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# Physico-chemical treatment for the degradation of cyanotoxins with emphasis on drinking water treatment- How far have we come?

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Highlights:

- Cyanotoxins removal are dependent on environmental parameters, mainly pH and Natural Organic Matters
- Chlorination and ozonation employed for cyanotoxin treatment may breach guideline values
- Membrane technology and photocatalysis operation involves high energy and maintenance
- Specific reaction pathway shifts oxidation process more towards sustainable approach

**ABSTRACT:**

Over the years, various physicochemical treatment processes, such as photocatalysis, membrane technology, ozonolysis~~,~~ and chlorination~~, etc.~~ have been tested at laboratory and pilot scale for the treatment of various cyanotoxins. Most of these treatment processes are also being commonly practiced in a drinking water treatment plants (DWTPs). However, the degree of treatment widely varies among cyanotoxin variants and is mainly governed by the source water characteristics,

operational parameters (temperature, pH, cyanotoxin level) ~~organic matter, etc.~~ which changes continuously in a DWTPs. Other common elements present in raw water, such as natural organic matter (NOMs), residual nutrients~~,~~ and metal ions~~, etc.~~ shows competitive behaviour with the cyanotoxins. Thus, a high demand in input energy is needed for unit operations, such as photocatalysis, reverse osmosis membrane and excess chemical requirement in terms of ozone, permanganate and chlorine (for ozonation and chlorination) which can breach the guidelines and increase the toxicity level. This review provides an insight into the effectiveness of major physico-chemical operations from simple to the advanced treatment level for the removal of different cyanotoxins along with their limitations and challenges in a DWTP. The goal of this review is to provide information on the possible reaction mechanism involved in the cyanotoxin treatment, accounting mainly for the toxicity, modifications in the process that happened over the years and the process feasibility. In future, hybrid technique assisted by UV, peroxides, among others promises to assist photocatalytic, ozonation and chlorination to undergo efficient cyanotoxin removal with reduced toxicity level. Also, persistence cyanotoxins, such as anatoxin and saxitoxin need further study.

**Keywords**: Physico-chemical treatment; cyanotoxin; reaction pathway; oxidation; drinking water

## 1. Introduction

Cyanobacteria are among the largest group of photosynthetic prokaryotes present in the terrestrial and aquatic environment and are capable of outcompeting other algae and microorganisms present in lakes, reservoirs, and ponds under favorable environmental conditions. These conditions favor the occurrence of phenomena known as cyanobacterial (or algal) blooms [1,2]. These blooms are a global concern and a threat to the aquatic environment as they deplete the dissolved oxygen level followed by the release of cyanotoxins. A lake in China (Lake Taihu: China`s third-largest lake

water) was found to be impacted by this phenomenon where dangerous cyanotoxins were released at an amount high enough to leave more than two million people without access to drinking water for over one week. Hence, when these cyanobacterial cells or dissolved cyanotoxins enter the DWTP (along with raw water), their treatment becomes necessary.

According to the World Health Organization (WHO) guidelines for the drinking water, the critical concentration of some cyanotoxins such as microcystin-LR (MC-LR) is even < 1 µg/L (WHO, 2009). Cyanotoxins and their metabolites are persistent in the environment and hence can directly enter the DWTP [3]. For example, the half-life of microcystin-LR (MC-LR) (secreted by *Microcystis aeruginosa*), is around 90 days and is known to be among the most toxic cyanotoxin present in the natural environment [4]. The half-life of saxitoxin (produced by *Anabaena sp*.) is around 9-28 days. Some of their by-products (such as gonyautoxins) have even a longer half-life of >90 days [5]. Apart from the aquatic organisms, serious health issues associated with these cyanotoxins extends to humans as well, ranging from acute (skin irritation, gastrointestinal) to chronic effects (kidney damage, liver damage, possible carcinogens) [6]. Table 1 shows various cyanotoxins produced by different cyanobacterial genera along with their $LD_{50}$ values. Continuous exposure to untreated source water for drinking water purpose may achieve a lethal dose within the human lifespan (Table 1). Hence, effective cyanotoxin removal is necessary for a DWTP to avoid any possible user-end problems (tap water).

Various conventional treatment options that are most commonly employed in a DWTP (such as ozonation, chlorination, filter adsorption media ~~etc.~~) have proven to be effective for various cyanotoxins removal at the lab-scale. ~~However,~~ Such lab-scale experiments outline their best performance under the most favorable conditions (neutral pH, mild temperature, less organic matter presence~~, etc.~~). Even if they show effective treatment potential, they have been challenged

