Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com

Review

Potential of biological approaches for cyanotoxin removal from drinking water: A review

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ARTICLE INFO

Keywords:
Cyanotoxin
Biodegradation
Attached growth process
Biofilm
Gene analysis

ABSTRACT

Biological treatment of cyanotoxins has gained much importance in recent decades and holds a promise to work in coordination with various physicochemical treatments. In drinking water treatment plants (DWTPs), effective removal of cyanotoxins with reduced toxicity is a primary concern. Commonly used treatments, such as ozonation, chlorination or activated carbon, undergo significant changes in their operating conditions (mainly dosage) to counter the variation in different environmental parameters, such as pH, temperature, and high cyanotoxin concentration. Presence of metal ions, natural organic matter (NOM), and other chemicals demand higher dosage and hence affect the activation energy to efficiently break down the cyanotoxin molecule. Due to these higher dose requirements, the treatment leads to the formation of toxic metabolites at a concentration high enough to break the guideline values. Biological methods of cyanotoxin removal proceed via enzymatic pathway where the protein-encoding genes are often responsible for the compound breakdown into non-toxic metabolites. However, in contrast to the chemical treatment, the biological processes advance at a much slower kinetic rate, predominantly due to a longer onset period (high lag phase). In fact, more than 90% of the studies reported on the biological degradation of the cyanotoxins attribute the biodegradation to the bacterial suspension. This suspended growth limits the mass transfer kinetics due to the presence of metal ions, NOMs and, other oxidizable matter, which further prolongs the lag phase and makes biological process toxic-free, albeit less efficient. In this context, this review attempts to bring out the importance of the attached growth mechanism, in particular, the biofilm-based treatment approaches which can enhance the biodegradation rate.

1. Introduction

Cyanobacteria are the blue-green algae which form blooms over various surface water sources such as lakes, rivers, and ponds. These microbial communities when subjected to environmental stress (high natural organic matter, metal ions), release cyanotoxins. Drinking water treatment plants (DWTPs) use conventional treatment processes to make these water sources fit for the public consumption. Different cyanotoxins impose various health issues in the human body if consumed for a prolonged period. Cyanotoxins are categorized as a neurotoxin (nerves) and hepatotoxin (liver) based on the target organ/parts of the human body it affects the most. For example, cyanotoxins, such as microcystins and nodularin are the hepatotoxins that primarily affects the liver cells, while anatoxins and saxitoxins are the neurotoxins which mainly affect the nerve cells. Other common human health issues include vomiting, diarrhea, skin irritation, and muscle tumors. These toxins have a very low critical concentration value that affect the human cells. The transformation of these compounds due to their reaction with the other background elements present in the drinking water, such as natural organic matter, chlorine and, ozone can further render the matrix toxic. These toxins also affect the skin (dermatoxins) or the biological membrane where the more hydrophobic cyanotoxins getattached to the skin cells easily, because of the higher surface activity. For example, microcystin variant: MC-LF and -LW interacts more easily

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https://doi.org/10.1016/j.ecoenv.2019.01.066
Received 8 September 2018; Received in revised form 20 January 2019; Accepted 22 January 2019
Available online xxx
0147-6513/

with the biological cells as compared to MC-LR (Vesterkvist and Meriluoto, 2003). Nodularin and microcystin show the similar chemical structure and they both inhibit protein phosphatases which are responsible for binding and blocking the sodium channel in the neural cells. The biotransformation of cyanotoxins has been found to be responsible for causing the signaling effect which relates to the toxicity of the main compound present in the environment matrix. The biotransformation products of microcystin and nodularin are glutathione and S-transferase, while that of CYN is Cytochrome P450. In all these cases, the exact mechanism of biotransformation pathway is not known. However, the possibility of toxin accumulation and storage is evident from these pathways (Wiegand and Pflugmacher, 2005).

Various physicochemical treatments commonly used in the DWTPs including ozonation, filtration, chlorination, can efficiently breakdown and degrade different cyanotoxins. Advanced treatment processes, such as reverse osmosis (RO) and photocatalysis can almost completely remove these cyanotoxins and are rapid. However, incomplete removal of certain persistent cyanotoxins, such as anatoxin and saxitoxin has become a major challenge. Due to their persistent nature, anatoxins and saxitoxins proceed at a slower degradation rate ($< 1\,M^{-1}\ s^{-1}$), where most of the used oxidants have weak oxidation potential (Rodríguez et al., 2007). Even the advanced treatment methods, such as photocatalysis or peroxides or even combination of UV-C/H_2O_2 have failed to achieve more than 50% degradation of such compounds (Tominaga et al., 2011; Tak et al., 2018). Further, the problem of the by-product toxicity persists with most of these physicochemical treatments. Some techniques, such as RO and photocatalysis are energy intensive and thus require high power input to overcome the effects (barrier) of environmental parameters, such as NOMs, salinity, and cyanotoxin concentration as these interfere with the cyanotoxin molecule making the overall process ineffective or less efficient.

A biological process, on the other hand, has advantages over physicochemical treatments, in being more economical, effective (bioadsorption), and most importantly it produces fewer toxic by-products. By-products toxicity becomes very important in relation to the drinking water treatment. Fig. 1(a) shows the chemical structure of the microcystin where all the small peptide structures forming the compound are numbered. The first step involved in the degradation of such a complex structure is hydroxylation followed by the linearization. Biological degradation mechanism comprises the linearization of the complex cyanotoxin structure (especially, microcystins and nodularin) into a series of simple oxidative products via protein-encoding genes, which renders the overall degradation toxic-free. For example, the hydroxylation followed by linearization in the microcystin compound occurs after the cleavage of the Adda (toxic peptide)-Arg bond. The biological process has the potential to fulfill the promise, especially by the *in-situ* bacterial species (where bloom forms), which over the years have shown tremendous success in removing most of these cyanotoxins (Ho et al., 20072007a; Somdee et al., 2013). However, the slow removal rate due to a longer lag phase (as compared to chemical processes) and continuously changing experimental or operating conditions, such as temperature, pH, gene expression. have been an area of concern with these methods. Further study on these parameters and proper validation of results, at least to the pilot scale level, is needed before employing any full-scale bioreactor operation in DWTPs. Also, modification of the existing treatment units in the DWTPs, such as sand filtration unit into a biosand filtration system, can not only effectively reduce the cyanotoxin level in the treated water,but also make it toxic-free and less time-consuming as compared to a solo biological process. Some bacterial species, such as *Sphingomonas sp., Arthrobacter* sp*., Rhodococcus sp*., have even shown a faster removal rate, which can adjust well to the changing environment (Chen et al., 2010; Chen et al., 2010; Neilan et al., 2014). Among the biological methods, attached growth process has the potential as a commercially feasible option in the form of the biological activated carbon filter, biosand filter, bioceramic filter, among others (Itayama et al., 2008).

This review discusses in detail about the biological degradation of cyanotoxins with emphasis on the effects of the chemical structure of cyanotoxins, bacterial population, and the environmental conditions, which affect their degradation. Bioreactor operation studies have been documented at the laboratory and the pilot scale. Many researchers have pointed toward the importance of the lag phase, which is a major parameter for the slow kinetics in a biological process. To the best of author's knowledge, this is the first review providing detailed insight into the possibility of using an attached growth mechanism for the removal of cyanotoxins. Biofilm aspects of cyanotoxin removal are discussed and conceptualized based on some available research studies. Also, the importance of gene analysis is critically discussed to investigate the cyanotoxin removal with reference to the drinking water treatment.

2. Biological treatment of cyanotoxin

Recent advances in molecular microbial ecology have proved that bacterial strains of genera, such as *Sphingomonas sp., Sphingosinicella sp., Arthrobacter sp., Brevibacterium sp., Rhodococcus* sp. and many others are capable of degrading different cyanotoxins within a period of hours to days (Kormas and Lymperopoulou, 2013; Manage et al., 2009). There is evidence that specific members of the planktonic bacteria including *Myxophyceae, Cyanophyta,* which are capable of cyanotoxin degradation, become more efficient in the presence of the toxic cyanobacterial water blooms (Kulasooriya, 2012). This simulates the cyanotoxin-degrading-microorganisms present (*in-situ)* in various water bodies (Mazur-Marzec et al., 2009; Feng et al., 2016). It might be due to an increase in the number of cyanotoxin-degrading genes. A detailed discussion on gene analysis related to cyanotoxin degradation is presented in Section 3.2.

Only a few degradation pathways have been proposed, to date, for the most common cyanotoxin, i.e., microcystin (Bourne et al., 19961996a; Chen et al., 2010). There is no sufficient information on the biodegradation mechanism of other cyanotoxins, such as nodularin, cylindrospermopsin (CYN) and anatoxin-a. However, nodularin has been reported to follow degradation through nda cluster genes found in some bacterial species such as N. spumigena strain AV1 (Jonasson et al., 2008). Other studies proved the delinearization of the nodularin molecule (demethylation pathway) due to the *in-situ* bacterial presence and some other known bacteria, such as *Arthrobacter* sp. strain 206, *C. bacterium* strain 309, *Methylarcula* sp. strain 369, *Cellulophaga* sp. strain 394 and, *Sphingopyxis* sp. USTB-05 (Mazur-Marzec et al., 2009; Feng et al., 2016). Anatoxins (AN) were reported to be degraded through epoxy-AN and dihydro-AN pathway. Water and carbon oxides were found as the end-products as depicted by hybrid quadrupole time-of-flight mass spectroscopy (James et al., 2005). Also, other cyanotoxins, such as CYN were reported to be degraded by Cylindrospermopsis raciborskii AWT205 that possess *cyrK and cyrO* gene (Mihali et al., 2007).

