kumar Asthana [a,*]

[a] Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi 221005, India
[b] Environmental Biotechnology Research Centre, Korea Research Institute of Bioscience & Biotechnology, Daejeon, Republic of Korea

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ABSTRACT

Increased cyanobacterial blooms, a source of cyanotoxins are linked with climate change and eutrophication in aquatic bodies, a major concern worldwide. Microcystins are potently hepatotoxic, nephrotoxic as well as carcinogenic. Thus microcystins are threat to tourism, agriculture and animal's health. However, there is a still lacuna in the knowledge of regulation of microcystins production. Presence of toxic and non-toxic cyanobacterial strains together and occurrence of various microcystin variants in aquatic bodies compounded the problem. Although several analytical techniques for microcystin detection such as bioassay, ELISA, HPLC and LC-MS etc. have been already prevalent, the development of biosensors offered rapid and accurate detection, high reproducibility and portability. Sequencing of *Microcystis* spp., opened the new vistas towards the development of biosensor at molecular and genetic level. This review incorporates the current trends in the development of biosensors for microcystin detection in the light of state-of-the-art techniques.

1. Introduction

Cyanobacterial bloom has significant hazard to public health as well as environment due to release of cyanotoxins into water supplies. The first incidence of cyanobacterial toxin poisoning was reported from an Australian lake in 1878 (Francis, 1878). In China, the highest incidence of primary liver cancer occurred in the areas with cyanobacterial contaminated water (Ueno et al., 1996; Kuiper-Goodman et al., 1999) and recently caused 60-deaths in Brazil due to presence of cyanotoxins in dialysis units (Pouria et al., 1998). The most common cyanotoxins are hepatotoxins (microcystins and nodularin), neurotoxins (anatoxin-a, anatoxin-a(s) and saxitoxins), cytotoxins (cylindrospermopsins), and dermatotoxins (aplysiatoxins and debromoaplysiatoxins) (Sivonen and Jones, 1999), which are produced by about 40 genera of cyanobacteria (Carmichael, 2001). Microcystins (MCs), most frequently produced by different species of cyanobacteria such as *Microcystis* (unicellular and colony forming), *Oscillatoria* (filamentous), *Anabaena* and *Nostoc* (heterocystous, filamentous and N_2-fixing) (Fig. 1) (Botes et al., 1984; Skulberg et al., 1993; Rinehart et al., 1994), are usually occurring in fresh water bodies throughout the world (Pearson et al., 1990; Ressom et al., 1994).

MCs are monocyclic heptapeptides having an unusual amino acid, Adda ((2S, 3S, 8S, 9S)-3-amino-9-methoxy-2, 6, 8 trimethyl-10-phenyldeca-4, 6-dienoic acid), which is essential for the expression of its biological activity (Fig. 2). To date, more than 90 congeners of MCs have been identified, showing variations in structure and toxicity (Carmichael, 1994; Sivonen and Jones, 1999). MCs are secondary metabolites produced by peptide synthetases (Robillot et al., 2000) and then released into a water body by cell lysis (McElhiney and Lawton, 2005). MC-LR, where L and R represent the variable amino acid leucine and

* Corresponding author. Tel.: +91 0542 6701101; fax: +91 0542 6701124.
E-mail address: asthana.ravi@gmail.com (R.K. Asthana).

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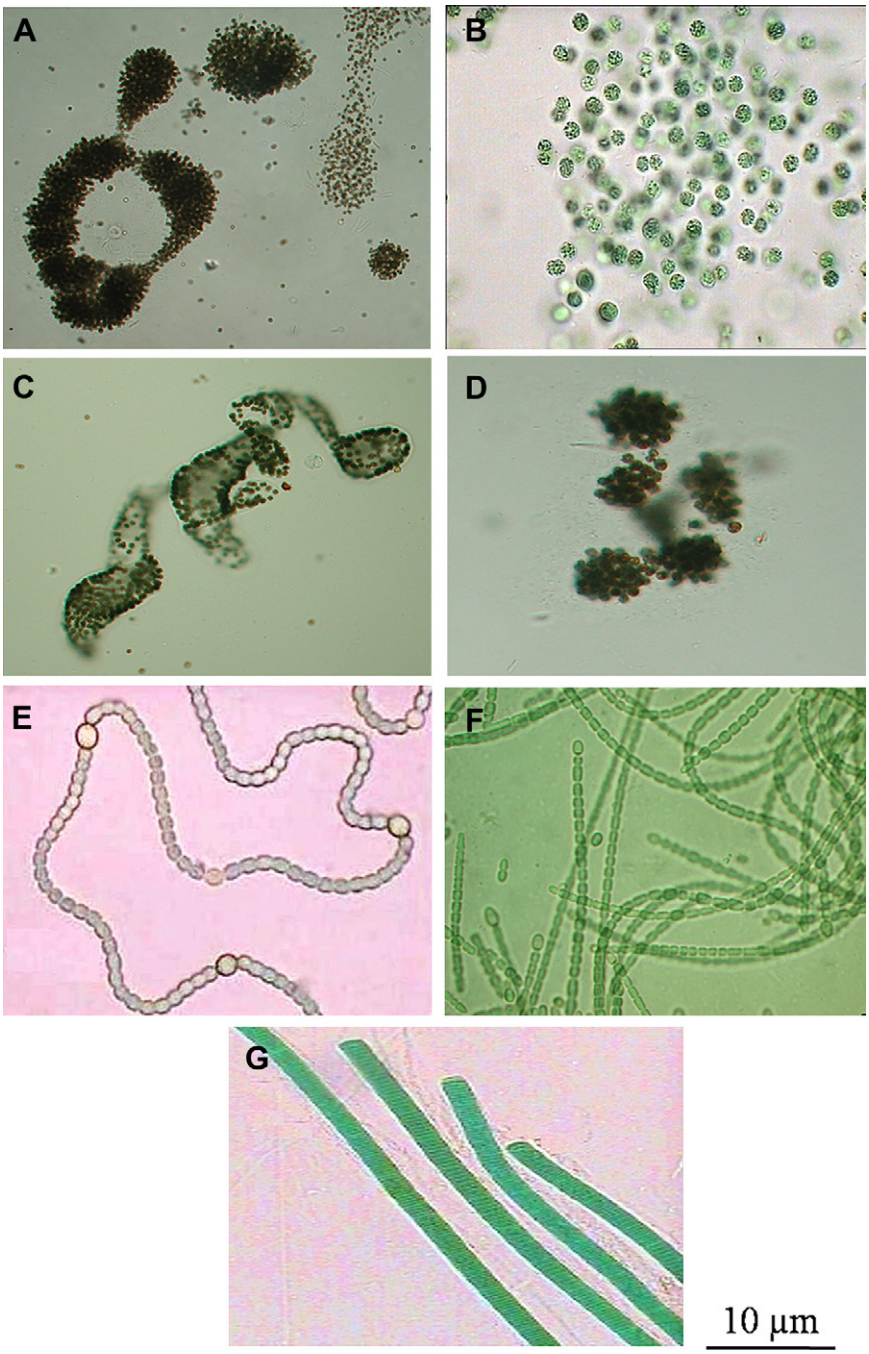

Fig. 1. Photographs of few microcystin producing cyanobacteria (at 100×) A) *Microcystis aeruginosa* (colonial) B) *Microcystis aeruginosa* (unicellular) C) *Microcystis wesenbergii* D) *Microcystis novacekii* E) *Nostoc spongiaeforme* F) *Anabaena flos-aquae* and G) *Oscillatoria princeps*.

arginine, respectively, was the first chemically identified MC and has been associated with most of the incidents of toxicity involving MCs (Fawell et al., 1993).