by the high energy footprint making them uneconomical and unsustainable (in the case of RO membrane and photocatalysis) or higher requirement of input chemical dose than normal, which breaches the guidelines of drinking water treatment (for disinfection and ozonation). The problem escalates especially during a summer-autumn season where bloom phenomenon is more prominent as compared to other seasons. During this phase of a year, DWTPs needs to be more cautious and potentially ready for the effective treatment of cyanotoxins. Thus, unit operations of DWTP may demand periodical adjustments apart from carrying out their general treatment objectives, because, at times, it becomes difficult to anticipate an algal bloom alert beforehand when no definite trend is noticed from the previous history. This review discusses some widely used physical-treatment treatment technologies, till date taking into consideration the cyanotoxins that can potentially be removed under different environmental conditions. Other reviews on the cyanotoxin removal provided a general overview of various oxidative processes including chlorination, ozonation, photocatalysis, etc [7, 8]. However, this review presents discussion ~~an overview~~ from simple to the advanced version of the above oxidative methods to understand the change in the behavior of various cyanotoxin degradation, toxicity level of the by-products, reaction mechanism under different environmental conditions (NOMs, pH, etc.). This review will highlight the importance of each physicochemical treatment that is generally practiced in a DWTP and will discuss the maturity of their usage achieved till date for controlling various cyanotoxin removal with relevant information based on the recent research work. Before discussing the various physicochemical treatments in detail, a ~~brief~~ overview of environmental conditions and various factors affecting the "bloom" phenomenon has been presented in the next section.

## 2. Environmental conditions and various factors affecting cyanobacterial bloom

The bloom phenomenon mainly occurs during the spring-autumn season in natural water bodies and can potentially cover a large surface area which is often associated with the release of cyanotoxins from live and dead cyanobacterial cells. Various environmental and nutritional conditions including salinity, level of nutrients, light intensity and turbidity level~~, etc.,~~ influences the cyanobacterial growth [9]. Different concentration as well as type of phosphorus and nitrogen largely influence cyanobacterial growth in ~~still~~ stagnant water sources (lake and reservoirs). ~~Li et al., (2014) [8],~~ It was reported that the highest growth rate (0.17 ± 0.01/day) for cyanobacteria (*Halomicronema hongdechloris*: isolated from a cyanobacterial community) was observed when nitrate is used as the nitrogen source as compared to other forms of nitrogen, such as $NH_4^+$, $NO_2^-$ [10]. Apart from the concentration, the ratio of nitrogen to phosphorus affects the algal culture growth [11]. It was found that the growth of *H. hongdechloris* was inhibited for N/P value <7.8 (growth rate 0.1 $day^{-1}$) or >780 (growth rate 0.125 $day^{-1}$) as compared to the ratio of 78 (growth rate 0.23 $day^{-1}$). Some studies have also shown the variability in cyanotoxin production even under limited phosphorus content (with growth rate: 0.1/day). For example, production of cyanotoxins, such as microcystin, anatoxin-a, and nodularin by *Microcystis sp., Aphanizomenon, and Nodularia,* showed a decrease. However, another study found an increase in microcystin under similar conditions [12]. In fact, the cyanotoxin production also showed dependency on the type of cyanobacteria categorized as nitrogen-fixing and non-nitrogen-fixing [13]. Hence, the municipality can investigate this situation for the condition prevailing in natural water sources before obtaining raw water, and channel it to the DWTP, especially during bloom conditions. This might help them to understand the situation that they need to deal beforehand and also to keep an inventory of the record for the next season or year.

Change in light intensity also influences cyanotoxin production in living cyanobacteria which changes the transcription start site of *mcyA* (common gene responsible for the breakdown of microcystin compound found in *Microcystis aeruginosa*) [14]. It was also reported that the initial induction of these genes in the transcription process can be observed under higher light intensity (30 µmol $m^{-2}.s^{-1}$)[14]. The growth of other cyanobacteria, *Nodularia spumigena* (nodularin-producing cyanobacteria) has also been reported to be dependent on light intensity (45–155 µmol photons $m^{-2}$·s-1), where co-transcription of *nda* cluster genes is responsible for encoding high light inducible chlorophyll-binding protein (HLIP) [15]. *Oscillatoria sp.* PCC 6506 and *Aphanizomenon Ovalisporum* producing cylindrospermopsin (CYN) too require high light intensity (85 µmol photons $m^{-2}·s^{-1}$) for their growth. In this case, the transcript gene: *cyr* regulates the growth activity where even lack of nitrogen favors their growth [16]. On another note, cyanobacteria have several defense mechanisms against the UV light exposure (especially *Anabaena*. Their ability to synthesize certain compounds such as mycosporine-like amino acids (MAAs) and scytonemin helps in absorbing the deleterious UV light without affecting their growth. Such properties of cyanobacteria enhance the bioactivity and cell accumulation without much influencing the photosynthetic evolution of oxygen. The photoautotrophic growth of cyanobacteria follows the trend of light limitation, light saturation, and light inhibition, which means the growth is enhanced by the high light intensity up to certain level and then mark a decrease when it achieves the saturation level [17].