Biodegradation of MC-LR (by *Sphingomonas* sp. ACM-3962) and subsequent formation of the metabolites have been reported due to sequential enzymatic hydrolysis of the peptide bonds (Imanishi et al., 20052005a; Zhang et al., 2016). The *mlr* gene cluster (*mlrA, mlrB, mlrC, mlrD*) plays a pivotal role in this sequential mechanism, forming at least three intracellular enzymes. The *mlrA* gene encodes an enzyme which is responsible for breaking the cyclic structure of MC-LR, which cleaves the Adda-Arg peptide bond (Fig. 1(a)). The *mlrB* gene encodes a putative serine peptidase, which is responsible for the degradation of the linear MC-LR molecule to tetrapeptide H-Adda-Glu-Mdha-Ala-OH, formed after hydrolysis step via *mlrA* gene. On the other hand, *mlrC* gene is responsible for the further breakdown of tetrapeptides into Adda or smaller amino acids whereas gene *mlrD* encodes transporter

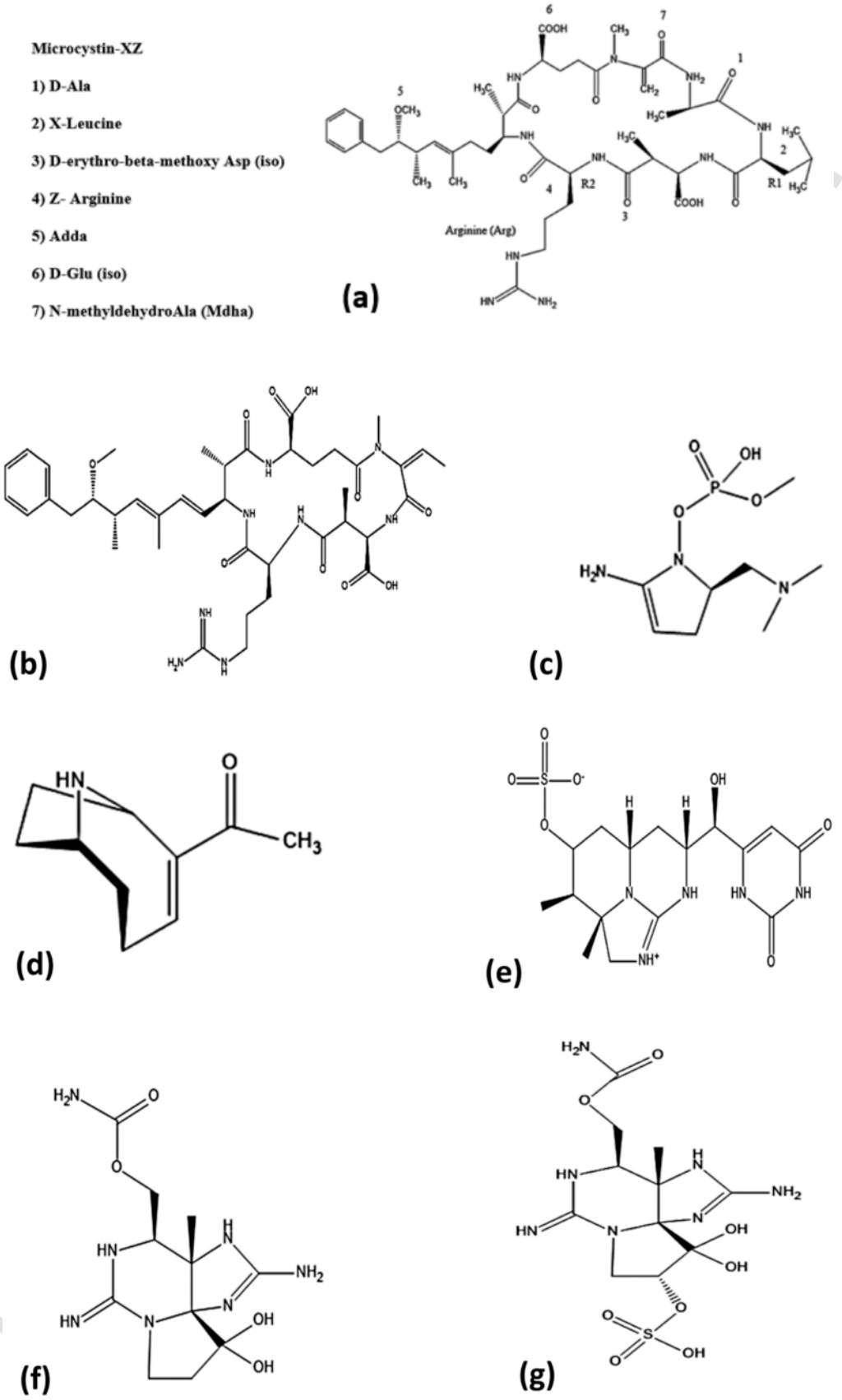

Fig. 1. Chemical structures of a) Microcystin-LR b) Nodularin c) Anatoxina(S) d) Anatoxin-a e) Cylidrospermopsin f) Saxitoxin g) Gonyautoxin (GTX).

proteins, which allow MC uptake into the cell (Edwards and Lawton, 2009). Nodularin has a similar chemical structure (Fig. 1(b)) as compared to MC-LR with an added pentapeptide [cyclo-(D-MeAsp-L-Arg-Adda-D-Glu-Mdha). Thus, generally, the bacteria, which degrade MC-LR are also good nodularin-degraders, possibly due to the encoding of *mlrA* genes responsible for the hydrolytic enzymatic activity (Edwards and Lawton, 2009). Biodegradation mechanism of cyanotoxins like CYN has been difficult to be generalized and is mainly unknown, possibly because they undergo some specific enzymatic pathway (Dziga et al., 2016).

Several bacterial species, capable of cyanotoxin biodegradation have been identified from various water bodies proving that the *in-situ* biological treatment of cyanotoxin holds a promise. Additionally, the formation of toxic metabolites is less extensive as compared to the physicochemical treatment technologies (Maghsoudi et al., 2015; Hu et al., 2012; Mohamed and Alamri, 2012). Some laboratory scale experiments have been tabulated in Table 1 comprising various cyanotoxins and the bacterial species involved in their degradation along with the half-life (of toxin degradation) and total time taken to degrade it. However, biological treatment has not been explored at the pilot or full scale, which may be due to the limited knowledge related to the degradation mechanism and the kinetics of toxin interaction with different bacterial species. Commercialization of biological treatment systems requires an understanding of the cyanotoxins biodegradation along with innovation in the design of the treatment technologies. As such, it is important to investigate the biodegradation mechanism and the rate of cyanotoxins removal in a systematic approach to develop an understanding of the parameters that influence them. Next part of the review will cover the biological aspects of cyanotoxins removal with exclusive emphasis on the biofilm/attached growth methods. The parameters that influence the biodegradation of cyanotoxins, such as chemical structure, bacterial population, and environmental condition are discussed below in the next sections (Sections 2.1, 2.2 and 2.3).

2.1. Biodegradation of cyanotoxins: the effect of bacterial population

Bacteria, isolated from the sediments or other particulate matter present in the water bodies, where cyanobacterial blooms had occurred, can biodegrade both extracellular and intracellular toxins (Bourne et al., 19961996b; Cousins et al., 1996). (Ishii and Abe, 2000) studied biodegradation of microcystin by incubating lake water (Lake Suwa, Nagano, Japan), which was previously found to be contaminated with cyanobacteria. Complete degradation of microcystin (50 mL batch mode with an initial concentration of 20 μg/mL) was found after the addition of bed sediment (by directly putting mud) from the lake. It was also reported that the bacteria isolated from the natural water bodies (lake water), previously known to be contaminated with cyanotoxins (microcystin primarily from *Microcystis* sp.) biodegraded MCs without showing any lag phase (Christoffersen et al., 2002). Also, multiple bacterial strains isolated from lake sediments have been found to be capable of degrading microcystin and other cyanotoxins (Rapala et al., 19941994a). Even the bacterial community (containing *Sphingomonas* sp. ACM-3926) obtained from anthracite biofilter unit (from DWTP) has shown > 80% MC-LR removal in 9–10 days post-enrichment process (Eleuterio and Batista, 2010). These results can potentially lead to an investigation of the *in-situ* bacterial community capable of cyanotoxin degradation. With respect to the DWTPs, investigation of bacterial community isolated from the top layer of the (sand) filtration unit could provide further information on the presence of gene clusters, their enzymatic activity and possible reaction mechanism (that can confirm cyanotoxin degradation with toxic-free metabolites).

Research exploring the *in-situ* bacterial degradation has shown that biodegradation is the most preferred removal choice for such toxins (especially, microcystin varieties) (Maghsoudi et al., 2015; Hu et al., 2012). However, bacterial degradation of CYN sometimes become unpredictable as shown by the results tabulated in Table 1, where the filtered and unfiltered lake water showed no degradation in the presence of the particulate attached bacteria (PABs). On the contrary, sludge treated water, if left unfiltered, resulted in degradation and if filtered, no degradation was observed (Maghsoudi et al., 2015). This explains the fact that the presence of the *in-situ* bacteria, if assisted with some essential metabolites (unfiltered case), can support the biodegradation of cyanotoxins. (Wormer et al., 2008) showed that CYN released from *Aphanizomenon ovalisporum* UAM 290 was not degraded even after 40 days by the bacterial community, previously exposed to the cyanotoxin environment. Additionally, a decrease in the dissolved organic carbon (DOC) was observed in the presence of toxins due to the continuous growth of the bacterial cells utilizing it, giving less priority to the toxins as a carbon source. However, if the concentration of a cyanotoxin is low(< 10 μg/L) in the source water (thus less interference), bioreactors with such bacterial community can be of great use as DOC removal forms an important aspect in the drinking water treatment.

2.2. Biodegradation of cyanotoxins: the effect of chemical structure

Microcystins are the most widely known cyanotoxins being researched to date. MC-LR is a variant, which is known to be the deadliest cyanotoxin in terms of toxicity level (Lone et al., 2015). They are commonly found in almost every cyano bloom water body, such as lakes, ponds, rivers and hence forms a common research aspect for most of the researchers (Zhang et al., 2016; Chen et al., 2016). They are produced by cyanobacteria of the genera *Anabaena, Microcystis, Nostoc* and *Oscillatoria* (Planktothrix) (Lyra et al., 2001) consisting of cyclic heptapeptide hepatotoxins [cyclo-(D-Ala1-X2-D-MeAsp3-Z4-Adda5-D-Glu6-Mdha7)] (shown in Fig. 1(a)). They are very stable in the environment and resistant to eukaryotes and many bacterial peptidases. Nonetheless, they can be naturally degraded by some *in-situ* aquatic bacteria found in rivers and reservoirs, as already discussed in the previous section. These bacteria can be found either in sewage effluent, lake water, lake sediment or river water. There are studies that observed no degradation of microcystin over a period of more than 3 months by an inoculum taken from the river (Kiviranta et al., 1991). This indicates the importance of identifying specific microorganisms that are capable of degrading microcystin. This might happen due to non-breakage of adda-arg bond in the microcystin structure and thus retain the ring-like structure (Dziga et al., 2016). Also, the presence of different carbon source present in distinct water ecosystem affects the microcystin degradation pathway where even an MC-degrading microorganism (previously reported to degrade MCs) may not degrade it. A study by Jones et al. (1994) (Bourne et al., 19961996b), isolated *Sphingomonas* sp. that led to the ring opening of MC-LR and produced a linear compound which was nearly 200 times less toxic than the parent compound.[1] The same bacterium, however, did not respond well in the case of cyclic pentapeptide: nodularin, indicating the influence of chemical structure and need of the enzyme specificity for the biodegradation of a cyanotoxin. Thus, the chemical structure of cyanotoxin becomes an important aspect when a bacterial species is utilized to study their degradation.

Little work has been done to date on biodegradation of other cyanotoxins, such as anatoxins, saxitoxins, cylindrospermopsin as compared to microcystins. Bacterial species, such as *Pseudomonas* sp. was reported to biodegrade anatoxin-a with a removal rate of 6–10 μg/L per 3 days (Kiviranta et al., 1991). Considering the degradation of anatoxin-a

[1] Mouse bioassay. Intraperitoneal injection of purified microcystin LR and degradation products into adult white mice was performed by Mandy Choice, University of New England.