Although the primary target of MC-LR is liver, more precisely of the serine/threonine protein phosphatases type 2A (PP2A) and type 1 (PP1A) (Mackintosh et al., 1990; Yoshizawa et al., 1990; Dawson, 1998; Kuiper-Goodman et al., 1999), it is also known to affect kidney (Nobre et al., 1999; Dias et al., 2009; Feurstein et al., 2009) and the gastrointestinal tract (Botha et al., 2004). In case of acute toxicity, it can lead to multi-organ failure (Giannuzzi et al., 2011). World Health Organization categorized MC-LR as probable carcinogen for humans (Grosse et al., 2006) and established a maximum permissible limit of 1 μg/L in drinking water (WHO, 1998). There is change in MCs production on temporal as well as spatial basis with limited knowledge of its regulation of production (Wood et al., 2010). In addition, toxic and non-toxic strains cannot be

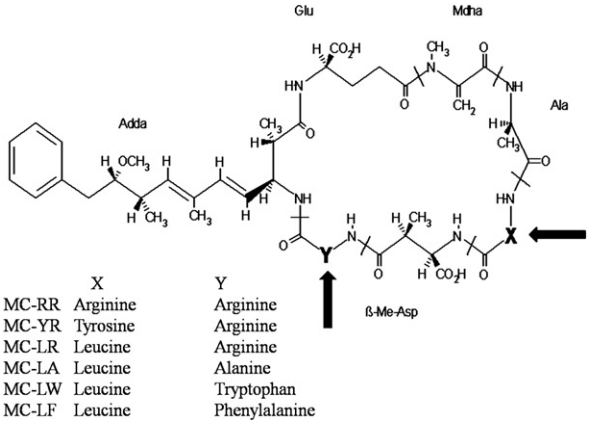

Fig. 2. Structure of microcystin and its variants (-X and -Y show two amino acid residues).

distinguished by simple microscopy as they can occur together. Thus, due to the strong toxicity and ubiquity of MCs, it was necessary to develop a fast, sensitive and reliable method to detect and quantify MCs in environmental samples. Recently, gene clusters involved in microcystin production from *Microcystis* (Nishizawa et al., 1999, 2000; Tillet et al., 2000), *Planktothrix* (Christiansen et al., 2003) and *Anabaena* (Rouhiainen et al., 2004) and even complete genome of *Microcystis aeruginosa* strains, NIES-843 (Kaneko et al., 2007) and PCC 7806 (Frangeul et al., 2008) have been fully sequenced. This has made possible to develop molecular method for identification of toxic species (e.g. conventional PCR, multiplex PCR) (Neilan et al., 1999; Tillett et al., 2001; Hisbergues et al., 2003; Vaitomaa et al., 2003; Kurmayer et al., 2002, 2003; Rantala et al., 2004; Al-Tebrineh et al., 2011) as well as quantification of toxic strains (real time PCR) (Vaitomaa et al., 2003; Rinta-Kanto et al., 2005; Baxa et al., 2010; Kim et al., 2010; Joung et al., 2011). Our group has also quantified toxic and non-toxic cyanobacteria for the first time in Indian water body using real time PCR (Srivastava et al., 2011).

Several analytical and biochemical screening methods are currently in use, but limited in terms of their potential application in routine environmental monitoring. The most widely used analytical and biochemical techniques for the determination of MCs in raw or drinking water are described in Table 1. Analytical methods often require time-consuming sample preparation procedures that usually involve the pre-concentration of considerable water volumes prior to analysis to reach the required sensitivity. Furthermore, the ability of these techniques to identify unknown MCs in environmental samples has been hindered by the lack of analytical standards for many MC variants (McElhiney and Lawton, 2005). Thus a simple, easy-to-use, rapid, robust, specific, sensitive and portable method may account for analyzing low concentrations of MCs, which can act as a better monitor for MCs to manage

Table 1
Comparison of methods currently available for analysis of microcystin variants.

Methods	Comments	Limit of detection	References
Whole cell bioassay	Measure LC_{50} value, qualitative, poor sensitivity and precision, ethical issues	25–150 μg/kg (mouse)	Falconer (1993), Campbell et al. (1994) and Sangolkar et al. (2006)
TLC	Requires standard, low sensitivity, very easy	0.01 μg	Pelander et al. (2000)
ELISA	Colorimetric, sensitive cross reaction, expensive	Polyclonal antibody – 2.5 μg/L Monoclonal antibody – 0.1 μg/L	An and Carmichael (1994), Nagata et al. (1995) and Metcalf and Codd (2003)
Protein phosphatase inhibition (PPI) assay	Colorimetric, fluorometric, very sensitive, false positives, expensive	Colorimetric – 0.25–2.5 μg/L Fluorometric – 0.1 μg/L	An and Carmichael (1994), Bouaïcha et al. (2002) and Rapala et al. (2002)
HPLC	Measure UV absorbance, ISO standard method, detection of individual variants, required standards, Expensive, required trained personnel	PDA/UV array – 100 μg/L MS – 0.1 μg/L	ISO (2005) and Lawton et al. (1994)
MALDI-TOF	Identification of variants, structural elucidation, matrix interferences	1 μg/L	Welker et al. (2002)
NEEIA	Rapid, sensitive, disposable, portable, useful for site monitoring	0.1 μg/L	Zhang et al. (2007)
Chl-a fluorescence based bioassay	Rapid, sensitive, useful for different variants of MC	0.01 μg/L	Perron et al. (2012)
Cl-immunoassay	Wide linear range, highly sensitive, suitable for natural sample	0.02 μg/L	Hu et al. (2008)
LC-MS	Mass spectrometry, simultaneous separation and identification of variants, sensitive and specific, very expensive	~0.02 μg/L	Lawton et al. (1994), Robillot et al. (2000) and Spoof et al. (2001)
LC-MS/MS	Identification of variants, sensitive and specific, expensive	0.0026 μg/L	Zhang et al. (2004)
Gas chromatography (GC and GC–MS)	Fluorescence detection, low sensitivity, tedious process	0.0043 μg/L	Sano et al. (1992) and Tanaka et al. (1993)
TRFIA	Broad working range, highly sensitive and efficient	5×10^{-6} μg/L	Lei et al. (2004)
Capillary electrophoresis (CE and CE-MS)	Laser induced fluorescence detection, low sensitivity needs further improvement	–	Bateman et al. (1995) and Li et al. (1999)
NMR	Structural determination of cyanotoxins, requires large amount of sample, requires pure sample, expensive	–	Harada et al. (1993)

the risk associated with the health. These requirements are fulfilled by biosensor, an analytical device that consists of a biological recognition system (often called bioreceptor), in direct contact with a transducer (Turner et al., 1987). Therefore, biosensors can be classified either by their biological recognition system or their signal transduction methods (Fig. 3).

In a biosensor, a bioreceptor is combined with a suitable transduction method which produces a signal after interaction with the target molecule of interest (Fig. 4). The presence of the biological element makes the biosensor systems extremely specific and highly sensitive, giving an upper edge over the conventional methods and bioassay in environmental sensing and detection. Over the years, a number of different natural and artificial biological elements have been used in biosensors; the most important ones are enzymes, antibodies, nucleic acids, whole cells and Molecularly Imprinted Polymers (MIPs). On the basis of these biological elements, biosensors are either catalytic (enzymes and whole cells) or affinity biosensor (antibodies and nucleic acids) (Tothill, 2001). During last decade, many review articles came into existence, discussing the role of biosensors in environmental monitoring such as for waterborne pathogens (Rasooly and Herold, 2006), endocrine disruptors (Rodriguez-Mozaz et al., 2005), heavy metals (Verma and Singh, 2002; Gooding et al., 2003) and food security (Amine et al., 2006). However, to best of our knowledge, there is a lack of comprehensive review especially with biosensor development related to MCs. Thus, in the present review, we aimed at discussing the recent trends and challenges in the development of biosensors for microcystin detection.