However, in general, the biomass productivity and the specific growth rate of the cyanobacterial cells (*C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa)* increases with the light intensity. On an average, 140 - 210 µE $m^{-2}$ $s^{-1}$ of light intensity has shown to favor the optimum growth of these cells. Further, these optimum light conditions which promote high photosynthetic activity also enhance

the removal of nutrients which also depends on the growth rate. An increase in the light intensity from 36 μE $m^{-2}$ $s^{-1}$ to 180 μE $m^{-2}$ $s^{-1}$ enhanced the nitrogen removal from partial removal to 100 % [18]. It has been seen that nitrate-nitrogen assimilation by cyanobacterial cells is higher than the phosphorus assimilation. However, a significant removal of phosphorus was also achieved (65.8 to 87.0% for *Chlorella kessleri*) when light intensity was increased (0 to 200 μE $m^{-2}$ $s^{-1}$) [19]. A future research on the nutrient limitation, growth kinetics and its effect due to the light intensity can further highlight interesting relationship among various cyanobacteria.

Similarly, temperature follows the same trend where high temperature enhances the photosynthetic $O_2$ evolution and biomass accumulation but decreases after a certain point (generally 40 °C). Generally, the cyanobacterial growth is maximum during the summer-autumn season (20- 30 °C) as compared to the spring (temperature <15 °C). ~~However~~ In contrast, Konopka et al., ~~(1978)~~ [17] showed that there was not much difference in the photosynthesis process that was observed at the low temperature (70 % of maximum) suggesting that low temperature cannot be responsible for the decreased bloom condition during spring or winter. Temperature can have an indirect effect on the toxicity of the bloom formed by the cyanobacterial cells. For instance, ~~Davis et al., (2009) [16]~~ it was found that the increase in temperature yielded more Microcystis cells having toxic genes (*mcyD*) in their cells (83 % of the experiment) as compared to the non-toxic Microcystis where only in 33 % of the experiment, growth was enhanced [20].

Non-algal turbidity (NAT) also plays a vital role in regulating the relationship between total phosphorus level in water bodies and the phytoplankton biomass growth resulting in varying cyanobacterial population. A possible reason may suggest that NAT reduces the light penetration, affecting cyanobacterial cell growth by creating light limiting condition. Another likely reason could be the fact that inorganic phosphorus binds to the non-algal sediment particles which may

become unavailable for the direct uptake by these cells. Phosphorus adsorption on to the sediments is a complicated mechanism to understand among various environmental factors and has been a topic of debate since long. Ligand exchange process (with methyl groups) and electrostatic attraction are the most common modes of explaining phosphorus binding with the non-algal sediments [21]. Also, the presence of calcium and other metal oxides favors the high fractionation of phosphorus in the sediments which affects the cyanobacterial cells uptake mechanism [22]. The light was also shown to be increasingly limited when NAT level exceeds 2.0 2/m [23]. This threshold value was identified to be the cut-off mark above which the researchers found less cyanobacterial biomass per unit of total phosphorus. ~~However,~~ Additional research on these studies may be required to understand the mechanism behind such limiting criteria. Other factors, such as pH levels, the concentration of carbonate and bicarbonate ions also affects the growth rate of cyanobacterial cells as they eventually control the cyanotoxin released by them [24]. For example, ~~Touloupakis et al., (2015) [18]~~ it was found that the productivity, growth and biomass yield of *Synechocystis sp.* PCC 6803 cultures (cyanobacteria) declined by 32%, 28%, and 26%, respectively when pH increased from 7.5 to 11 [24]. Even the low concentration of carbon dioxide favors the growth of cyanobacterial cells as they become competitive under these circumstances because they use very effective $CO_2$ concentrating mechanism [25]. This mechanism allows the uptake of bicarbonates and $CO_2$ with subsequent accumulation of inorganic carbon [26]. Considering biological factors, such as the presence of zooplankton cells, they sometimes do not easily digest cyanobacteria, thereby, increasing their level under sufficient nutrient conditions available in raw water. Algae and another microorganism (especially bacteria) have been found to synergistically affect physiology and metabolism. This mutual relationship helps in cyanobacteria growth [27]. Moreover, it has been found that horizontal gene transfer from bacteria to algae too

helps in adapting them to extreme climatic conditions [28]. This possibly could be the reason for bloom formation and their long persistence in lakes and other water bodies. An interesting study comprising more than 180 heterogenous bacteria observed for the changes in the cyanobacteria: *Anabaena* (non-toxic) and *Microcystis* (toxic-forming). It was found that more than 100 strains affected the cyanobacterial growth in either way. A bacterial strain *Herbaspirillum* JO59 was found to inhibit the growth of non-toxic Anabaena while enhancing the growth of Microcystis. On the other hand, *Sphingomonas* LI2 produced the opposite effect [29]. These synergistic effects have also shown influence on the photosynthetic activity of the cyanobacterial cells. An allelopathic influence on this phytoplankton by the aquatic macrophytes (*Myriophyllum spicatum*) was studied using various polyphenols: pyrogallic acid (PA), gallic acid (GA), ellagic acid (EA) and (+)-catechin (CA). Some polyphenols changed the whole electron transport system of *M.aeruginosa* where photosynthetic activity was hampered more due to PA and GA (19 % and 41 % , respectively) [30]. Although control of cyanobacterial remains the major challenge, some pulmonates (*Radix swinhoei*) and submerged plants (*Potamogeton lucens*) showed a decrease in chlorophyll a, total nitrogen, total phosphorus and the potassium permanganate index by 76.2, 51.4, 55.6 and 31.6%, respectively [31].