Table 1
Biodegradation performance of different cyanotoxins[4,5,6].

Cyanotoxin	Bacteria	Lag period; Initial Concentration	Half-life of toxin	Full degradation period	Degradation efficiency	Reference
MC-RR	*Bacillus flexus* SSZ01	NSD; 10 mg/L	1.7 days	5d	100%	(Alamri, 2012)
MC-LR	*Sphingomonas* isolate NV-3	NSD; 25 μg/mL	< 1 d	3 d	100%	(Somdee et al., 2013)
MC-RR	*Sphingomonas* CBA4	200 μg l^{-1}	18 h	36 h	100%	(Valeria et al., 2006)
MC-LR	*S. acidaminiphila* MC-LTH2	NSD; 21.2 mg/L	5.5 d	7 d	100%	(Neilan et al., 2014)
MC-RR	*S. acidaminiphila* MC-LTH2	3 d; 39.2 mg/L	5.5 d	7 d	100%	(Neilan et al., 2014)
MC-LR	Bacillus sp.	NSD; 2.15 mg/L	18 h	24 h	100%	(Hu et al., 2012)
MC-LR (lake water)	Particulate attached bacteria (PAB)	4-6d; 10 μg l^{-1}	5.4d	14d	100%	(Maghsoudi et al., 2015)
MC-LR (Filtered lake water)	(No PAB presence)	12d; 10 μg l^{-1}	14.6d	24d	100%	
Sludge	(PAB)	< 2d; 10 μg l^{-1}	2.7d	19d	100%	
MC-LR (Filtered sludge)	(No PAB presence)	6d; 10 μg l^{-1}	8.8d	21d	100%	
MC-YR(lake water)	(PAB)	4-6d; 10 μg l^{-1}	5.4d	14d	100%	
MC-YR (Filtered lake water)	(No PAB presence)	12d;10 μg l^{-1}	13.9d	24d	100%	
MC-YR (Sludge)	(PAB)	< 2d;10 μg l^{-1}	2.3d	19d	100%	
MC-YR (Filtered sludge)	(No PAB presence)	6d; 10 μg l^{-1}	8.5d	21d	100%	
MC-LY (lake water)	(PAB)	4-6d;10 μg l^{-1}	8.5d	8.5d	100%	
MC-LY (Filtered lake water)	(No PAB presence)	12d;10 μg l^{-1}	------	------	-----	
MC-LY(sludge)	(PAB)	< 2d;10 μg l^{-1}	2.8d	19d	100%	
MC-LY (Filtered sludge)	(No PAB presence)	6d; 10 μg l^{-1}	-----	-----	-----	
MC-LW (lake water)	(PAB)	4-6d;10 μg l^{-1}	8.8d	8.8d	100%	
MC-LW (Filtered lake water)	(No PAB presence)	12d;10 μg l^{-1}	----	----	------	
MC-LW (Sludge)	(PAB)	< 2d; 10 μg l^{-1}	3.4d	19d	100%	
MC-LW (Filtered sludge)	(No PAB presence)	6d; 10 μg l^{-1}	-----	-----	------	
MC-LF (lake water)	(PAB)	4-6d; 10 μg l^{-1}	8.3d	8.3d	100%	
MC-LF (Filtered lake water)	(No PAB presence)	12d; 10 μg l^{-1}	---	---	------	
MC-LF (Sludge)	(PAB)	< 2d; 10 μg l^{-1}	3d	19d	100%	
MC-LF (Filtered sludge)	(No PAB presence)	6d; 10 μg l^{-1}	----	----	-----	
CYN (lake water)	(PAB)	4-6d; 3 μg l^{-1}	---	---	-----	
CYN (Filtered lake water)	(No PAB presence)	12d; 3.25 μg l^{-1}	---	---	------	
CYN (Sludge)	(PAB)	< 2d; 3 μg l^{-1}	6.1d	17d	Approx 95%	
CYN (Filtered sludge)	(No PAB presence)	6d; 4.25 μg l^{-1}	---	---	----	
MC-LR	*Stenotrophomonas sp.*	NSD; 700 μg/L	13 h	24 h	100%	(Chen et al., 2010)
MC-RR	*Stenotrophomonas sp.*	NSD; 1700 μg/L	11 h	24 h	100%	(Chen et al., 2010)
MC-LR	L.Rhamnosus LC-705	NSD;100 μg l^{-1}	15 h	24 h	Approx 60	(Nybom et al., 2012)
MC-LR	B.Longum 46	NSD;100 μg l^{-1}	12 h	24 h	Approx 70	(Nybom et al., 2012)
MC-LR	*Bacillus sp.*	NSD; 100 μg/L	2d	4d	100%	(Kansole and Lin, 2016)
MC-LR	*Bacillus sp.*	2-7 d; 220 μg/L	2d	12 d	> 74%	(Kansole and Lin, 2016)
		NSD; 100 μg l^{-1}	4d	7d	100%	
		NSD 100 μg l^{-1}	6d	8d	100%	
MC-LR	Sphingopyxis genes (isolate LH21)	NSD; 25 μg l^{-1}	> 10d	12d	100%	(Ho et al., 20072007a)
		NSD; 10 μg l^{-1}	3d	4d	100%	
		NSD; 3 μg l^{-1}	1d	2d	100%	
	Sphingomonas ACM-3962	NSD; 25 μg l^{-1}	12d	15d	100%	
		NSD; 10 μg l^{-1}	4d	7d	100%	
MC-LA	Sphingopyxis genes	NSD; 30 μg l^{-1}	11d	12d	100%	

[4] NSD: No significant delay
[5] ------- Not biodegraded
[6] ------- Not biodegraded

Table 1 (*Continued*)

Cyanotoxin	Bacteria	Lag period; Initial Concentration	Half-life of toxin	Full degradation period	Degradation efficiency	Reference
		NSD; 10 μg l^{-1}	3d	4d	100%	
		NSD; 5 μg l^{-1}	1d	2d	100%	
	Sphingomonas ACM-3962	NSD; 30 μg l^{-1}	14d	15d	100%	
		NSD; 10 μg l^{-1}	7d	9d	100%	
MC-RR	Sphingopyxis sp. USTB-05	1d; 50 mg/L	2d	3d	100%	(Zhang et al., 2010)
Microcytin-LR	*Microbacterium, Ochrobactrum anthropi*	NSD; 250 μg/L	10 d	> 30 d	84%	(Ramani et al., 2011)
NOD^^	Natural microbial population	4-5d; 1 μg ml^{-1}	15d	15d	100%	(Edwards et al., 2008)
MC-LR	*Pseudomonas aeruginosa*	< 1d; 1 μg/L	> 15 d	24 d	100%	(Ramani et al., 2011)
MC-LR and MC-LA	Sphinopyxis sp.	NSD; 2.17 mg/L(MC-LR) and 3.27 mg/L(MC-LA)	< 2 d (both) (8d)	8d	100%	(Ho et al., 20072007b)
MC-LR	Morganella morganii	NSD; 20 μg/L	6d	9d	100%	(Eleuterio and Batista, 2010)
MC-LR	*Arthrobacter spp.,*	ND; 5 μg/mL	ND	3 d	84% (2 days), 100% (3 days)	(Manage et al., 2009)
MC-LR	*Brevibacterium sp.*	ND; 5 μg/mL	ND	3 d	23% (2 days); 100% (3 days)	(Manage et al., 2009)
MC-LR	Stenotrophomonas maltophilia	NSD; 5 μg/mL	5 d	10 d	100% (10 days)	(Idroos et al., 2017)
MC-LR	*Ralstonia solanacearum*	1d; > 25 mg/L	2 days	3 d	100% (3 days)	(Zhang et al., 2011)
MC-LR	*Rhodococcus sp.*	ND; 5 μg/mL	ND	3 d	64% (2 days); 99% (3 days)	(Manage et al., 2009)
CYN	*Bacillus strain* (AMRI-03)	NSD; 300 μg/L	3.5 d	6 days	100%	(Mohamed and Alamri, 2012)
		NSD; 100 μg/L	3.75 d	7 days		
		NSD; 10 μg/L	5.5 d	8 days		
CYN	*Aeromonas* sp.	1 d; 3000 μg/L	13d	14 days study (< 50% degradation)	< 50%	(Dziga et al., 2016)
CYN	Aphanizomenon ovalisporum UAM 290	100 μg/L	----	40 days (almost no degradation)	< 5%	(Wormer et al., 2008)

*Probiotic bacterial cells; #enzymatic presence shown faster degradation than probiotic bacterial cells; PAB particulate attached bacteria;
^^ One of the many results database; NSD: No significant delay.

in the cyanobacterial bloom (crude extracts of *Anabaena* sp. strain 123), it was reported that the bacteria present in sand and mud sediments, (obtained from lake Tuusulanjarvi, Finland) degraded it from 2.4 μg/mL to < 0.010 μg/mL in 3 weeks under a batch-mode system (Rapala et al., 19941994b). Saxitoxins are globally less significant and found locally in and around Australia, where *Anabaena circinalis* produces a range of saxitoxins. Their biodegradation is less studied as compared to the other cyanotoxins.

Moreover, for different cyanotoxins, due to the difference in chemical structure and enzymatic degradation pathway, the toxicity level of the formed by-products, ranges from less toxic to even more toxic than the parent molecule. For example, (Bourne et al., 19961996a) observed that the biodegradation of MC-LR by *Sphingomonas* sp. produced 200 times lesser toxic by-products (dosage up to 250 μg/kg was non-toxic to mice). On the other hand, it was observed that the biological removal of saxitoxins led to their biotransformation to gonyautoxins (GTX) because of structural modifications, which was found to be more toxic[2] than the parent saxitoxin (Kayal et al., 2008).

(Donovan et al., 2008), isolated bacteria from the digestive tracts of blue mussels (*Mytilus edulis*) and cultured them using marine agar which responded well for saxitoxin removal, degrading almost 100% within 1–3 days. A study by (Tang, 2012), found biodegradation rate of different cyanotoxins in the order, MC-LR (2.6×10^{-2}/h) > cylindrospermopsin (9.6×10^{-4}/h) > saxitoxins (not detected) based on pseudo-first-order rate constants. Thus, under similar experimental conditions, saxitoxin was not degraded owing to a different chemical structure and specific enzymatic degradation pathway. This suggests that either the bacteria capable of degrading saxitoxins are few in numbers or they feed on other primary substrates available in natural water matrices, such as NOMs. (Edwards et al., 2008) reported that various cyanotoxins, such as nodularin, MC-LF, and MC-LR found in different lakes and one river water source (Table 1), were removed by the indigenous bacterial population (already present as a part of bloom) variably under similar experimental conditions. The half-life of 5 days for MC-LR; 9 days for MC-LF and 15 days for nodularin and complete removal in 12 days for MC-LR; 15 days for MC-LF and 18 days for nodularin were reported. Nodularin showed the slowest removal rate as compared to both types of microcystin with almost no degradation in one of the source water.