2. Recent advances in biosensor for microcystin detection

2.1. Enzyme based biosensor

Although the use of canaries in coal mines could qualify as the first biosensors, the area of biosensors research started with the development of enzyme electrodes for glucose detection (Clark and Lyons, 1962). In enzyme-based biosensors, the biological element is the enzyme which reacts selectively with its substrate (Guilbault et al., 2004). Enzymes are the most used biocatalytic elements, enabling the detection of analytes in various ways. Since enzymatic reactions are followed by the consumption or production of various species, transducers can easily detect as well as correlate these consumed or produced species to the substrates. In another way, activation (Bontidean et al., 2000; Kamtekar et al., 1995, 1996) or inhibition (Amine et al., 2006; Sole et al., 2003) of an enzyme by a substrate can avail in detection of the substrate concentration. Enzymes can be used either directly in catalytic biosensor or as markers in affinity biosensor. MCs are hepatotoxic in nature, having toxicities that are several orders of magnitude greater than most nerve agents (Carmichael, 1996; Falconer et al., 1981). Inhibitory effects of MCs towards specific enzymes raised the possibility to develop a rapid and sensitive enzyme biosensor (Sadik and Yan, 2004; Campas et al., 2005, 2007; Campas and Marty, 2007).

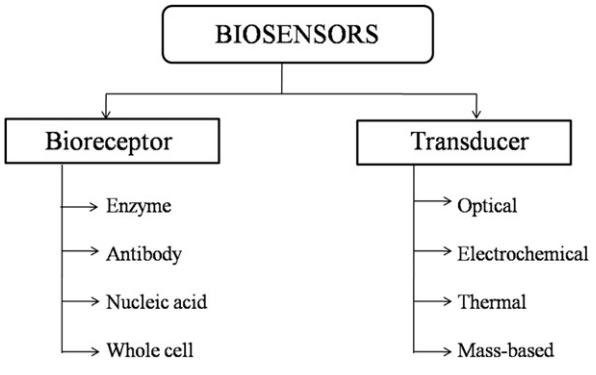

Fig. 3. Types of biosensors.

2.1.1. Optical biosensors

Optical biosensors have provided a highly effective technique for sensitive, selective and nearly immediate detection of a vast variety of analytes. Optical detection is usually based on the measurement of luminescent, fluorescent, colorimetric or other optical signals produced by the interaction of biorecognition element with target analyte. The fluorescent optical biosensors are based on the measurement of fluorescence produced by the target analyte which is proportional to its concentration. The fluorescence produced by small proximity changes between two fluorescent molecules is measured by Fluorescence Resonance Energy Transfer (FRET) (Grant et al., 2001). Fluorescence can be produced by many natural and synthetic compounds. Non-fluorescent cellular compounds e.g. protein, can be made fluorescent by labeling with an extrinsic fluorophore, which causes the molecule to be fluorescent by absorbing energy of a specific wavelength and then re-emitting energy at a different wavelength. Derivatives of rhodamine, coumarin, cyanine and fluorescein are the commonly used fluorophores. Fluorescein isothiocyanate (FITC), a reactive derivative of fluorescein, is the most common one used in various applications. In MC-LR, no fluorophore group is present, thus by introducing a FITC molecule into the MC-LR, a fluorescent MC-Cys-FITC conjugate was synthesized (Sadik and Yan, 2004). The developed biosensor was based on competitive binding between the native microcystin and its fluorescent analog with immobilized alkaline phosphatase enzymes. Firstly PP1A enzyme was immobilized on optic fiber and then unlabeled MC-LR and labeled MC-Cys-FITC were allowed to compete for the limited binding sites provided by PP1A. The resultant signals were inversely related to the concentration of unlabeled MC-LR, which varies according to the concentration. The problem of cross-reactivity between MC variants and other cyanotoxins are of major concern during the analysis. The developed sensor was tested against other cyanotoxins and was able to resist interference and was highly sensitive for MC-LR.

2.1.2. Electrochemical biosensor

PP inhibition can be detected by different techniques including radioisotopic method and colorimetry. Radioisotopic method is limited because of sophisticated labeling

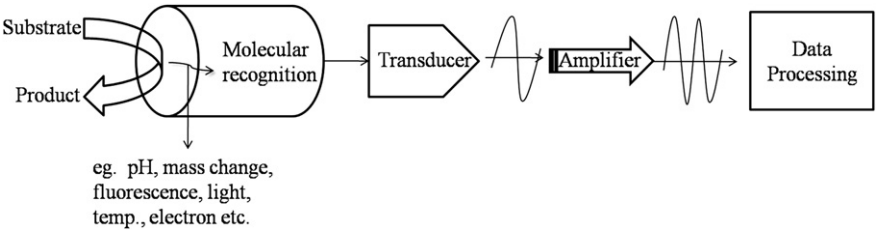

Fig. 4. A schematic diagram of the biosensing principle.

procedures and hazardous problems (Craig et al., 1993; Sim and Mudge, 1993; Jones and Orr, 1994). Colorimetry test is most common, simple, cost effective and sufficiently sensitive (An and Carmichael, 1994; Wong et al., 1999) and has been employed in development of an electrochemical biosensor for microcystin detection in water samples (Campas et al., 2005, 2007). In recent years, electrochemical biosensors hold a leading position among the bioprobes and also hold a great promise for the task of environmental monitoring as well. These electrochemical techniques are very complementary to optical detection methods such as fluorescence, the most sensitive optical technique. Use of enzymatic approach for the detection of toxins is limited and not commercialized because of fast inactivation of enzymes when not kept under specific conditions. So, immobilization of enzymes came about to solve several problems such as loss of enzyme activity, maintenance of stability and shelf life of the biosensor. To do this, several immobilization methods like carrier binding (binding of enzymes to water-insoluble carriers), cross-linking (intermolecular cross-linking of enzymes by bi-functional or multi-functional reagents), covalent immobilization and entrapping (incorporating the enzymes into the lattices of a semi-permeable gel or enclosing into a semi-permeable polymer membrane) have been investigated. Campas et al. (2005) used the most common and extensively used method, covalent immobilization of the enzyme by using sol–gel entrapment, glutaraldehyde immobilization and polyvinyl alcohol containing styrylpyridinium groups (PVA-SbQ) and found that the latter method was fast with good reproducibility. As an immobilization support, Screen-Printed carbon Electrodes (SPEs), Maxisorp microtiter wells and Ultrabind modified polyethersulfonate affinity membranes were used and the highest immobilization yields were obtained with microtiter wells, while the highest operational and storage stabilities were achieved with carbon SPEs and membranes, respectively. To develop an electrochemical biosensor, several phosphorylated substrates were used; good results were achieved with the commercial enzyme and α-naphthyl phosphate, *p*-aminophenyl phosphate and catechol monophosphate as enzyme substrates. The developed biosensor was simple, rapid and can be commercialized.

Campas et al. (2007) developed an enzyme-inhibition based amperometric biosensor for the detection of MCs in natural water samples. Electrochemical results obtained from amperometric biosensor were compared to conventional colorimetric PP1 assay. The developed sensor was found to be 20-times less sensitive than colorimetric PP1 assay, but provided a large working range. Although the sensitivity of the developed sensor was very low, it could be used for screening and monitoring of MCs. In the developed sensor, less reproducible SPEs were used. The reproducibility and limit of detection (LOD) of biosensor could be improved by using new types of electrodes.