Thus, these cyanobacterial cells producing harmful cyanotoxins can be held responsible due to the combination of many such factors which can be tracked down for different drinking water sources based upon the history they possess (for at least past 10-15 years). This way, their dynamic behavior can be understood with the surroundings to establish a potential strategy for pre-treatment of raw water sources linked to the nearby DWTP. ~~Hence,~~ An effective treatment system or modification in the existing DWTP unit operations can be proposed in the future for the enhanced cyanotoxins removal. Different physicochemical treatment methods are discussed in the following

sections performed mainly at laboratory-scale for the removal of various cyanotoxins with insight into the DWTPs.

## 3. Conventional and advanced physicochemical treatment methods

Different physicochemical treatment processes including photo-catalytic operation, membrane separation, ozonation, and chlorination have been successfully applied for the removal of extracellular as well as in-bound toxins. Table 2 shows various conventional and advanced treatment methods used for the treatment of different cyanotoxins along with their major shortcomings and removal efficiencies. It will be further investigated in details of each of these methods for the removal of various cyanotoxins with an insight into the DWTP.

### 3.1 Photocatalytic method

Fast and efficient removal of cyanotoxins can be obtained through oxidation processes, such as the photo-catalytic technique~~, ~~and ozonation~~, etc.~~ which are quite prevalent these days in the modern DWTPs. In fact, photocatalytic oxidation at laboratory-scale has proven successful in the removal of various cyanotoxins, such as microcystins, anatoxin, cylindrospermopsin (CYN), etc. Figure 2 shows different cases of photocatalytic method of treatment depicting $TiO_2$ and MC-LR as the photocatalyst and a cyanotoxin molecule representatives, under no dopant (Figure 2 (A)), oxidants presence (Figure 2 (B)), NOMs presence at pH 7 and pH < 7 (Figure 2 (C) and (D), respectively) and metal-doped $TiO_2$ (Figure 2 (E)).

Generally, oxidation reactions involve the production of hydroxyl radical ($OH^{\cdot}$) through a chain of photoreaction (oxidation reaction, Figure 2 (A)). These hydroxyls radical on production oxidizes the persistent and stable cyanotoxin compound. Use of metals, such as iron or silver nanoparticles (equation 4 and 5) doped ~~with photocatalysts~~ into $TiO_2$ matrix for photocatalytic application

promotes the excitation of electrons from the valence band (VB) to the conduction band (CB) via an intermediate energy level mechanism (Figure 2 (E)). This enhances the photocatalytic activity and reduces the energy band gap (between VB and CB) thereby allowing efficient redox reaction with a decrease in the recombination rate between electrons and the holes. Due to the reduction of an oxygen molecule into oxygen radical molecule (equation 1) and formation of hydroxyl radical (equation 2, Figure 2 (A)) due to oxidation of water molecule, combines to effectively degrade the micropollutant present in water (cyanotoxins). Some general photochemical reactions are mentioned below in Equations 1 to 8:

$O_2 \dashrightarrow O_2^{\cdot}$ (1)

$H_2O \rightarrow OH^{\cdot}$ (2)

$OH^{-} \dashrightarrow OH^{\cdot}$ (3)

$H_2O_2 + h\upsilon \rightarrow 2OH^{\cdot}$; $Fe(OH)_2 + h\upsilon \rightarrow Fe^{2+} + OH^{\cdot}$ (4)

$Ag_3PO_4 + h\upsilon \dashrightarrow Ag_3PO_4 (e^- + h^+)$ (5)

$e^- + O_2^{-} \rightarrow O_2^{-}$ (6)

$h^+ + H_2O \dashrightarrow H^+ + OH^{\cdot}$ (7)

$H^+ + OH^{-} \rightarrow OH^{\cdot}$ (8)