Biodegradation of hepatotoxins, such as CYN has been studied to a limited extent. Given the fact that these are water-soluble cyanotoxins and are a major threat to the aquatic environment, due to their stable and persistence nature (Bláha et al., 2009), it is imperative to screen potential bacterial species that might be capable of biodegrading these cyanotoxin species. Also, as discussed earlier, the various enzymatic degradation pathways (based on chemical structure) for different cyanotoxins is responsible for biodegradation via specific bacterial species (Pearson and Neilan, 2008).

Various kind of cyanotoxins needs different parameters to be optimized for their enhanced degradation. For example, the initial concentration of these toxins for studying the batch degradation by specific bacterial culture is one of the most crucial factors (Table 1) affecting the degradation rate (measured as cyanotoxin degraded/day). Higher

[2] Concentrations of the saxitoxins were determined by calibration of the peak areas with that of certified reference standards (Institute of Marine Biosciences, National Research Council, Canada). Conversion factors (Oshima, 1995) were used to express the toxicity of the sum of the variants as saxitoxin toxicity equivalents (STX-eq) due to the differing toxicities and concentrations of the individual saxitoxin variants (as read in manuscript with reference no 41).

the initial toxin concentration (Table 1), the higher will be the degradation rate as evident from various studies (Hu et al., 2012; Mohamed and Alamri, 2012; Saito et al., 2003). Biodegradation rates for different microcystins have been reported as high as 2.99 mg/L/d for microcystin-RR when the initial concentration was 3000 μg/L (Hu et al., 2012) to as low as 0.00125 mg/L/d for CYN for the initial concentration of 300 μg/L (Mohamed and Alamri, 2012). These biodegradation rates are much slower as compared to the degradation of cyanotoxins via chlorine oxidation process, where degradation rate revealed as high as 80,000 mg/L/d (Tsuji et al., 1996). However, the use of chlorine is challenged by the production of toxic by-products (Kull et al., 2004). Table 1 shows some studies where the degradation rate can be related to the initial concentration of various cyanotoxins. Higher biodegradation rate is expected at a higher initial concentration of cyanotoxins because of an increase in *mlrA* genes (in the case of MCs), which are primarily responsible for toxin degradation (Lezcano et al., 20162016a). Smith et al. (2008) (Hu et al., 2012) hypothesized this understanding further and stated that higher toxin concentration acts as an inducer which activates the genes responsible for the synthesis of enzymes involved in the degradation. To support this understanding, a study by (Mohamed and Alamri, 2012), where CYN degradation by *Bacillus* sp. (isolated from the lake that experienced algal bloom) was studied. At an initial CYN concentration of 10–300 μg/L, highest biodegradation rate (50 μg/L/d) was obtained at 300 μg/L and the lowest (1.25 μg/L/d) at lowest toxin concentration (10 μg/L). As can be seen from Table 1, different cyanotoxins under distinct bacterial species responded variably in terms of degradation rate based on the initial concentration of cyanotoxins (due to different genes involved in each of them: *mlrA*, *nda*, *stx*, *cyn* for microcystin, nodularin, saxitoxins, and cylindrospermopsin, respectively). The initial concentration of cyanotoxin becomes an important criterion especially when it comes to drinking water treatment. Knowing the fact that the high concentration (> 300 μg/L) of cyanotoxin present in source water proceeds at a faster degradation rate (as discussed above), such high concentration is not expected in a real scenario. Most time of the year, even in the peak summer-autumn condition, the concentration hardly reaches 30 μg/L (Shang et al., 2018). It will be very interesting to study in future, the aspect of the toxin-degrading gene (eg.: *mcyE* in case of MCs) at "low" concentration to evaluate for their persistence that will pre-determine an effective cyanotoxin treatment.

2.3. Biodegradation of cyanotoxins: the effect of environmental conditions

Environmental conditions, such as temperature, pH, organic matter, affect cyanotoxin degradation. (Park et al., 2001) reported that an increase in temperature from 5 °C to 30 °C led to an increase in microcystin-biodegradation rate by 7 mg/L/d. In another study by (Wang et al., 2007), they observed complete MC-LR degradation at the initial concentration of 5 μg/L (spiked) in just 3 days at 33 °C, while it took 6 days at 22 °C. Akin to microcystins, CYN degradation is also dependent on temperature and pH. For example, it was found that at 25 °C and 30 °C, CYN was fully degraded in 7 days as compared to only 20% degradation at 10 °C for the same duration while no degradation was observed at a pH of 11 as compared to 100% degradation within 7 days at neutral pH (Mohamed and Alamri, 2012). Also, the same dependence on CYN degradation (by *Aeromonas* sp.) with temperature and pH was reported, whereas at 20 °C and 30 °C, the degradation was higher than 40% as compared to 7% at 4 °C, while degradation of mere 6.5% at pH 9.5 to over 40% at pH 6.5 was obtained (Dziga et al., 2016).

Presence of biodegradable organic matter affects the cyanotoxin removal too, as they are in competition for biodegradation by bacteria, thereby lowering toxin removal efficiency. (Eleuterio and Batista, 2010) observed that the presence of 30 mg/L of acetate carbon suppressed the biodegradation of MC-LR at an initial concentration of 100 μg/L using *Sphingomonas sp*. ACM-3926 (SACM). Biofilter bacterial culture and bacteria enriched from lake water source (two lake water sources: Lake Mead and anthracite biofilter fed with Colorado river (LAAFP)), resulted in the biodegradation rate of 12.1 μg/L/d, 7.2 μg/L/d and 5.3 μg/L/d for SACM, LAAFP and Lake Mead bacterial culture, respectively. However, without the presence of acetate, the biodegradation rate was found to be higher (13.8 μg/L/d, 12.5 μg/L/d and 11.2 μg/L/d correspondingly). This was attributed to the repression of microcystinase synthesis (*mlrA* gene) by acetate, which was easier to metabolize as compared to cyanotoxins (Eleuterio and Batista, 2010).

Nutrient conditions also play a key role in cyanotoxin degradation. It was reported that under various nutrient conditions (glucose, phosphate, and nitrate), cyanotoxin degrading-bacteria, isolated from biofilm (of a water treatment unit) were capable of complete biodegradation of MC-LR at the initial concentration of 100 μg/L within 10 days (Li et al., 2012). However, with the use of glucose or phosphate, the biodegradation was delayed by 3 days. On the other hand, the addition of dual nutrients (glucose with nitrate) alleviated this inhibitory effect. Lower bacterial activity in the presence of nutrients was attributed to the formation of microcystin metabolic products, resulting in reduced enzymatic activity of microcystin-degrading bacteria. However, in a recent study by (Idroos et al., 2017), it was shown that the presence of phosphate and nitrate ions enhanced the biodegradation rate of MC-LR (phosphate: 0.02 ppm increased degradation rate from 0.3 μg/d to 0.4 μg/d (33% increase) while nitrate ions increased the degradation rate by almost 100% (from 0.2 to 0.4 μg/d) at nitrate concentration of 0.45 ppm. In general, under the nutrient conditions, bacterial biofilm can even sustain their degradation activity during winter. The presence of nutrients influences the MCs biodegradation, especially after a bloom collapse when the MCs gets released. It is due to the fact that cell debris or other nutrients from water columns may later serve as an alternative carbon or nitrogen sources (Lezcano et al., 20162016b). Under these conditions, both *mlr*+ and *mlr*- genes present in the bacterial community (isolated from natural water sources) may respond differently in the degradation of MCs. (Lezcano et al., 20162016b) studied the biodegradation effect in the presence of different nutrient conditions:1.9 mg/L of TN (MSM), 0.05 mg/L of TN (reservoir water and R2A medium) with mlr^+ gene strains (identified as Sphingopyxis sp.) and mlr^- gene strain (*Paucibacter toxinivorans*) isolated from MCs degrading bacterial communities. They not only found difference in the degradation behavior between *mlr*+ strains (100% degradation in 6 h: 1 mg/L initial MCs concentration) and *mlr*- gene strains (more than 120 h), but also the degradation rates also varied, as reported highest, lowest rate: 171 μg/L/h, 37 μg/L/h and 21 μg/L/h, 8 μg/L/h, for mlr^+ and mlr^- strains, respectively. Even some cyanotoxins, such as anatoxins-a were naturally degraded by aquatic macrophyte present as the natural factor in the native environment. A study by (Kaminski et al., 2015) found that the presence of *Lemna trisulca* in the aquatic environment decreased the biomass concentration of *Anabaena flos-aquae* along with the reduction in anatoxin concentration (by 78.6% in media and 99.9% in cyanobacterial cells) at the expense of phosphate ions uptake (when N/P increased from 21.2 from 14.7).

Under the nutrient-limited conditions, biosynthesis of cyanotoxins via toxic strains showed almost 10 times higher toxicity than the nutrient-rich condition ((Pimentel et al., 2014))NtcA (global nitrogen regulator) has been described as a potential component for controlling the MCs synthesis. These strains in the long run might become more resistant to the nutrient limiting conditions and may pose difficulty in their degradation as well as toxicity reduction. In a DWTP, such nutrient limitation condition is prevalent which successively decreases for each treatment units (Volk and LeChevallier, 1999). Considering a case where nutrient limited cyanobacterial cells reside over the top layer of the biosand filter in a DWTP, the treatment might become a challenge.

In this case, the real challenge will be to study the symbiotic relationship between the cyanobacterial cells (which are resistant and capable of producing more toxic cyanotoxin) with other residing bacterial communities. The situation can become worse if the source water is assisted by high NOMs or other foreign intrusions as the biofilter under the nutrient-deprived condition, not only have to deal with the released cyanotoxin (which is 10 times more toxic than normal) but also the higher concentration of NOMs. Downstream treatment units, such as disinfection and post-ozonation can further form toxic metabolites if a large concentration of MCs is present in the filtered effluent. Thus, in a DWTP unit, a regulatory check on the growing population of such toxic cyanobacterial strain needs to be performed to provide an overall toxic-free filtered water under nutrient-limited conditions.