Electrochemical biosensors are the most sensitive detection techniques and various strategies were developed to amplify the electrochemical signals. Substrate recycling is one of the most popular and can be easily implemented in enzyme electrodes for electrochemical signal amplification and achieved by using 1) an oxidizing or reducing agent (Uchiyama et al., 1993; Hasebe et al., 1994, 1995), 2) oxidation or reduction of the substrate on the electrode surface (Martín and Domínguez, 1999; Coche-Guérente et al., 1999) and 3) enzymes (Limoges et al., 2006). Campas et al. (2008) used a novel strategy based on enzymatic recycling for improving the LOD of above said amperometric biosensor. They used *p*-aminophenyl phosphate as enzyme substrate and diaphoreses and NADH oxidase as amplifying enzymes. The detection principle was based on the dephosphorylation of non-electroactive dephosphorylated phenolic compounds by PP2A and the oxidation of the corresponding electroactive dephosphorylated phenols to quinones on the electrode surface. The enzymatic activity of PP2A was inhibited by MCs and thus oxidation current intensity decreased proportionally to the MCs concentration. The amplification strategy reduced the LOD of amperometric biosensor from 37 μg/L to 0.05 μg/L, which shows higher sensitivity of this approach. In some reports, two recycling approaches were used simultaneously to further enhance the sensitivity (Huang et al., 1998). Thus by using this approach, a more sensitive biosensor can be designed.

2.2. Immunosensor

Immunological techniques are highly sensitive for the detection of low level concentration of analytes in complex matrices (Killard et al., 1996; Luppa et al., 2001). Enzyme-linked immunosorbent assays (ELISAs) using monoclonal and polyclonal antibodies are specific and sensitive method for microcystin monitoring, but having problems of cross-reactivity among MCs and nodularin congeners (Chu et al., 1990; Nagata et al., 1995). To overcome this problem, immunosensors were introduced which are faster, cheaper, portable, specific and highly sensitive. In

immunosensor antibodies or antibody fragments are used as a molecular recognition element for specific analytes (antigens) and provide concentration-dependent signals. The main advantage of immunosensors over other immunological methods (such as ELISA) is the regeneration and reusability of the sensing surface, which ensures the reliability of a sensor system (Homola, 2003).

Immunosensors are more versatile than enzyme-based biosensor as antibodies exhibit very specific binding capabilities for specific structures, which result into more selectivity and sensitivity. Affinity constant between the antibody and antigen are very high than other biomolecules including enzymes, which makes immunosensors advantageous over the enzyme based biosensors. Antibodies can be generated to bind to a wide range of compounds and this selective binding is the basis of the detection. However, use of immunosensors is somewhat limited because each compound needs specific antibodies to be developed and characterized. On the basis of signal transduction method, immunosensor may be divided into four types; electrochemical, optical, piezoelectric and thermometric (Luppa et al., 2001). Further, based on operating principle electrochemical biosensors are assorted into potentiometric, amperometric and impedimetric biosensors.

2.2.1. Electrochemical immunosensors

In electrochemical immunosensors, electrochemical detection of immunoreactions is carried out with electroactive labels, with enzyme labels or directly without the label (Fig. 5). Campas and Marty (2007) fabricated an amperometric immunosensor for the detection of MCs, based on the affinity between the microcystin and the corresponding monoclonal and polyclonal antibodies. They designed competitive direct ELISAs, by immobilizing both monoclonal and polyclonal antibodies on microtiter wells and screen-printed graphite electrodes which results into faster and sensitive immunosensor. In recent years, SPEs have attracted an increasing interest for the development of biosensors because of low-cost fabrication and mass production. SPEs have miniaturized dimensions which allow all immunological steps by using only a few drop of reagent and thus reducing its consumption. In electrochemical biosensor a redox mediator is required to provide electrical contact between the enzymatic label and the electrodes. The important features essential for the function of redox mediators are fast electron-transfer rate, low redox potential, sufficient chemical stability of the reduced and oxidized form and low reorganization energy (Marcus, 1993). 5-Methyl-Phenazinium Methyl Sulfate (MPMS) was used as a redox mediator to provide electrical contact between the enzymatic label and the electrodes. Optimization of experimental parameters and performance of monoclonal (MAb) and polyclonal antibodies (PAb) were compared by colorimetric method. It was found that the MAb sensor provides lower limit of detection, while PAb sensors were more reproducible and reliable. This assay was capable of detecting microcystin in the ng/L level, but it needed the labeled enzyme and thus analysis was rather complicated with relatively high cost. In this view a direct or label-free immunosensing techniques seemed to be more appropriate.

Label-free immunosensors detect affinity interaction by monitoring changes in electronic or interfacial properties owing to the antigen–antibody complex formation on the electrode surface. One of the detection principles that have been successfully applied to detect the changes is capacitive measurement (Berggren and Johansson, 1997; Berggren et al., 1998; Bontidean et al., 1998). Capacitive immunosensors measures the changes in dielectric properties when an antibody–antigen complex is formed on the surface of an electrode (Berggren et al., 2001; Gebbert et al., 1992). In a capacitive immunosensor, the immobilization of the recognition element is crucial for the immunocomplex formation as the recognition film formed must be an insulating layer in order to prevent the possible interference from electroactive species in the electrolyte solution (Berggren et al., 2001; Hu et al., 2002). Loyprasert et al. (2008) developed a label-free capacitive immunosensor using self-assembled thio-urea monolayer incorporated with silver nanoparticles on gold electrode. Self-assembled thio-urea monolayer is a cheaper thiol reagent with low environmental impact and can strongly adsorb on gold surface (Holze and Schomaker, 1990; Ubaldini et al., 1998) was used for immobilization of antibodies (anti-MC-LR). Silver nanoparticles were incorporated into gold electrode itself which enhanced the response capability of gold electrode and a more sensitive biosensor was derived.

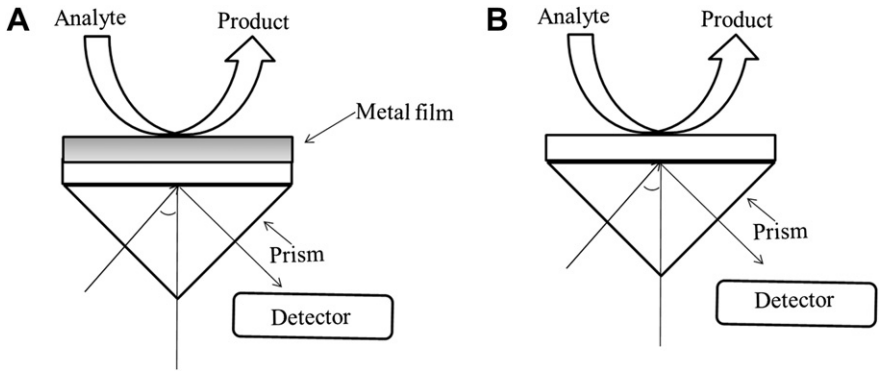

Fig. 5. Labelled immunosensor A) sandwich format and B) competitive format.

Silver nanoparticles are easy in preparation, having good biocompatibility and relatively large surface area (Katz and Willner, 2004; Wang, 2005; Luo et al., 2006). The modified thio-urea gold electrode was simple to prepare and was used to determine MC-LR with high sensitivity and a detection limit of 7.0 pg/L, lower than the prescribed WHO limits under optimum conditions. The modified electrode was reused up to 43 times and reduced the cost of analysis.

Recently a label free amperometric immunosensor for rapid detection of MC-LR was prepared by immobilizing antibody on a gold electrode coated with L-cysteine-modified-gold-nanoparticles (Tong et al., 2011). A stable film of immobilized antibodies was prepared by combining gold nanoparticle (GNP) and self-assembled-monolayer (SAM). GNP is a very attractive material for the construction of biosensor as it offers a large number of advantages including easy preparation, large surface area, excellent biocompatibility, simplicity, accuracy and non-pollution (Valera et al., 2008; Wang et al., 2008). GNP provides a suitable microenvironment for biomolecules immobilization retaining their biological activity and by facilitating electron transfer between the immobilized proteins and electrode surface. Immobilization of GNP layers onto electrode surface was done by covalent bonding between gold nanoparticles and surface functional groups including $-SH$, $-NH_2$ and $-CN$ (Mena et al., 2005). Similarly SAM can be used as specific functionalized surface for the immobilization of gold nanoparticles and enzymes (Wu and Hu, 2006). In present biosensor hydroquinone was used as the electron mediator because of its good electrochemical activity, structure and stability. The developed label free immunosensor was simple, reproducible, stable, economical and very sensitive with a LOD of 0.03 μg/L.