Use of Titanium oxide ($TiO_2$) as a photocatalyst is very common in the application at the lab-scale and use of different novel dopants has also been tried in recent years to improve the efficiency of the former. For instance, ~~El-Sheikh et al., (2014) [24] obtained~~ MC-LR was removed (0.5μM, 10

mL) in just 5 h~~hours~~ (0.587/h) using sulfur-nitrogen-carbon doped $TiO_2$ photocatalyst[1] (dose: 0.5 g/L) as compared to un-doped $TiO_2$ catalyst sample (0.0232/h) [32]. The key electrochemical reaction ~~followed in this study~~ is shown as equation 1-3. Pelaez et al., ~~(2012)~~ [33] showed the successful removal of other common cyanotoxins viz. cylindrospermopsin, MC-RR, MC-LA, MC-YR (0.5 μM) within 3 h~~hours~~ of light exposure (using two 15 W fluorescent lamps) using NF-$TiO_2$-P25 nanoparticles as a photocatalyst (borosilicate glass reactor and dose: up to 15 g/L). However, the use of light makes the overall photocatalysis operation cost-intensive. Sometimes even prolonged exposure to light energy is not sufficient for effective degradation of cyanotoxin compounds and by-products which demands high energy input. For example, ~~Lawton et al., (1999) [26],~~ it was observed that six out of seven reaction products (mainly dehydroxylated products of the main MC-LR molecule) formed during the photocatalytic reaction failed to undergo further degradation after a prolonged exposure (100 minutes) [34]. Some studies even reported the usage of solar light that helped in reducing the energy footprint [33]. For example~~, Pinho et al., (2015) [27] used~~ $TiO_2$ photocatalysis method (dose: 200 ppm) was used for the successful destruction of MC-LR and CYN (300 μg/L) through solar radiance (under 6 h~~hours~~, following equation 1-2) [35]. ~~However~~ The use of solar light may not be efficient at times. For example, Fotiou et al. [36] reported complete CYN degradation through commercially available $TiO_2$ photocatalysts, Degussa P25 and Kronos-vlp7000 within 15 minutes and 40 minutes, respectively under UV-A and within 40 minutes and 120 minutes under solar light irradiation. This highlights that to have sustainable degradation of CYN, prolonged treatment is required which makes the photocatalytic process an energy-intensive option.

[1] Borosilicate glass petri dish (Pyrex, 60 mm diameter x 15 mm (h)) using 15 W fluorescent lamps: light intensity (1.33 mW/cm$^2$)

The major oxidizing species formed during the photocatalysis process (for example $TiO_2$ with UV-A light) is hydroxyl radical where they perform substitution of a hydrogen atom or hydroxyl addition. Reaction intermediate formed during the photocatalytic treatment of cyanotoxin has been documented in very few studies. Some hydroxyl substituted molecule includes m/z 1011.5 and 1029.5 during the MC-LR degradation [37]. These hydroxylated intermediates are the first step to the linearization of the molecule. Removal of a neutral molecule, such as $H_2O$, ammonia, $CO_2$ indicated the aspects of mineralization (from m/z 1012.6 to 765.3). Oxidation of "adda[2]" molecule can also occur to produce lower toxicity of the formed MCs by-products [38]. Other cyanotoxins, such as CYN has been shown the same mechanistic pathway of hydroxyl radical attack (m/z 432, starting from dehydroxylate m/z 450). However, it was observed that hydroxyl radical is prominent in attacking nitrogen atom rather than carbon. This seemed justifiable as apart from carbon mineralization (into $CO_2$), nitrate ions have also been observed [36]. Reduced toxicity of CYN degradation can be linked to the opening of the urea moiety depicting m/z 375. Various other cyanotoxins, such as anatoxin, nodularin, and saxitoxin needs a detailed study on the reaction intermediate formed from the photocatalysis. A complete understanding of the reaction intermediates (involving mineralized products) can be beneficial for the DWTP for all kinds of cyanotoxins to employ photocatalytic treatment with confidence.

High energy usage is a common problem associated with photocatalysis along with other factors, such as skilled supervision requirement, strict experimental conditions (for example: frequent pH adjustments), by-products toxicity and most importantly, difficulty in characterizing the by-products formed limits photocatalytic process in becoming a primary choice for the water treatment systems [39]. In one of the studies, a brine shrimp bioassay test for the MC-LR degraded

[2] One of the peptide structure in MC-LR structure

products showed that the lethal concentration of the residual MC-LR increased from 2 µg/ml (initially) to 27.5 µg/ml and > 50 µg/ml at 4 minutes and 30 minutes, respectively [34]. Also, the mineralization of MC-LR by photocatalytic oxidation was sometimes found to be as low as 10%. This can be related to the change in the degradation pathway as further discussed later in details in section 4.