The presence of metal ions also affects the biodegradation of cyanotoxins. Not many studies have been reported on cyanotoxin biodegradation in the presence of the metal ions, to date. However, some metal ions, such as Fe^{3+} (in the form of $FeCl_3$) with a concentration of 100 mg/mL was reported to be effective in MC-LR decomposition with 50% decrease in its concentration within 10 min (Takenaka and Tanaka, 1995). Also, it was reported that these aluminum and iron ions potentially reduced quantification level of the microcystin with maximum reduction shown to be 32% and 82% with metal ions concentration of 1.2 mg/L and 0.1 mg/L, respectively (Oliveira et al., 2005). MC-LR undergoes complete removal after 1 h of treatment with Fe^{6+} ions (20 mg/L) pointing at the strong oxidizing property of this ion which breaks the peptide ring of the toxin (Yuan et al., 2002). Thus, the strong oxidizing property of metal ions along with enzymes secreted by cyanotoxin-degrading bacteria, might enhance the oxidizing property of these metal ions and quickly linearize the cyanotoxin cyclic structure (Kappler, 2011; Karigar and Rao, 2011). However, in a DWTP, use of metal ions mainly comes into play during the coagulation/flocculation step. Use of ferric chloride as a coagulant is prevalent in DWTPs. A dose of 0.2–0.6 M of ferric chloride has been found to be effective for the coagulation purpose (turbidity removal) which depends on the quality of the source water undergoing treatment (Tang and Stumm, 1987; Kalavathy et al., 2017). Considering the above statements and the requirement of Fe ion for the complete MCs removal, the residual concentration of these ions needs to be ensured after the coagulation/flocculation step. These residual concentrations might affect the biodegradation efficiency of the biofilter present as a next treatment unit in-line with the flocculation/coagulation-sedimentation step. Not only this, but the final effluent needs to be ensured that it falls within the water quality guidelines (< 0.3 mg/L or < 2 mM). A wide gap in relation to the influence of metal ions on cyanotoxin-degrading bacteria needs to be filled and thus more studies and research is required for the same.

3. Bioreactor operation for cyanotoxins removal

Biodegradation of cyanotoxins has mainly been studied at the lab scale in the form of batch bioreactors. However, at pilot scale limited to no work has been done (Romero et al., 2014). Also, apart from microcystin, there have not been much-documented studies for other cyanotoxins especially in the context of the immobilized/fixed biofilm reactors. Variety of bioreactors successfully employed at laboratory scale include a biological sand filter, biological activated carbon filter and other simple designed bioreactors (Itayama et al., 2008; Keijola et al., 1988). These bioreactors with respect to their configuration, biodegradation efficiency and types of bacteria involved are discussed in this section.

(Tsuji et al., 2006)) investigated the degradation of MC-LR and microcystin-RR (600 μg in 3-liter water) by strain B-9 (closely related as *Sphingomonas sp*.) with the aid of immobilized carrier (polyester) bioreactor. Both microcystin variants achieved complete degradation within 24 h. However, no study was reported using immobilized reactors, and researchers relied more on the bioreactors in the form of biofilter which proved to be successful for cyanotoxin removal (both at lab scale and pilot scale). Biofilter works mainly on two principles viz. adsorption and degradation. Biological interference (bacterial cells or biofilm formation over the carrier media) has been shown to enhance the adsorption property of a filter media. For example, in a pilot-scale study, biological activity on GAC favored the removal of anatoxins not only proving its adsorption, but also the biological process being an important aspect in achieving overall toxin degradation (UK WIR, 1995). Many authors have reported the versatility of biological filter based on GAC that has shown successful MCs degradation (Wang et al., 2007; Ho and Newcombe, 2007). (Newcombe, 2002) noted that biodegradation in GAC filters resulted in a significant microcystin removal ($> 80\%$). Biological influence on physical adsorption enhanced the efficacy of the filters in removing cyanotoxins. For example, (Wang et al., 2007) found sterile GAC column able to remove MC- LR and MC- LA to only about 70% and 40%, respectively. But later, with bioactivity intrusion i.e. bacterial cell growth over GAC showed an increase in their overall removal up to 90% and 70%, within 38 days of operation, respectively. The removal of microcystin through a rapid sand filter was also examined where it was found that the biological degradation was the primary removal mechanism rather than the physical adsorption (Wang et al., 2007). A laboratory scale evaluation of cyanotoxin removal by microcystis and planktothrix was studied using a slow sand filter, where 80% and 30–65% removal was reported, respectively. Since filter alone cannot be expected to remove all the extracellular toxin, biosorption with some biotransformation could be the dominant mechanism during the process (Keijola et al., 1988). (Bourne et al., 2006) studied MC-LR degradation spiked at 50 μg/L (continuous mode of operation where settled water of 20 L volume was transferred daily) in six slow sand filters at pilot scale. After inoculating the filter with *Sphingomonas* sp., complete removal of MC-LR was obtained within 6 days with a faster degradation rate as compared to the non-inoculated filters.

With reference to the DWTPs, (sand) filtration unit holds the only possible treatment aspect where the biological system can be introduced. Also, biological degradation has been proven to undergo toxin-free water treatment as compared to other physicochemical treatments. However, the above-discussed studies critically failed to analyze the duration it takes for the cyanotoxin treatment. In a DWTP, the residence time of 15–25 min is generally followed for the filtration unit operation. Owing to this constraint, it might be difficult for the attached microorganisms to avail enough contact time between them and the cyanotoxin molecule. Also, the plant operator needs to continuously monitor the release of cyanotoxin-degrading genes (eg.: *mlrA* for MCs) post biofilm maturation because the effective treatment is limited by the time period as discussed earlier. Enough gene copies (for eg.: *mlrA* gene copies for microcystins) will ensure the degree of cyanotoxin treatment required to have better control over the biological process (Zhu et al., 2014).

This aspect of the hybrid approach (physical and biological) can also be considered, if adsorbing media is improved. Such improvement in the widely used filter media (prominently sand and activated carbon) can be improvised to provide a more effective surface area enabling enhanced biological degradation by eliminating the limitation posed mainly due to lag phase. Also, the cyanotoxin degrading-bacteria must have the potential to form a stable, long-term biofilm. For an attached growth process, adsorption behavior is essential to evaluate before using them in a bioreactor. (Ho et al., 2006) assessed biological sand filtration (attached biofilm) in a laboratory column for MC-LR and microcystin-LA removal at an initial concentration of 20 μg/L. Biodegradation was found to be the primary mechanism (removal through adsorption was secondary). Bacteria present in the biofilm (formed due to continuous-fed reservoir water over the sand collected

from DWTP) resembled with *Sphingopyxis* sp. C- 1 (NCBI accession number AB161685) and next match to *Sphingomonas* sp. ACM-3962 (NCBI accession number AF411068), both found to encode *mlrA* gene. Though the lag period lasted for 3 days, still complete biodegradation was achieved in about 4 days. Also, biofilm formation varies from surface to surface (Garrett et al., 2008). For example, Somdee et al. (2013) (de Freitas et al., 2013), showed that *Sphingomans* (isolate NV-3) bacteria capable of degrading MCs could form a biofilm on ceramic material followed by polyvinyl chloride (PVC), polystyrene, stainless steel, and glass coupon too. They used an internal airlift loop (ceramic honeycomb support) bioreactor to degrade microcystin. Both continuous and batch processes were studied and 100% microcystin degradation was reported. For the same initial concentration (25 μg/mL), in a batch experiment, MC-LR achieved full degradation in just 30 h whereas, in a continuous process, it took 36 h. Thus, not much difference between the mode of operation was observed for the biofilm-based reactor to treat MCs. With further improvement in the process by decreasing the lag period through cell culture enrichment (discussed in Supplementary file, Fig. S1), the cyanotoxin removal can be rapid or be suitably studied with more effective adsorptive surfaces (such as bentonite, clay or activated carbon).

(Miller and Fallowfield, 2001)) analyzed half-lives of microcystin and nodularin due to adsorption, desorption, and degradation involved during biodegradation using different soil media (clayey soil, sandy soil). It was found that degradation (among the three processes stated above) showed the highest half-life for both the toxins, suggesting that this process was likely to be the rate-limiting step. This again brings attention to the fact that, the degradation can limit the rate of complete toxin removal when complemented with adsorption (Wu et al., 2011). It may also be possible to reduce this limitation by decreasing the lag phase in the bioreactor (as discussed earlier) which is an important parameter to study bacterial degradation of cyanotoxins. This parameter may also be used in biofilm (attached growth system) methods for the cyanotoxins removal. Lag phase and its impact on suspended growth methods of cyanotoxin removal are discussed further in the next subsection and also in Table 1.

3.1. Importance of lag phase in biological treatment of cyanotoxins

The length of the lag phase is influenced by the type of bacteria involved in the biodegradation as well as the type of cyanotoxins. After the lag phase, once the degradation commences, it usually takes 2–10 days to achieve complete removal, for most of the cyanotoxins (Table 1) (Maghsoudi et al., 2015). However, these results also depend on the initial toxin concentration, temperature, and type of the source water subjected to treatment (Bourne et al., 19961996b; Lahti et al., 1997b1997). Lag phase plays a key role in deciding the operation of a bioreactor. This phase can be as high as months and as low as minutes. It can be reduced by taking help of the toxin-degrading bacteria obtained from the natural environment. It can be expected that these *in-situ* bacterial communities, will be able to degrade toxins along with other organic compounds (Christoffersen et al., 2002). For example, (Zhang et al., 2010) showed that MC-RR was degraded by *Sphingopyxis* sp. USTB-05, isolated from lake water showing very high removal rate (16.7 mg/L/d) with just 1 day of lag phase for the total degradation period of 3 days (Table 1). While (Edwards et al., 2008)), studied six different lake water samples to evaluate MC-LR degradation, found that every lake water sample showed different lag phases, ranging from 0 days to 10 days. It stated that source water condition containing different organic matter might influence the lag phase differently for the same toxin being studied.

Even nutrient limitation can shift the metabolic pathways of the bacteria responsible for the cyanotoxin degradation into some other secondary activity before the primary activity is resumed (Cherry and Thompson, 1997). A biofilter may face these events based on the change in seasons where source water may change its characteristics. A sudden change in the nutrient limitation can prolong the formation of a fresh cycle of biofilms. However, the effects of a biofilm system formed over the filter media might be under nutrient deficiency for a few micrometers (< 100 μm) (Anderl et al., 2003). Rest zone (depth of biofilm thickness) of the formed biofilm might still work for the cyanotoxin degradation. A new perspective of this observation can also be linked to the strain-type of bacteria forming biofilm and be responsible for cyanotoxin degradation. The role of gram-negative and gram-positive bacteria cannot be overlooked especially, if diffusion of oxygen in the biofilm zone is limited by the nutrient or substrate availability. A thick (> 55 nm) peptidoglycan layer in the cell structure for the gram-positive bacteria can resist the oxygen diffusion along with the cyanotoxin molecule to undergo less effective degradation. However, gram-negative bacteria can allow the diffusion a lot easier with lesser resistance due to lower thickness of the cell wall altogether (< 10 nm) (Mai-Prochnow et al., 2016). Such resistance in the diffusion of the substrate and molecular oxygen is important to explore and relevant to the cyanotoxin degradation as the anoxic zones (deeper biofilm zone) may not be able to mineralize the toxin along with the organic matter properly.

Apart from the organic matter, the lag phase and ultimate degradation also depend on the kinds of cyanotoxin being studied. For example, in the same study, nodularin and MC-LF degraded slowly as compared to MC-LR (12 days as compared to 18 days and 15 days for nodularin and MC-LF respectively) with the reported lag phase of 3 days each for nodularin and MC-LF whereas no lag phase was observed for MC-LR. This lag phase effect can be further improved by acclimatizing these bacteria to sequential concentrations of the cyanotoxins. Table 1 shows some studies (showing lag period) which considered a range of bacteria capable of degrading different cyanotoxins, such as microcystin variants (LR, RR, YR, LW, LA, LF), nodularin and cylindrospermopsin (CYN), anatoxins and saxitoxins.