Sun et al. (2010) used a simple, highly sensitive, label free and mediatorless approach for the fabrication of impedimetric immunosensor by immobilizing polyclonal anti-MC-LR on a self assembled L-cysteine monolayer to detect ultra-trace level of MC-LR in water. L-cysteine, as a self-assembly template have good electro-analytic properties (Zhang et al., 2008). To enhance the response and achieve a more sensitive system, GNPs were incorporated into modified electrodes. In optimum conditions the fabrication method had wide linear range, low detection limit, good recovery and high reproducibility. Additionally, the fabrication approach was simple and very rapid as it took only 15 min in the whole detection.

Current enhanced security and health concerns led scientists and researchers to develop numerous rapid biodetection assays. These assays and their reagents will need to be active and stable for longer period of time. Multiple analyte screening in a single assay may bypass such problems because of higher throughput compared with single-target systems, decreased sampling errors, easy inclusion of internal controls, as well as less reagents and consumables. Quantum dots (inorganic semiconductor nanoparticles) based biosensor provides multiple analyte screening in a single assay and have been previously used as fluorescent labels. Quantum dots (QDs) encapsulated with polymers is water-soluble, biocompatible and has ability to integrate with proteins, nucleic acid or subcellular components. Yu et al. (2009) used quantum dot/antibody (QD/Ab) probe immunological recognition of MC-LR as well as for electrochemical transduction. QDs responses were amplified and converted to an electrochemical signal by measuring the cadmium ions released from QD and recorded with Square Wave Stripping Voltammetry (SWSV). QD/Ab probe showed considerably high sensitivity in a wide working range (0.227–50 μg/L) as well as low limit of detection (0.099 μg/L). This probe works on electrochemical signal where direct transduction into electronic signals ascertained its use as a miniaturized portable device.

In the past few years carbon nanomaterials have received a great deal of attention in research activities because of very high activation barriers to their structural rearrangement providing extra stability even in their unfunctionalized forms. In past 2–3 decades various structures of carbon nanomaterials (carbon nanotubes, carbon nanoparticle, and carbon nanofiber) have been used in sensitive detection of various analytes. Among all carbon nanomaterials, carbon nanotubes (CNTs) are of great importance for the development of advanced biosensor because of their nano-dimensions, rich electronic-states, large surface area, high mechanical strength and excellent chemical and thermal stability. The Single Walled carbon Nanotubes (SWNTs) coated paper as a sensor for MC-LR toxin detection was shown by Wang et al. (2009). They impregnated regular filter paper strips with SWNTs and antibodies. In the developed sensor carbon nanotubes form a dense percolation network which allows antibody–antigen complex formation. Due to high sensitivity of SWNT–SWNT in charge transfer the conductivity of the network exhibits strong dependence on the presence of analytes. The presence of the target analyte, i.e., MC-LR alters the current flowing through the electrode and hence, the conductivity of SWNT-paper composite used in the detection of MC-LR concentration. The developed sensor has low limit of detection and time consumed for detection is about 28 times less than for conventional ELISA. Besides carbon nanotubes, Single-Walled carbon Nano-Horns (SWNHs) is also used in immunosensor development. SWNHs are spherical aggregates of thousands of graphitic tubule closed ends with cone shaped horns (Yuge et al., 2004; Zhang et al., 2009), having large surface area, excellent conductivity and highly defective horns (Yang et al., 2005; Urita et al., 2006). On comparing with SWNTs, SWNHs showed better sensitizing effect as immobilization matrices. A sensitive carbon nanohorn sensitized electrochemical immunosensor for detection of MC-LR was developed by Zhang et al. (2010). The designed immunosensor had good performance with high sensitivity, excellent stability and fabrication reproducibility.

2.2.2. Optical immunosensor

2.2.2.1. Surface Plasmon Resonance Immunosensor. In the last two decades Surface Plasmon Resonance (SPR) biosensor technique has received a great research interest. SPR biosensors are based on the optical method in which, changes in refractive indexes are measured as analyte binds to the surface. Among optical methods, SPR is currently the most used technique because of its fast response, real time analysis and ability to detect the multi-analytes at a time

(Myszka, 1997, 1999; Baird and Myszka, 2001; Homola et al., 2002). In recent 2–3 years various SPR immunosensor has been developed for microcystin detection using different approaches (Hu et al., 2009; Herranz et al., 2010; Vinogradova et al., 2011). An indirect inhibitive SPR immunosensor was fabricated by immobilizing a bioconjugate of bovine serum albumin and MC-LR on carboxymethylated dextran covalently attached to a gold surface (CM-5) sensor chip (Hu et al., 2009). CM-5 sensor chip is a general-purpose chip for interaction analysis involving all types of biomolecules and having regeneration property. Hu and his coworkers developed time-resolved fluorescence (TRF) and chemiluminescence techniques for MC-LR detection which were more sensitive than the developed SPR immunosensor (Lei et al., 2004; Hu et al., 2008). Although the sensitivity of SPR immunosensor was low, it offered several advantages as it was simple, rapid and can be used for repeated analysis (more than 50-assays).

Further, a SPR immunosensor based on a competitive inhibition format was developed in which the analyte MC-LR was covalently immobilized onto the surface of a gold SPR chip functionalized with an amine-SAM (Herranz et al., 2010). In the developed biosensor MC10E7 monoclonal antibodies were used which showed group specificity for [4-arginine]-MCs, thus biosensor exhibited high degree of cross-reactivity among MC-LR, -RR and -YR, and allowed the quantification of the total concentration of the three most common MCs in water samples. The configuration of the SPR instrument used included a four-channel chamber, thus allowed the measurement of four samples simultaneously. The developed immunosensor showed high stability, reusability (at least 40 assay–regeneration cycles with the same chip) and excellent precision.

SPR optical biosensors are useful in the detection of small molecules of biomedical, food and environmental interest (Sankaran et al., 2007). In modern era blue green algae (BGA) are popularized as healthy nutritionally balanced natural sources of vitamins, minerals and amino acids and are harvested for same. However, the occurrence of toxic species during harvesting can result in the contamination of BGA products. In various studies contamination of the BGAs with MCs has been reported (Gilroy et al., 2000; Lawrence et al., 2001; Lawrence and Menard, 2001) and a regulatory limit of 1 mg/kg for MCs in BGA-containing products has been established by the Oregon Health Division and the Oregon Department of Agriculture (Gilroy et al., 2000). A competitive inhibition SPR immunobiosensor using a monoclonal antibody to detect MC-LR levels ($<$1 mg/kg) in BGAs food supplements, using a simple sample preparation was developed (Vinogradova et al., 2011).

2.2.2.2. Evanescent Wave Fiber-optic Immunosensors. Evanescent Wave Fiber-optic Immunosensors (EWFI) are rapid, specific, sensitive, cost effective and suitable for real-time on-site detection and have been applied to detect a wide variety of pollutant. Conventional EWFI have the large size with an optic component which makes it costly and requires crucial optical alignment restricting its use as portable device. Long et al. (2008) developed a simple, compact and portable prototype set up of EWFI with a single-multi-fiber optic chamber for detection of 2,4,-D (a well known pesticide) as well as MC-LR, simultaneously. Instead of continuous tapered or straight probes, combination tapered fiber probes were produced by tube-etching method in which both the transmission of the excitation light and the collection and transmission of fluorescence was achieved which resulted into a significant signal enhancement. The reusable fiber optic probe was developed by covalent attachment of the MC-LR-OVA (recognition element) to a self-assembled monolayer formed onto the probe and the detectability values were comparable to the other analytical ones.