Photocatalytic process for cyanotoxin removal is highly affected by the change in pH of the surrounding environment. For example, Zhang et al.~~, (2014)~~ [40] achieved maximum MC-LR degradation (initial concentration: 9 ppm) rate at pH 5.01 ($Ag_3PO_4$ photocatalysts system[3]; dose 26.6 ppm) with pseudo-first-order kinetic constant, *k* value of 1.52 $h^{-1}$ and a removal efficiency of 99.98% in 5 h. The kinetic constant and overall degradation reduced further to 0.18 $h^{-1}$ and 59.19%, respectively when the pH was increased to 11.96. Change in pH influences the hydrophobicity of cyanotoxins, such as MC-LR which increases with a decrease in pH, preferentially allowing such compounds to move towards the catalyst surface from the bulk solution. On the other hand, under basic conditions, MC-LR showed very low adsorption on the catalyst surface. This explains the fact that pH influences the catalyst activity and cyanotoxin solubility which hampers the overall photocatalysis operation. Otherwise, DWTP might need to set up a neutralization tank just before the photocatalytic chamber to have an effective cyanotoxin treatment. Thus, problems related to pH variation can be solved, but the presence of other substances, such as NOMs and other organic matter can further decrease the removal efficiency of cyanotoxins. These inconsistencies in removal efficiency due to the influence of the process

[3] [3] 500 W xenon lamp as a light source following equation 5-8

conditions (e.g., pH, NOMs~~, etc.~~), and toxic by-products formation during photocatalysis challenges its commercial viability.

Other studies for different cyanotoxins using photocatalysis have been tabulated under Table 2. From the reported studies, it can be seen that the presence of NOMs are principally held responsible for the ineffective toxin removal (as it requires additional energy to remove the cyanotoxin in the given time period). Figure 2 (C) and (D) shows the effect of NOMs under different pH conditions where NOM particles absorb UV light and act as a scavenger for the hydroxyl radical (responsible for cyanotoxin degradation) formed due to the oxidation process. Under low pH, the effect of surface adsorption of NOMs inhibits the MCs molecule interaction with the catalyst surface. ~~Hence,~~ Apart from the scavenging action (which occurs at all pH), adsorption of foreign compounds in the form of NOMs and other oxidants, such as peroxide molecules (Figure 2 (B)) can interfere with the photocatalyst surface affecting the removal of cyanotoxin molecule. This makes the cyanotoxin molecule remain stable for a longer period of time demanding more operation time and hence more investment of energy. From Table 2, it can also be observed that all the studies have been performed at lab-scale with operational volume which is too low (< 20 mL) to extrapolate the results at least to the pilot scale. Moreover, preparation of the catalyst surface which can be made durable enough for a prolonged period is questionable. Further, a constant monitoring of the amount of energy consumed, and efficiency achieved must also be tracked down simultaneously from time to time which itself can add an operational burden on the plant operator.

### 3.2. Other oxidation methods: Ozonation and Chlorination

Use of chemical oxidants, such as ozone, chlorine, chlorine dioxide, chloramines, and permanganate have been effective for most of the cyanotoxins (especially, microcystins) [26]. An

ozonation study for the removal of different cyanotoxins viz. MC-LR, CYN, and anatoxins achieved approximately 95% oxidation at 0.25 mg/L, 0.38 mg/L and 0.75 mg/L of ozone dose, respectively [41]. These concentrations were lower as compared to the concentration at which the harmful by-products were detected. Such an ozone dose is compatible with the DWTP operation too as they fall within the safe dose ranges (0.4 mg/L at low NOMs level is safe for pre-treatment of raw water in DWTPs) [42]. Some studies have even shown non-formation of bromates (bromates: not acceptable in drinking water treatment) even in ammonia free water which strengthens the use of ozonation in drinking water plant for the cyanotoxin removal. Generally, bromide level in natural water sources may vary between 10-1000 µg/L and thus can be problematic for the human health if not treated properly (WHO recommendation of bromate: 25 µg/L). Various bromo-organic-by-products in form of bromoform~~,~~ and bromopicrin, ~~etc.~~ can be lethal for the human health [43].

Ozone treatment is widely used in a DWTP and is also considered to be a good option for cyanotoxin removal, having an added advantage in not letting the release of toxin from the cyanobacterial cells at low ozone dose (up to 0.6 mg/L) [44]. Less than 1 mg/L of ozone dose is quite common in DWTP as mentioned earlier. In another instance, Liu et al.~~, (2010)~~ [45] investigated MC-LR removal (initial concentration of 100 µg/L) with UV treatment for a duration of 5 minutes (2.6 $mW/cm^2$) followed by ozone dosage of 0.2 mg/L where they achieved a final MC-LR concentration of 1 µg/L. With higher ozone dose of 0.5 mg/L (permissible ozone dose in a DWTP), MC-LR concentration decreased further to 0.1 µg/L (< WHO guideline value). Ozonation proceeds with toxic by-products formation in the form of formaldehydes, other aldehydes, and ketones. High degradation efficiency of MC-LR is achieved via more oxidative force in form of $H_2O_2$ along with ozone dosage applied ($H_2O_2/O_3$: > 90 % in < 1 min while only