It is advisable to carry out the enrichment of bacteria present in lake water or sediment samples to enhance their degradation capability which reduces lag phase and hence the time for achieving full/saturated degradation (Ramani et al., 2011; Kumar et al., 2018). Enrichment also helps to screen out the potential bacteria (one or more kind of them) out of various possible species present in the source water (e.g. from lake or river, reservoir) for degrading cyanotoxin (Valeria et al., 2006). After enrichment, it is possible to isolate one or two strains that eventually turn out to be a potent toxin-degraders. (Valeria et al., 2006) found that initial enrichment took almost 14 days to degrade microcystin RR (initial concentration 200 μg/L), but after acclimatization, by successive sub-cultures, it took just 3–4 days to achieve complete degradation. Also, Smith et al. (2008) (Hu et al., 2012) reported rapid degradation of CYN by natural bacteria (found in the dam water) within 6 days, after three successive enrichment while it took 20 days for complete removal without enrichment. Investigating the biodegradation capacity of various bacterial species isolated from natural water, sediments, and existing filtration systems will provide insight into the design of bio-treatment process with a reduced lag phase that can achieve higher biodegradation rate for a variety of cyanotoxins in a bioreactor. An important aspect that also comes handy while designing the bioreactors, is the hydraulic retention time (HRT), which forms an integral part in any continuous bioreactor operation. The HRT study needs to be properly analyzed and correlated with the lag phase. For example, if HRT of the proposed/modified bioreactor is less than the lag period of the biodegradation process, the whole process may be just 10–20% efficient, while the other way around it can be relatively much more efficient. The future studies can be done on the continuous bioreactor where a different dose of various cyanotoxin can be tested with distinct HRTs. For the plant operator, it will be easy to channelize the

continuously flowing water depending on the lag phase and HRT relationship.

An important aspect of the lag phase in a DWTP can be framed regarding the biofiltration treatment unit. For successful long-term operation of these biofilters, a proper study on backwashing effect must be done to evaluate the biofilm stability which governs the biofilter performance. It is expected that a significant portion of biofilm coverage is affected during this operation and hence the next maturation period of the bacterial community will depend on the lag phase of the native or the spiked bacterial community employed for the cyanotoxin treatment. A gene analysis throughout the filtration media depth might be an interesting topic of research in future which can help in analyzing the maintenance of gene copies which are responsible for the cyanotoxin degradation (discussed in detail in Section 3.2).

3.2. Importance of gene activity for the removal of cyanotoxins

Another perspective of looking at the biodegradation of cyanotoxin (in particularly the MCs) is to understand the gene responsible for its synthesis (*mcy* genes) and degradation (*mlr*+ genes) individually. The *mcyE* gene is widely chosen as the representative for the toxicity and toxin synthesis (for MCs) as it incorporates the ADDA moiety into the MCs structure (Fig. 1a). An in-depth study of the relationship between *mcyE* genes and *mlr*+ gene can further illustrate the mechanism of MCs degradation and efficiency of the MC-degrading bacteria in the presence of different biotic and abiotic factors. Increasing concentration of MCs and hence *mcyE* genes assist in triggering the *mlr*+ gene bacterial community, which is primarily responsible for the degradation of the dissolved MCs (Lezcano et al., 2018). Use of algicides followed by bacterial degradation of MCs could be beneficial for the DWTP dealing with a high concentration of cyanobacterial cells producing it. It is because these *mlr*+ genes can intrigue the cell lysis as well as can contribute to the MCs degradation later (Zhang et al., 2012). However, the temporal shift in MCs producing and MCs degrading bacterial community needs further assessment to form a more reliable relation among biotic as well as the abiotic factors to account for the overall MCs degradation.

Especially for the DWTP, which employ pretreatment operation and deals with the seasonal cyanobacterial influence (present in raw water intake), this information can be very vital for the cyanotoxin treatment. Various environmental factors (pH, temperature) stresses the toxic microcystis strain to synthesize more *mcyE* genes and thus any significant change in the DWTP operation may lead to cell lysis and hence production of unwanted excess cyanotoxin thereby further affecting the normal operation (Park et al., 2017). Microscopic examination of cyanobacteria provides information about the most general strains present in the water sample. However, quantification of toxic-synthesis gene present in various known cyanobacteria, such as *mcyE* (microcystin), *anaC* (anatoxin), *cyrJ* (cylindrospermopsin), *ndaF* (nodularin) better depicts the possibility of toxicity level in drinking water. A study comprising over 300 benthic cyanobacteria revealed a novel cyanobacterium (*Nostoc sp*.) which showed characteristic toxic fragment (ADDA) similar to the most common ADDA fragment producer known till date, i.e., *microcystis* sp. Another study revealed the presence of non-heterocystous filamentous cyanobacteria (*Anabaena* (benthic form), Calothrix and *Nostoc* from the *Nostocales* and Geitlerinema, Leptolyngbya, Limnothrix, Lyngbya, Oxynema, Phormidium, and Pseudanabaena) present in three different drinking water reservoirs (Gaget et al., 2017). Toxins as high as 739 ng/mg (dry weight of benthic cyanobacteria) were reported and it was highlighted that the benthic species presence could not be ignored and should be considered for the water quality monitoring program. Likewise, the DWTP needs to cope up with the algal metabolites which may demand high dose/energy for their effective removal and thus an absence of routine monitoring may put the quality of treated water at risk which otherwise can be solved by gene analysis (*mcyE* for MCs or *cyrJ* for CYN). The gene analysis can serve as an important parameter in knowing beforehand the degree of cyanotoxin treatment required if monitored in the raw water source before it enters the DWTPs. However, this study needs to be further verified with several instances, where lack of knowledge regarding cyanotoxin production does not allow the modification or adaptation of the treatment which might be necessary for the future. Thus, biological degradation can be beneficial in eradicating the root cause of cyanotoxin exposure through gene analysis and treatment by employing suitable bioreactor.

In fact, gene study of the sample is critical (both *mlrA* and *mcyE* gene for MCs) and could highlight the importance of the degree of cyanotoxin treatment needed as more energy/dose is required to overcome the toxic as well as the non-toxic fragment within a cyanotoxin molecule. However, sometimes high ratio of MCs and *mcyE* genes (MC: *mcyE*) can reach beyond 300 and may not indicate the toxicity (Lezcano et al., 2018). Hence, a dynamic study is necessary to understand the toxicity level behavior for the cyanobacterial population. Molecular techniques, such as polymerase chain reaction (PCR) and reverse transcriptase PCR can analyze the target cyanotoxin synthesis genes (*mcyE, cyrJ, anaC, ndaF*) to indicate the degree of toxicity in an environmental sample. These techniques can even detect the genes when more than two cyanobacterial variants are present where detection of one kind of gene is needed (genera-specific PCR) (Gobler et al., 2007). Also, cyanotoxin production in bloom may not always be constitutive as a significant rapid-up and down-regulation of genes (for example *mcyE* for MCs) is possible. In one of the studies, these significant changes have even been observed just 2 h from the earlier sampling rising microcystin value from < 100 pg/cell to > 2000 pg/cell (where a change in temperature between two samples was just 1.3 °C) (Wood et al., 2011).

The *mcyE* gene showed a good relationship between the amount of *Microcystis* sp. and thus these findings indicate the possibility of extracellular microcystin which might have acted as a signaling molecule. Such rapid increase-decrease of regulation in the environment needs proper understanding, which will assist the DWTP to counter the health risk, which might otherwise go unnoticed. Further, real-time monitoring can even lead to the development of different model studies, which may enable the DWTP operators to quantify the toxicity of the sample. Fig. 2 (A) and (B) represents the condition of upregulation and downregulation of the genes affecting the final toxicity of the treated water in a DWTPs, respectively. In future, gene-based cyanotoxin degradation (eg.: *mlrA* for MC-LR degradation) can also be planned by "installing" them (in a cyanobacterial chassis) in the photoautotrophic bacterial community itself (Dexter et al., 2018). This method could possibly prolong the *in situ* biocatalytic stability considering the cyanotoxin degradation. This technology of gene incorporation might be advantageous for the bioadsorption (sand filter) in a DWTP. Frequent backwashing in a sand filter operation where biofilm gets sheared can be developed again with more potential without any issues of lag phase or attempt of rebuilding or regulating the *mlrA* gene copies responsible for cyanotoxin degradation.

4. Conceptualization of cyanotoxin removal based on the different modes of bacterial growth

Many studies have already been reported by utilizing suspended growth biodegradation (Table 1). Mainly, they are useful at laboratory scale to check the performance of cyanotoxin-degrading bacteria, which later can be employed at a larger scale too. However, DWTP usually employs biological process units in the form of filters (mainly fixed-bed filters) categorized under attached growth process. Following section mainly focuses on the attached growth process along with generalized hypothesis related to the removal of cyanotoxins. Not much of