A quantitative immunoassay method is based on antibody–antigen binding which is mainly dependent on van-der-Waals forces and hydrophobic interaction. In real water samples different type of organic and inorganic compounds are present which may affect the antigen–antibody binding because of alteration in van-der-Waals forces and hydrophobic interaction. These effects may lead to false positive or negative results in immunoassay, which is called as matrix effect. Matrix effect is described as the difference between the mass spectrometric response for an analyte in a standard solution and in a biological matrix (Kebarle and Tang, 1993). Unwanted interferences caused by matrix effect are highly variable and difficult to predict in the newly developed advanced immunoassay technology. Thus, to investigate the influence of different interfering agents (pH, humic acid, PBS, copper ion) on the sensitivity and stability of the MC-LR fluorescence immunoassay an Evanescent Wave All-fiber Immunosensor (EWAI) was developed (Long et al., 2009a). It was found that PBS and humic acid did not affect the immunoassay at all, while pH (6–8) was favorable for an MC-LR immunoassay. Low concentration of copper ion was not affecting the immunoassay but at higher concentration (5–10 mg/L) the fluorescence signals detected by EWAI were reduced. They also proposed that influence of copper ion on the immunoassay could be minimized by adding chelating reagent EDTA to the pre-reaction mixture. Further, the design of EWAI was improved and a new advanced and highly reusable immunoassay system, Trace Organic Pollutant Analyzer (TOPA), portable in nature with less optical components was developed (Long et al., 2009b). The fiber optic probe surface was produced by covalently immobilizing a MC-LR-OVA conjugate onto a self assembled thiol-silane monolayer of fiber optic probe. The MC-LR-OVA immobilized fiber optic probe was highly resistive to non-specific binding of proteins, and had very low cross-reactivity against other compounds structurally similar to MC-LR. The immunosensor had the low limit of detection that is 0.03 μg/L with a detection range of 0.1–10.1 μg/L and the immunosurface showed high regeneration power (more than 150 assays) with 20 min of surface regeneration time.

2.2.2.3. Luminescent immunosensor. A portable device based on a capillary ELISA technique in combination with a miniaturized fluidic system i.e. Chemiluminescence Multichannel Immunosensor (Cl-MADAG) was developed for semi-quantitative on-site determination of MC-LR

(Lindner et al., 2009). The developed immunosensor was able to detect a minimum concentration of 0.1 μg/L of MC-LR in real water sample. In immunoassays, if very low concentration of MC-LR is present, the antigen-immobilized system shows a very weak or hardly detectable signals, which is caused either due to non-sufficient surface coating with the antigen or because of damage of the respective coated surface. This problem can be minimized by carrying out the immunoassay in microplates where many parallel and control assays are conducted at a time. The newly established assay format was advantageous because the antigen-immobilized assay format allows the internal control for integrity of the coated surface as well as for the assay itself. On generation of very weak signals, the functionality of the test can be controlled by conducting a second immunoassay using antibodies and strong signals can be generated.

2.2.2.4. Fluorescent immunosensors. A highly sensitive and simple approach based on color changeable polydiacetylene (PDA) liposomes for detecting MC-LR was developed by Xia et al. (2010). A MAb of MC-LR (anti-MC-LR) was embedded onto the PDA vesicle surfaces; a substitute for an enzyme, by an EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/NHS (*N*-hydroxysuccinimide)) reaction and a MC-LR recognition vesicle (PDA-anti-MC-LR) was formed. PDA vesicles are unique conjugated polymers which mimic biological interfaces and show a stress-sensitive color changes because of alteration in PDA configuration (Lee et al., 2009). Another important feature of PDA is their fluorescent property, nonfluorescent in the blue phase and fluorescent in the red phase (Park et al., 2007). These color changeable and fluorescent property of PDAs have been employed for developing the sensors to detect virus, *Escherichia coli*, nucleic acids, toxins, etc. (Charych et al., 1993; Ma et al., 1998; Wang and Ma, 2005; Wang et al., 2006; Kolusheva et al., 2008; Deng et al., 2009). On addition of different concentrations of MC-LR, PDA confirmation was altered because of specific immunoreactions and led to a color change from blue to purple, violet, pink and red with increased concentration of MC-LR. This method has LOD of 1 ng/ml and color change was detectable with naked eye.

2.2.2.5. Immunoarray biosensor. A compact and portable analytical immunoarray providing an excellent multiple assay platforms for clinical as well as environmental samples was developed by Long et al. (2010). Analytical microarray are powerful tools for high-throughput and rapid analysis of multiple analytes (Seidel and Niessner, 2008; Blicharz et al., 2009) and are used in a number of bio-analytical applications, such as disease diagnosis, drug discovery, proteomics assay, environmental monitoring and detection of biological warfare agents (MacBeath, 2002; Belleville et al., 2004; Ahn et al., 2006; Kopf and Zharhary, 2007; Blicharz et al., 2009). Antibody and hapten microarrays are specific quantitative analytical techniques in which antibodies/antigens are used as specific biological recognition elements and can detect numerous analytes in low sample volumes simultaneously (Epstein et al., 2003; Ahn et al., 2006; Seidel and Niessner, 2008). An optic fiber-based immunoarray biosensor for two target analytes, MC-LR and trinitrotoluene (well known explosive compound used in the preparation of landmines) was developed through immobilization of two types of hapten conjugates MC-LR-OVA and NB-OVA onto the same fiber optic probe (Long et al., 2010). The developed sensor was able to detect two small pollutants MC-LR and TNT simultaneously within an analysis time of about 10 min for each assay cycle. The proposed fiber based optic immunoarray biosensor can be further expanded for multi-analyte detection simply by immobilizing more haptens on the same immunoarray.

2.2.3. Piezoelectric immunosensors

Quartz Crystal Microbalance (QCM) is one of the best alternatives to SPR as a label free method for the detection of immunological reaction (Aberl et al., 1994; Kosslinger et al., 1995). The physical principles of QCM and SPR are very similar, although there are some fundamental differences (Fig. 6) (Kosslinger et al., 1995). The QCM immunosensor

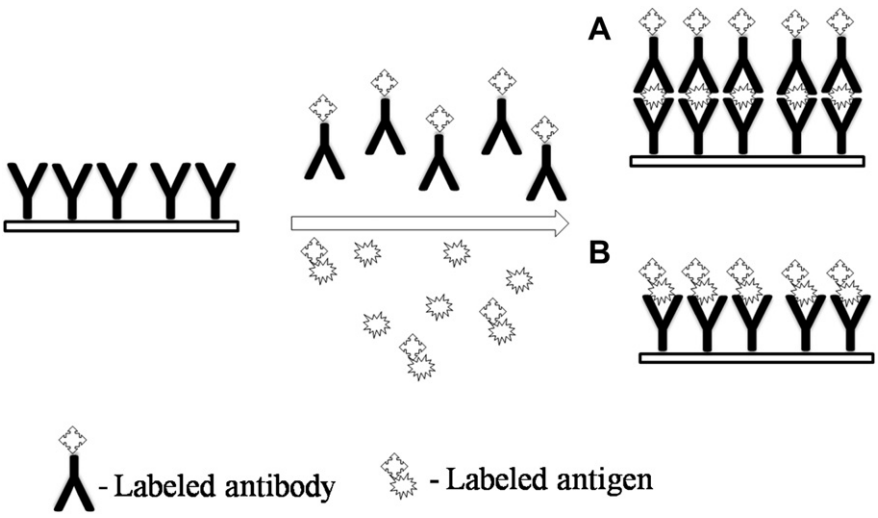

Fig. 6. Schematic diagram for A) surface plasmon resonance and B) evanescent wave measurement.