$O_3$: 60 % in 30 min) but at the expense of producing toxic by-products [46]. It was observed that at a lower molar ratio of ozone and MC-LR (40:1), $H_2O_2/O_3$ treatment produced an equivalent biotoxicity of 0.04 ppm $Zn^{2+}$ concentration as compared to 0.008 ppm when only $O_3$ treatment was followed. This trend did not change much at a higher ratio, where the latter showed biotoxicity of 0.01 ppm $Zn^{2+}$ concentration while former showed 0.05 ppm. Thus, a balance between effective MC-LR degradation (or other cyanotoxins) and biotoxicity level needs to be taken care of in the DWTP operation. ~~Chang et al., (2015) [39] too revealed the~~ An effective removal of MC-LR by UV/$O_3$ treatment at low ozone level (48 µg/L) and increase ozone level (76 µg/L) was achieved where inclusion of UV parameter enhanced the MCs removal by > 40 % and > 20 %, respectively. ~~However, This study qualitatively (~~Evidence from mass spectra showed complete cleavage of the adda side chain molecule (represents toxicity) and thus ~~previously discussed study is~~ contradicted the previously discussed study. Moreover, the $O_3$/UV treatment showed the stability in MCs removal efficiency in the presence of high NOMs (> 4 mg/L) to about 85 % as compared to 60 % when only ozone was used.as the treatment. This might prove to be very effective, practical and apt for treating cyanotoxins (MCs in particular) in the presence of NOMs as it has been the most common and important challenge for all the physicochemical treatment processes. Raw water (with high NOM and MCs) entering the pre-treatment unit (pre-ozonation) will be treated effectively in a DWTP and will ensure toxic-free water discharge to the next subsequent operational units.

Meanwhile, the intermediate by-products formation during each reaction step is a toxic component, which requires an additional treatment for their removal [48]. Adda fragment molecule is a characteristic part of microcystins and protein phosphates are inhibited by these molecules. Thus, by-product molecules comprising “adda” fragment are a sign of toxic metabolites [49].

Toxic metabolites mainly consist of adda-fragment masses of m/z values: 192, 208, 232, 248 and some higher molecular masses of 796 and 836 [50].

Further, the presence of high NOMs in untreated raw water had been a major challenge for the ozonation system due to its competitive nature to react with ozone [51]. Akin to photocatalysis process, ozonation too is sensitive to the pH of the surrounding environment. For example, under alkaline conditions, ozone has lower oxidation potential (1.24 V) as compared to acidic conditions (2.07 V), which allows the hydroxyl radical to decompose the ozone molecules under the basic conditions and hence acts as an inhibitor radical for the cyanotoxin removal. The increase in ozone decomposition within a short pH window ranging between 7.5 and 9 can even deviate from the result by 45% (of unoxidized MC-LR) in solution [48]. Thus, for the drinking water treatment containing cyanotoxins, ozonation might not always be a variable option (widely applied as a pre-treatment step in form of pre-ozonation) and may incur great challenges if overall balance is not attained. Chlorination also shows the effective removal of cyanotoxins where a dose of up to 3 mg/L showed complete MC-LR degradation [52]. However, the removal varies for other cyanotoxins, especially anatoxins, ~~whereas in one study~~ where only 15% of anatoxins was found to be oxidized for the same chlorine input. The formation of disinfection by-products at high chlorine dose can further make the overall cyanotoxin removal ineffective (as the usual dose is taken up by NOMs presence). Hence, the DWTP dealing with anatoxin might have to choose a different alternative apart from chlorination (or even ozonation as discussed earlier). Other oxidants such as chlorine, chloramines, and chlorine dioxides have also been found to be ineffective for some varieties of cyanotoxins, particularly anatoxin where they become highly pH dependent at some stage of the treatment [53]. Also, chlorine and chloramine showed variable removal efficiency for different cyanotoxins. ~~However~~ Chloramines have an advantage over

chlorine usage in the DWTP (especially water containing high NOM), as the latter forms comparatively higher disinfection by-products than the former. Use of chloramines reduces down the concentration of THMs and other chloro/bromo analogues and ensures better safety for the public. However, ~~Nicholson et al., (1994) [46]~~ it was found that the use 20 mg/L of monochloramine was only able to remove 17 % of cyanotoxin extracts (from the *M.aeruginosa*: mostly MC-LR) in 5 days whereas chlorination showed non-detectable concentration of cyanotoxin extract (MC-LR) at a dose of 2 ppm and contact time of 30 minutes [54]. Chloramines have weaker oxidizing potential as compared to hypochlorous acid/hypochlorite ion and usually proceeds with the slower kinetic rate for MC-LR, CYN or anatoxins ( $< 1\ M^{-1}\ s^{-1}$) especially when NOM is in the background [51]. Moreover, chloramines usage may demand more molar ratio requirement for the cyanotoxin treatment. For example, ~~Banker et al., (2001) [47]~~ it was showed that chlorine required less molar ratio (CYN: Chlorine = 1:1) as compared to the chloramine (CYN: Chloramine = 1: 2) to remove toxicity level of CYN which was duly determined by the formation of 5-chloro-cylindrospermopsin (non-toxic) [55].