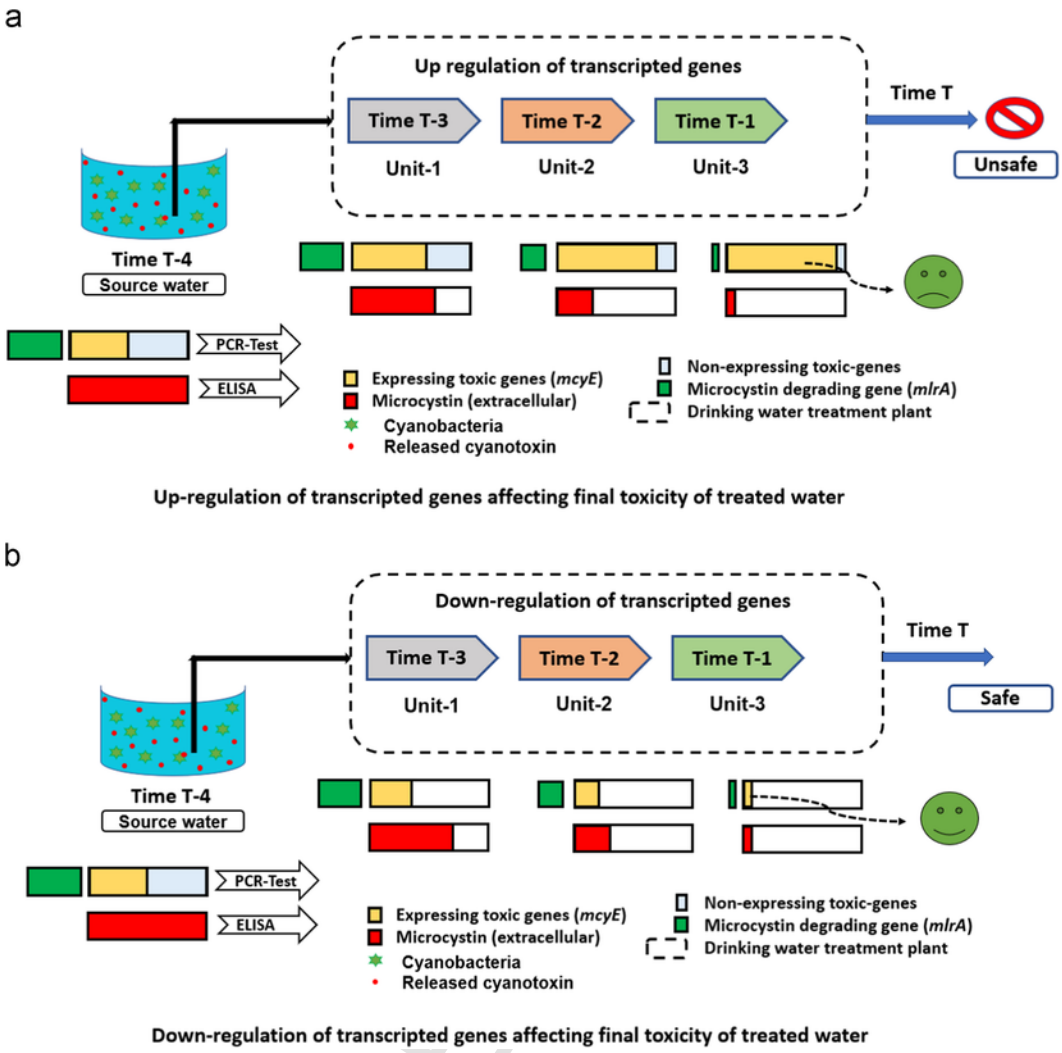

Fig. 2. (A) Up-regulation of genes (B) Down-regulation of genes in a drinking water treatment plant.

the research work has been reported so far on the biofilm aspects of cyanotoxin removal and hence most of the discussion will be hypothesized based on some basic theories to understand their removal.

4.1. Biodegradation of cyanotoxin: attached growth vs suspended growth

Both suspended and the attached growth process can be employed in a bioreactor operation for the removal of cyanotoxins (Kayal et al., 2008; Bourne et al., 2006). Successful biological treatment is influenced by microflora management, type of substrate in the system, energy maintenance requirement, stability to resist shock load and capacity of biomass to degrade the compound of interest (cyanotoxins) in a bioreactor (Yeon et al., 2011; Comparison of Suspended Growth and Attached Growth Wastewater Treatment Process, 2015). Fig. 3 shows a conceptualization of suspended and attached growth mechanism of bacterial cells for the removal of cyanotoxins. In suspended growth, bacteria are supposed to obtain nutrition and oxygen from their growing vicinity where they are exposed to large medium (liquid) volume/surface ratio, which is generally not enough and essential for the substrate degradation. On the other hand, attached growth mechanism sets an optimal surface for the bacteria to grow, intake of food/nutrition in and around a definite area near to their micro-colonies (as hypothesized in Fig. 3). This way, it ensures a specific balance between the space where the bacteria grow and the ease in performing their metabolic activity (Comparison of Suspended Growth and Attached Growth Wastewater Treatment Process, 2015).

The green color in Fig. 3(a) represents the extracellular polymeric substance (EPS) matrix link or connected with two or more (exaggerated) bacterial cells (red color) in a suspended growth mechanism. The larger void space can be expected between this linkage and may lead to insufficient participation of the cyanotoxin molecules. On the other hand, Fig. 3(b) represents the attached growth mechanism where region/zone of biofilm is expected to involve cyanotoxin molecules effectively and in association with degradation. Relatively lower void space between two media carriers (red color) can be expected between these influence zones (shown in green color) created by the bacterial attachment. Illustrating further, degradation of these molecules is expected to get affected mainly by: a) shear resilience of extracellular polymeric substance matrix (EPS) (forms during bacterial growth utilizing substrate), b) adhesion surface (for proper initial bacterial growth and resilience to counter shock loads) and, c) degradation zone offered by the bacterial community.

Some studies on biofilm suggested that EPS assembly mechanism and export are relatively conserved, and require polysaccharide copolymerase and other such proteins, which can be obtained by the cyanobacteria present in and around the degrading bacteria. This releases cyanotoxin as a consequence (Cassier-Chauvat et al., 2016). These copolymerases might influence EPS matrix during the bacterial

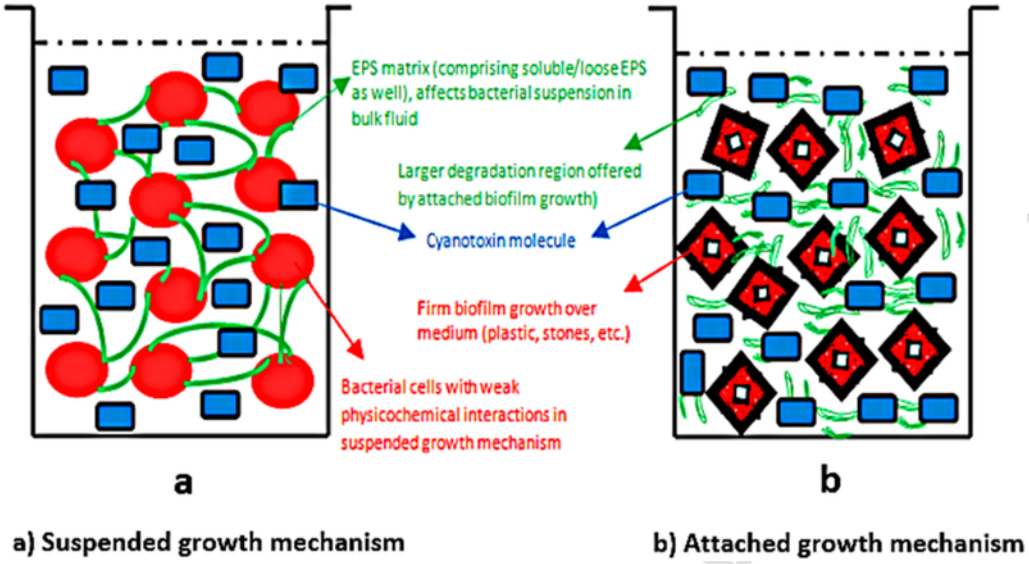

Fig. 3. a) Suspended vs. b) attached growth bacterial processes for the removal of cyanotoxins (Hypothetical picturization).

growth, such that their formation is supported by other nutrients available around biofilm (suited in attached growth biofilms). One of the studies also revealed that the presence and absence of these nutrients (nitrogen, phosphorus, trace metal) influences the production of *mlrA* genes in degrading cyanotoxins (Lezcano et al., 20162016b). Attached growth process where biofilms are primarily composed of microbial cells and EPS (constituting 50–90% of the total organic carbon of biofilms) (Carrasco et al., 2006), and EPS matrix (with context to co-polymerase, other nutrient sources). It can be understood that the attached growth bacterial concept in a bioreactor has the possibility to play an important role in the degradation of cyanotoxins. Also, the attached growth process is expected to undergo a faster degradation rate than the suspended growth mechanism (Carrasco et al., 2006; Stewart, 2003).

4.2. Are biofilm systems a good option for the cyanotoxins removal?

As explained earlier, adsorption and degradation play a key role in the biodegradation, and thus good biofilm formation is an important phenomenon which must be carried out effectively by adsorption on to a proper surface, allowing efficient cyanotoxins (or substrate) utilization/degradation. However, a study showed that a biofilm need not be very thick, especially for the anoxic zone creation (anoxic zone in a biofilm is followed by aerobic zone). Cluster radius which is defined as the minimum size needed to deplete the solute in the biofilm needs to be at least 25 μm. This ensures the co-existence of both aerobes and anaerobes microorganisms in the biofilm matrix. Rusten et al.,al. (Rusten et al., 2006). This co-existence can allow each other to work in a close association which may prove effective in degrading cyanotoxin molecules. Under the aerobic zone of biofilm, cyanotoxin molecules are expected to get degraded to lower molecular mass byproducts (around the molecular weight (M.W.) 200–400) and based on the effective diffusivity D_e/D_{aq} value of 0.25*[3] (where, D_e is the effective diffusion coefficient in biofilm and D_{aq} is the diffusion coefficient in pure water), the solutes of M.W. ranging 150–350 can be diffused through the biofilm (Stewart, 2003; Rusten et al., 2006). The diffusion of smaller M.W. products of cyanotoxins is expected to be taken care of by the anoxic/aerobic biofilm zone and further degradation of them is expected. Also, the diffusion of solutes may depend on the type of biofilm system being subjected to degrade particular cyanotoxins. Not only this, but these aerobic/anoxic regions in the biofilm can facilitate denitrification too which is essential in a DWTP.

Biofilm formed on the media carriers based on their movement within the bulk system (fluid surrounding it) can be divided either as a fixed bed or moving bed. In the moving bed system, the biofilm bacterial cells are under continuous movement resulting in a well-mixed fluid system. This might facilitate transport of cyanotoxin inside the biofilm due to the convection process (bulk flow of fluid) rather than the diffusion (random molecular motion). While in a fixed bed system, diffusion may not be limited and solute (cyanotoxins), still penetration inside the biofilm may largely depend on this phenomenon (Rouhiainen et al., 2010). Under these conditions, the diffusion distance might increase and effective solute transfer from bulk to biofilm region may become limited (also due to the stagnancy of the system, high EPS and biomass density effect around the media carriers). Thus, for larger cyanotoxin molecule (around M.W. of 1000 for microcystin for example), moving bed system can prove quite effective in their removal.

In a recent study by Kohler et al. (2014) (Misra et al., 2014), use of gravity-driven membrane (GDM) was used for the removal of microcystin with continuous passage of water sample (artificial Microcystis aeruginosa bloom in lake water). The biofilm formation took 10–15 days to form over the membrane and it took 15 days to remove MC level to below 1 μg/L level with less than one-week post-establishment of the stable biofilm over the membrane. In fact, this system of water purification has been in running mode in some countries and the studies have shown the possibility of remediating algal bloom water containing microcystin by biofilms. GDM filtration method helped in the removal of harmful chemicals from raw water, particularly the cyanotoxins/cyanobacteria as recently shown by (Silva et al., 2017). This biomass-amended treatment was hypothesized to show the potential formation of prime-biofilms for rapid cyanotoxin degradation. However, since the cyanobacterial bloom is a transient phenomenon, thus, understanding the role of mature biofilm and finding the relation of its behavior with changing cyanotoxin level needs critical evaluation in the future. With respect to the biofilm treatment of cyanotoxins in a DWTP, a study by (Shimizu et al., 2013) showed that biofilm recovered from biological treatment facility (apparatus and honeycomb tubes) has *mlrA* gene in them all year round (these genes are previously reported to be the microcystin degraders; as also discussed in the previ-

[3] *(depends on the biomass density in the biofilm and physiological property of solutes).

ous sections). Thus, enhancing the biofilm growth of these bacteria carrying microcystin degrading genes (*mlrA*) can prove to be an effective and economical solution for the cyanotoxin removal.