comprises a quartz crystal with an antigen or antibody immobilized on its surface. A novel QCM-based enhanced piezoelectric immunosensor for MC-LR detection by double amplification was developed by Xia et al. (2011). QCM is used as a transducer in immunoassay because of its low cost, high portability and wide and routine adaptability (Kurosawa et al., 2004, 2006; Hao et al., 2009; Baldrich et al., 2008). Intrinsic sensitivity of QCM is relatively low which can be improved by surface modification using liposome layers (Lee et al., 2005) and mass amplification using gold nanoparticles (Tang et al., 2006; Chu et al., 2006). In developed immunosensor primary amplification was done by surface modification method using a synthetic dendritic novel surfactant, bis(amido-ethyl-carbamoylethyl) octadecyclamine (C18N3). C18N3 is extremely biocompatible with antibodies, which maintain the activity dependent on its outward-facing, multi-amine head and well structured double membrane mimicking the liposome. To enhance the sensitivity, secondary amplification was implemented with anti-MC-LR gold nanoparticles conjugates as probes. Han et al. (2011) had also developed a QCM-based method for the detection of MC-LR by an AuNP-amplified sandwiched immunoassay. Stepwise surface modification of the sensor by adding antibodies and the toxin was specifically characterized by QCM analysis. The system was not able to detect very low concentration of MC-LR but after addition of AuNP the developed immunosensor detected picogram levels of MC-LR.

Microcantilever biosensors are label-free and can detect small amount of molecules. Cantilever-based immunosensors are fabricated by immobilizing a monolayer of receptor molecules on one side of the cantilever and then the induced mechanical bending caused by antigen–antibody specific binding is detected through several signal transducer techniques. Ding and Mutharasan (2010) proposed a real time Piezoelectric-Excited Millimeter sized Cantilever (PEMC) immunosensor for the MC-LR detection with enhanced immunoassay sensitivity. Piezoelectric cantilever biosensors have high mass-change sensitivity and have been used for label free detection of proteins and pathogens (Craighead, 2000; Maraldo et al., 2007). This cantilever-based biosensor approach have special advantage as it does not require labeled reagents, sample preparation or pre-enrichment step and can be mass produced, having compatibility with automated high throughput assay formats. PEMC sensor is composed of two layers, a PZT (lead-zirconate titanate) which acts both as an actuating and as a sensing element, and a nonpiezoelectric layer made up of glass (surface for antibody immobilization).

2.2.4. NMR-based immunosensor

Now a day's magnetic nanoparticle are extensively used in various fields because of their intrinsic magnetic features. A stable and sensitive immunosensors based on the relaxation time change of magnetic nanoparticles for the detection of microcystin was developed (Ma et al., 2009). In this method target analyte, MC-LR competed with the antigens on the surface of the magnetic nanoparticles which in turn influenced the formation of aggregates of the magnetic nanoparticles making it sensitive and fast. In the presence of different concentration of target analyte the magnetic relaxation time of the magnetic nanoparticles was changed by different degree, and determined by magnetic relaxation images and transverse relaxation time of magnetic nanoparticles. This method was sensitive and simple in operation, including only mixing of magnetic nanoparticle solution with the sample solution and determination of result through instrument.

2.3. Nucleic acid biosensor

In recent years, nucleic acids have been extensively used into a wide range of biosensors and bioanalytical assays, due to their wide range of physical, chemical and biological activities (Wang et al., 1997; Bagni et al., 2006; Rogers, 2006). In nucleic acid based biosensors, sensing elements are oligonucleotides, with a known sequence of bases, or a fragment of DNA or RNA. Nucleic acid biosensors are either based on the highly specific hybridization of complementary strands of DNA/RNA molecules or play the role of a highly specific receptor of biochemical/chemical species. Nucleic acid biosensors are of major interest owing to their great promise for obtaining the sequence-specific information in a faster, simpler and cheaper manner compared to the traditional ones. Unlike enzymes or antibodies, nucleic acid recognition layers can be readily synthesized and regenerated for multiple uses. Nucleic acid biosensors can be more sensitive and specific when combined with polymerase chain reaction (PCR) methods (Bell and Ranford-Cartwright, 2002).

2.3.1. Electrochemical DNA biosensor

A fully automated DNA detection system using a specific DNA probe based on sequence polymorphism within the 16S rDNA of *Microcystis* spp. was developed (Matsunaga et al., 1999). Specific DNA sequences were determined by sequence data analysis and were designed for the detection of *Microcystis* spp. Genosensors are based on the highly specific hybridization of complementary strands of DNA or RNA molecules in which a sequence specific probe (short chains of DNA/RNA) immobilized onto the transducer surface, works as a biorecognition molecule. In automated DNA detection system, two specific DNA probes, one conjugated on magnetic particles and other digoxigenin labeled, were designed for *Microcystis* spp. detection. These specific probes were able to discriminate a difference of a few base pairs in oligonucleotide DNA probe.

An electrochemical DNA biosensor for the rapid detection of specific gene related to *Microcystis* spp. was developed (Yan et al., 2001). The sensor was fabricated by the immobilizing a 17-mer DNA probe, complementary to a specific gene sequence related to *Microcystis* spp. on a gold electrode through specific adsorption. The DNA probe was used to determine the amount of target gene by using methylene blue (MB) and ruthenium bipyridine ($Ru(bpy)_3^{2+}$) as electrochemical hybridization indicators. The detection limit using this approach was as 9.0×10^{-11} M. In addition, these indicators were capable of selectively discriminating against mismatches. This new developed system was a rapid detection method for the identification and quantitation of *Microcystis* spp. genes without cell culture and PCR amplification. A similar type of

electrochemical DNA biosensor was developed by Erdem et al. (2002). A specific DNA probe based on 16S rDNA was designed and immobilized onto carbon paste electrode (CPE). The CPE modified probe was very specific for *Microcystis* spp. and was able to discriminate even two-base mismatch.

Screen Printed Carbon Electrodes (SPCEs) is gaining a high attraction in research area of electrochemical sensor because it is cheap, easy-made and disposable (Cardosi and Birch, 1993; Millan et al., 1994; Lucarelli et al., 2002; Teng et al., 2009), but the application of SPCEs in the DNA sensor is very limited (Liu et al., 2006; Yang et al., 2009). Lan et al. (2010) used SPCEs as the base electrode in developing a disposable electrochemical DNA biosensor for *in situ* determination of *Microcystis* spp. Carbon-surface of SPCEs are non-functional in nature, so, they introduced AuNPs to the electrode surface for linking the thiolated-DNA, complementary to the specific sequence of *Microcystis* spp. on the electrode by Au–S bonds. AuNPs are able to adsorb the DNA molecule strongly and increased the quantity of the DNA immobilized on the electrode and finally enhanced the electrochemical signals and sensitivity of DNA biosensor (Cai et al., 2002; Zhuo et al., 2005). To detect the hybridization of specific-sequence of *Microcystis* spp. DNA in developed biosensor, methylene blue was used as the redox indicator and DNA immobilization and hybridization was characterized by cyclic voltammetry and differential pulse voltammetry.

In electrochemical DNA biosensor DNA hybridization is detected by using redox indicator such as MB and RuBP. Despite their high potential in DNA hybridization detection, redox probes require high redox potential, which often destroy the DNA complementarity. Therefore, an alternative electrochemical DNA biosensing technique for detecting DNA hybridization is needed. K'Owino et al. (2007) introduced the concept of Metal-Enhanced Detection (MED) for the determination of DNA–DNA reaction and genetic mismatch detection in *Microcystis* spp. using silver ions. In metal enhanced detection, metallic films are deposited as a continuous or as a monolayer on to a solid electrode, or even electrostatically held which enhances the rate of electron transfer by reducing the distance between the donor and acceptor species and leads to label-free assays during DNA hybridization. This label-free metal enhanced DNA biosensor was successfully applied in detecting two-base-pair mismatches in the gene sequences of *Microcystis* spp.

2.3.2. SPR-DNA biosensor

Aptamers are short fragment of single-strand DNAs (ssDNAs)/RNAs selected *in-vitro* and can bind with a broad range of target molecules like amino acids, drugs, proteins and other molecules with high affinity and specificity. These aptamers are screened from extremely complex libraries of nucleic acids through a process called Systematic Evolution of Ligands by Exponential enrichment

Table 2
An evolution in the development of microcystin biosensors.

Biosensor	Major advances	References
Enzyme Based Biosensor	Introduction of optical biosensor based on competitive binding and fluorescence detection	Sadik and Yan (2004)
	Immobilization of enzymes to increase the stability of electrochemical biosensor	Campas et al. (2005)
	Development of an amperometric biosensor based on enzyme inhibition reaction	Campas et al. (2007)
	Use of substrate-recycling for electrochemical signal amplification which improves the sensitivity	Campas et al. (2008)
Immunosensor	Development of electrochemical immunosensor based on competitive direct ELISA	Campas and Marty (2007)
	Introduction of evanescent wave fiber optic in developing immunosensors	Long et al. (2008, 2009a,b)
	Development of label-free immunosensor using SAM surface impregnated with nanoparticles	Loyprasert et al. (2008) and Tong et al. (2011)
	Use of inorganic semiconductor nanoparticles (QDs) for probe designing	Yu et al. (2009)
	Use of carbon nanomaterials as an immobilization matrix to increase the stability	Wang et al. (2009) and Zhang et al. (2010)
	Introduction of SPR optical immunosensor	Hu et al. (2009), Herranz et al. (2010) and Vinogradova et al. (2011)
	Development of chemiluminescence multichannel immunosensor	Lindner et al. (2009)
	Development of NMR based immunosensor	Ma et al. (2009)
	Development of label-free and mediatorless immunosensor	Sun et al. (2010)
	Introduction of fluorescent immunosensor based on color changeable PDA liposomes	Xia et al. (2010)
	Development of immunoarray biosensor which is used for multiple analyte analysis	Long et al. (2010)
	Development of PEMC immunosensor based on microcantilever	Ding and Mutharasan (2010)
	Development of QCM based piezoelectric immunosensor	Xia et al. (2011) and Han et al. (2011)
Nucleic acid biosensor	An automatic DNA detection system using DNA probe specific for *Microcystis* spp.	Matsunaga et al. (1999)
	Use of hybridization indicator in developing electrochemical DNA biosensor	Yan et al. (2001)
	Development of SPR-DNA biosensor based on DNA aptamers specific for microcystin	Nakamura et al. (2001)
	Use of carbon paste electrode in developing electrochemical DNA biosensor	Erdem et al. (2002)
	Use of metal enhanced detection in DNA biosensor	K'Owino et al. (2007)
	Use of screen printed carbon electrodes as base electrode	Lan et al. (2010)

(SELEX). Nakamura et al. (2001) developed a SPR biosensor based on screened DNA aptamers having capability to bind microcystin very specifically. Screening was done by the *in vitro* selection method of twelve rounds and obtained a sorbent specifically suitable for microcystin detection. The sensitivity and precision of microcystin detection was not as high compared with the methods reported previously and can be further improved by using high affinity aptamers.

2.4. Others

Biological molecules used in the development of biosensors are usually very specific and sensitive for the target analytes. Because of poor stability, high cost and time-consuming production of these biomolecules, Chianella et al. (2003) developed a sensor based on artificial receptor by using Molecular Imprinting Technique (MIP). MIP is an attractive tool for synthesizing highly selective polymeric receptor (Ramstrom and Ansell, 1998). In MIP, complementary cavities of target molecule with the right shape and functionality are formed. In developing a MIP based biosensor, very specific for MC-LR, computer simulation and molecular modeling for the design of a recognition element was used. The required low detection limit (1 μg/L) of MCs (WHO, 1998) requires a sensor with a very high sensitivity and also sample preconcentration. Coincidentally, molecularly imprinted polymers are also used in Solid-Phase Extraction (SPE). Previously in several studies, MIPs have been used in successful extraction and pre-concentration of target compounds from complex biological and environmental matrices (Muldoon and Stanker, 1997; Mullett and Lai, 1998; Bjarnason et al., 1999; Andersson, 2000). Similarly, the developed artificial MIP receptor was also used in a double role both as SPE material for MC-LR pre-concentration and as recognition receptor in a piezoelectric sensor and the minimum detectable amount of toxin was 0.35 nM.

It is apparent that multidisciplinary approach was adopted in the evolution of biosensors for MCs detection including use of nanotechnology (Table 2). However, unfolding of crux associated with MCs regulation in the environment may lead to development of models to make advanced biosensors.

3. Conclusion

The analysis of MCs is most commonly carried out by various conventional techniques, but none of them is ideal as these are expensive, time-consuming and often requires sample preparation, concentration of sample prior to analysis and trained personnel. For complete analysis of the MCs, the combination and comparison of these methods are required. Therefore, in order to satisfy the need of daily examination, emergency quick monitoring and to circumvent above said problems, biosensors emerged as valuable tools. Most of these developed biosensors are specific for MC-LR, the most potent variant of MC. For MCs analysis, firstly an optical biosensor based on competitive binding of MCs to a fluorescent analog was introduced which was very specific for MC-LR analysis with good working range and low LOD value. Later on, biosensors based on enzyme inhibition were introduced. These enzyme based biosensors are still limited because the enzyme is inhibited by other compounds present in the sample and thus, these sensors are not usually able to discriminate various cyanotoxins (e.g. nodularins) in the same sample and can only be used as screening tools. Besides the problem of cross reactivity, commercialization of enzymatic biosensors is also limited because of denaturing property of enzymes.

Cross reactivity among MC-variants and nodularin overestimated results. Thus, immunosensors, were introduced since they have more versatility, stability and selectivity, capable of detecting MCs in the ng/L level. Immunosensors based on labeled enzymes are very sensitive but this method is rather complicated with relatively high cost. In this sense label-free immunosensors seem more advantageous as they are easier in operation and less complicated. Further, to increase the sensitivity of immunosensors, various immobilization surfaces were used which include SPEs and modified SAM surfaces. With the development of nanotechnology, various nanomaterials, e.g. GNP, silver and carbon nanoparticles have been used in biosensor and enhanced the LOD of biosensors. For the detection of toxins in various samples, numerous rapid biodetection assays are required. Thus to avoid the above said problem, quantum dots based multiple analyte assay techniques were introduced. Similarly, SPR biosensors are also gaining a high attention from researchers and scientists because of its fast, real time and multi-analyte detection at a time. As an alternative to SPR immunosensors, QCM based immunosensors were introduced which are lower in cost than SPR and highly portable.

As harmful algal blooms (HABs) release toxins after death and decay into the surrounding environment, detection of toxin genes, in early stage of HABs prior to toxin release, might be useful in forecasting of HAB events as well as in water quality management. Nucleic acid biosensor fulfills this demand by detecting the specific DNA sequences and having high sensitivity, fast response, easy handling and low cost, compared to other types of biosensor. For microcystin detection, various genosensors and aptamers were developed in which sequence specific probes of DNA/RNA were used. These developed nucleic acid biosensors were applied for the analysis of gene sequences of *Microcystis* spp. and mismatches of even one base pair in the gene-sequences as well.

Thus it seemed that a coordinated approach involving more than one type of biosensors discussed in the text should be used together to solve the complex problem of MCs variants.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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