Biofilm application is mainly beneficial when accompanied by high surface adsorption media such as fine sand, charcoal and, activated carbon. These media are commonly used in DWTPs, mainly sand. For an effective biofilm formation over the top of an adsorbing media (filtration units), seeding of columns with cyanotoxin degraders is necessary to bring out an effective degradation of cyanotoxin. It has been found that this seeding procedure (*Sphingomonas* sp.) enhanced the degradation of geosmin (an algal toxin) by over 38% whether added to an active or inactive pre-existing biofilm community (McDowall et al., 2009). This can significantly reduce the lag phase needed for the onset of the effective cyanotoxin degradation in the form of biosand filter of biological activated carbon filter applied at a full-scale DWTP. However, in future, the relationship between the biofilm formation, their stability, and interaction with other heterogeneous bacteria, especially those which are present *in-situ* in the filtration unit of the DWTPs will be very informative. Biofilm formed by phototrophic bacteria (*Oocystis lacustris*; colonial green alga *Protoderma* sp. and diatoms *Navicula* sp. and *Achnanthes sp*.) can degrade microcystins at a very faster rate (Babica et al., 2005). (Babica et al., 2005) found that cyanobacterial biofilm has the potential to degrade the released microcystins at a significantly faster rate (complete degradation within 120 h) as compared to its absence (only < 25% degradation). Also, the presence of other substrates, such as casein and glucose did not affect the degradation rate, highlighting an effective degradation mechanism carried out by the phototrophic bacteria present in the bloom resulting in complete microcystin degradation and at a higher rate (as compared to other studies which showed longer lag phase: 3–21 days and degradation phase: 6–25 days) (Bourne et al., 19961996b).

4.3. Biofilm zone significance for the degradation of microcystin

The biofilm zone significance for the removal of cyanotoxins is discussed with respect to the microcystins as they are most dominant and commonly found in the environment. At present, the main mechanism considered for the attenuation of microcystins (MCs) in the environment is aerobic degradation. This mechanism has been reported to occur in various habitats including fresh water, lakes and drinking water (Center for earth and environmental science, 2017; Ho et al., 20072007b). Pathway for aerobic degradation of MCs has already been reported to produce non-toxic by-products (Wu et al., 2011). MCs have also been reported to undergo degradation under anoxic environment. (Holst et al., 2003) indicated that the MCs degradation under anoxic condition could further be stimulated by the nitrate addition to decreasing the MCs concentration from 100 μg/L to 13 μg/L as compared to reduction to 20 μg/L (within a day), when only glucose is added. They also reported that the addition of glucose (along with nitrate) promoted the metabolic activity of the bacteria which had a positive effect during anoxic biodegradation of MCs in sediment slurries from 100 μg/L to 9 μg/L. This is contradictory to the normal behavior shown during aerobic degradation of MCs as discussed earlier. Also, not many mechanistic studies have been carried out for the biodegradation of MCs under anoxic conditions and hence, the toxicity of the by-products remains unknown.

However, the anoxic conditions in a biofilm stimulate the activity of bacteria (in the presence of terminal electron acceptor like NO_3^-). The fact that the degradation rate of MCs remains unaffected by the presence of another carbon source (glucose), biofilm system can be a promising approach (as biofilm consists of anoxic/aerobic zones). Fig. 4 illustrates the proposed dynamics of diffusion and the biological activity of biofilm in the presence of microcystin. Microcystin and other organic matter present in the water source are expected to be degraded in the aerobic zone of biofilm. By-products generated will include car-

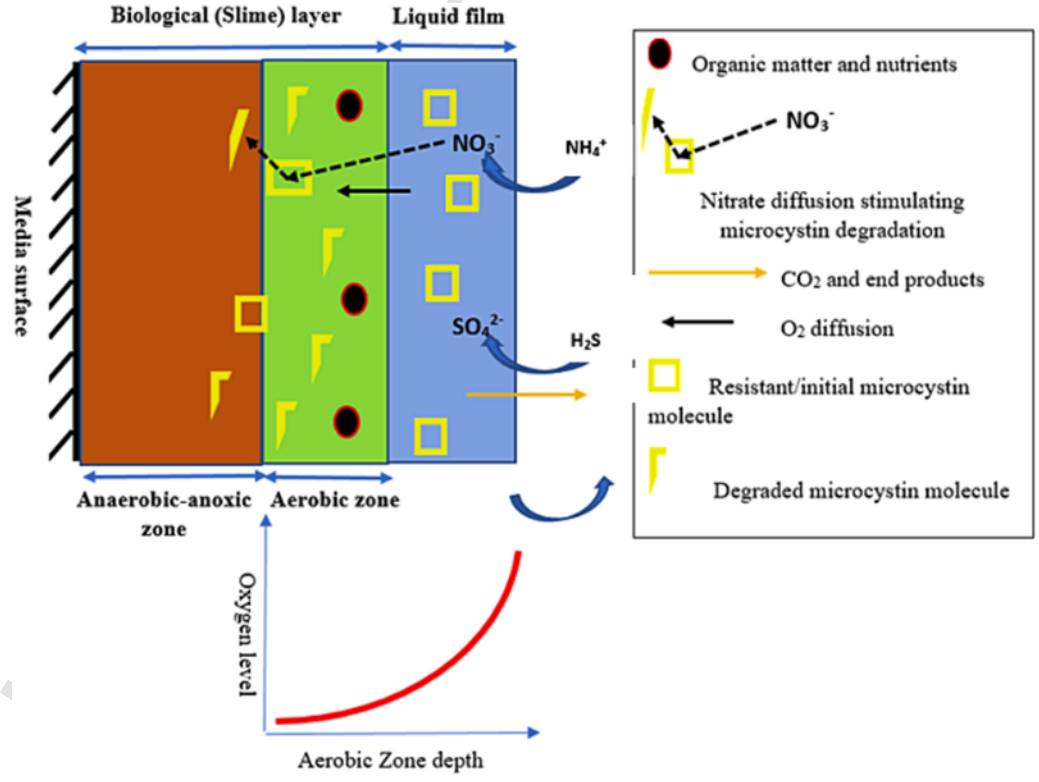

Fig. 4. Proposed dynamics of diffusion and biological activity of biofilm in presence of microcystin molecules.

bon dioxide release. All other inorganic molecules, such as ammonium and sulfide will be converted to nitrogen dioxide and sulfur dioxide, respectively. The key parameter is the resistant MC molecules which are either difficult to degrade or remain partially degraded under the aerobic zone of the biofilm. These molecules are expected to further diffuse at the interface of the anaerobic (or anoxic) and aerobic zone of biofilm. With the depletion of oxygen at the interface of two zones, the presence of nitrate molecules can further stimulate the degradation of these partially degraded MCs and non-degradable MC molecules (Holst et al., 2003). Also, the presence of organic matter (degraded or remained undegraded, present near aerobic zone) is unlikely to have any negative impact as discussed earlier by (Holst et al., 2003). These adsorbed biofilms have also been observed to dramatically increase the microbial activity by undergoing physiological modifications, associated with the promotion of certain genes (Dagostino et al., 1991). Further, the biofilm also changes the bacterial cell surrounding which facilitates higher nutrient uptake, oxygen utilization by limiting the invasion of inhibiting substances (Ghosh et al., 1999). Hence, a study on biofilm aspect of cyanotoxin removal can be helpful not only in reducing the toxicity of the by-products but also in creating an adaptable environment for them to carry out the degradation, which is not evident for physicochemical treatment or under the suspended growth mechanism.

5. Future perspectives and conclusion

Biological processes have shown an immense promise for a wide spectrum of cyanotoxin-degrading bacteria. Biodegradation of cyanotoxins is rarely accompanied by any harmful by-products (reduce toxicity level-up to 160–200 times). The method is energy saving and delivers high-efficiency removal for almost every kind of cyanotoxin. However, the biological process is time-consuming. Suspended growth mechanism requires continuous aeration/movement of the media and it takes time in efficiently degrading the cyanotoxins (sometimes $>$ 20 days). Hence, a large volume is often required to maintain higher hydraulic retention time (HRT) in a bioreactor. Thus, upscaling of such reactors becomes uneconomical, if employed in a DWTP unit. On the other hand, use of filter techniques (involving different media) has shown promise in toxin removal and provided effective treatment when the microorganisms were involved in association with the adsorbing media (attached growth). However, the degradation rate still needs to be improved before scaling up the bioreactors at the pilot level. The bacteria, such as *Sphingomonas, Rhodococcus, Arthrobacter, and Brevibacterium*, have shown a quick degrading ability of cyanotoxins at par with some conventional and advanced treatment facilities which rely either on chemical breakdown (producing even more toxic co-products) or expensive elemental input.

Modification of bioreactors based on attached growth biofilm process can be a promising approach in future due to the provision of higher resistance against improper conditions, shock loads by organics or hydraulics as compared to bioreactors involving suspended growth (where higher maintenance along with high nutrient supply is needed to sustain microorganisms' growth). Also, the *in-situ* bacteria derived from different units of the DWTP and other natural water sources (lakes, reservoir, sediments) has shown positive results in degrading cyanotoxins. This may allow the possibility of a common filter set-up for the DWTPs in future which can simultaneously carry out the filtration as well as the cyanotoxin removal. Easy availability of bacterial source, cost-effectiveness and non-complexity in experimental conditions can lend wider acceptance of these biological methods. Gene analysis can further suggest information on the upregulation and downregulation of the transcript genes which might affect the final toxicity of the treated water.

Other molecular level techniques, such as gene-specific polymerase chain reaction (PCR) or reverse transcript PCR can also help in identifying the cyanotoxin-degrading genes, especially when more than two kinds of cyanotoxins are present in the source water to be treated. Also, there is a significant research gap between suspended method and attached growth method for cyanotoxin removal where former is employed at the laboratory level without upscale application and latter is applied both at the laboratory as well as pilot scale. Chemical methods for the cyanotoxin treatment create toxic by-products which may go through sludge line and final effluents thereby necessitating further treatment. In future, stricter cyanotoxin regulations may come into force for different environmental compartments. Thus, this global issue of cyanobacterial bloom needs to be effectively and sustainably tackled. Biological methods (especially attached growth methods) can fulfill this promise considering an overall green ecology and maintenance of quality drinking water.

Uncited references

(Imanishi et al., 2005b; Simpson, 2008; Harrison et al., 2007; Wolyniak DiCesare et al., 2012)

Acknowledgment

The authors are sincerely thankful to the Natural Sciences and Engineering Research (Discovery Grant 23451), Genome Québec, Genome Canada (Grant 6116548-2015) and ATRAPP (Algal blooms, treatment, risk assessment, prediction, and prevention) for the financial support. Authors would also like to thank the research team for constant support and timely suggestions. The views or opinions expressed in this article are exclusively those of the authors.

Conflict of interest

All authors agree that there is no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2019.01.066

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