~~Further to note,~~Other cyanotoxins, such as anatoxin-a and saxitoxins are resistant to chlorination. This can mainly be attributed to the structural differences among different cyanotoxins [56]. Even after 30 minutes of contact time and changes in pH, they did not show any effect. On the other hand, CYN was found to be effectively oxidized by chlorine (4 mg/L dose) at neutral pH [57]. Also, the dose of chlorine is still higher (4 mg/L) than the usually recommended input of 2-3 mg/L. ~~Another study by Rodriguez et al., (2007) [33]~~ Also, it was revealed that ~~showed that~~ approximately 1.5 mg/L of chlorine dose was enough for complete oxidation of cylindrospermopsin (CYN), while 3 mg/L of chlorine was only able to remove 8% anatoxin [41]. ~~Hence~~Thus, chlorination is not effective to deal with all the variety of cyanotoxins and high dose

might be needed (>2-3 mg/L) that poses a danger to surpass the guideline for the drinking water system.

Moreover, oxidation of CYN by chlorine is accompanied by the formation of trihalomethanes (TTHM) at a detectable concentration of 150 µg/L. These TTHM levels are above the EU 1998 guidelines (100 µg/L) and thus can be detrimental to human health if present in drinking water. On the other hand, ~~a study by Blette et al., (2008) [50] provided an~~ information on over 190 water samples containing microcystins that were treated via ozonation and chlorination process revealed that the mean THMs level in chlorinated and ozonated water ~~was~~ found to be 45.1 ± 3.0 µg/L and 18.6 ± 2.2 µg/L respectively [58]. Both these values were found to be under guidelines values of 80 µg/L (U.S. EPA) along with the microcystin concentration that falls below the WHO guideline.

The oxidation rate of various toxins varies in response to their chemical structure too. For example, the oxidation of anatoxin-a by ozone was found to be relatively slower than the peptide hepatotoxins (such as microcystin variants). However, an acceptable removal efficiency ( 92%) was obtained in both cases [59]. On the other hand, removal of anatoxin-a at the initial concentration of 20 µg/L in raw water resulted in only 15% removal using 15 mg/L of chlorine (for 30 minutes) [60]. Other oxidants, such as aqueous chlorine and calcium hypochlorite at 1 mg/L dose were found to effectively remove 90% of the cyanotoxins, such as nodularin and microcystins [54]. ~~In the same study,~~ Also, chlorination via sodium hypochlorite was shown to achieve only 40% removal of MC-LR under similar experimental conditions.

Thus, the variation in removal efficiency and degradation rate pose a challenge for cyanotoxins removal by using chlorination in the water treatment plant. Moreover, the accumulation of various oxidant by-products formed during the chemical reaction requires further treatment and is not

desirable economically. These untreated by-products, when released into water bodies, affect the health of the aquatic organisms [61]. By-products in the form of trihalomethane and haloacetic acids get enhanced especially due to the presence of low levels of natural organic matter (NOM) which is quite common for more than 90% of the DWTP. Higher contact time (CT) in treatment via chlorination results in an enhanced removal of toxin but at the expense of TTHMs formation and haloacetic acid. Interaction of cyanotoxins, such as MC-LR with chlorine or other chlorine agents has been shown to form dichloro-microcystin followed by hydroxylation, resulting in the formation of dihydroxy-microcystin. The chlorinated-microcystin by-products might be more toxic than their parent compound [34]. A major disadvantage of using chlorination apart from the harmful by-products formation also lies in the operational difficulties because several parameters, such as optimum chlorine dose, proper contact time and pH needs to be optimized, which is difficult to achieve with respect to the variety of cyanotoxins and different degradation rates [35].

The pH dependence and rate constant (second-order reaction of cyanotoxins and chlorine) can further be explained based on the dissociation parameter of the cyanotoxins. For instance, the reaction between $OCl^-$ and non-dissociated CYN was found to be negligible at higher pH because the latter concentration must be lower to allow complete oxidation of the former and thus becomes an important criterion. For example, ~~Rodriguez et al., (2007) [33]~~ it was studied that at constant temperature (20°C), reaction rate constant for chlorine and CYN interaction, increased from 2.39 x $10^3$/s to 81.0 x $10^3$/s as pH increased from 4 to 7.1 and all the way down to 1.02 x $10^3$/s at pH 8.4, when studied at different concentrations of chlorine and CYN [41]. Additionally, the chlorinating agents, such as chloramines and chlorine dioxide have been found to be less effective as compared to chlorine and ozone usage for the removal of microcystin, cylindrospermopsin,

anatoxin-a, and saxitoxin (USEPA, 2017) [62]. Some other studies on chlorination and ozonation treatment of various cyanotoxins have been summarized in Table 2.

### 3.3 Membrane methods

Membrane filtration has been proved efficient for the removal of both intracellular and extracellular cyanotoxins. Processes, such as nanofiltration (NF), reverse osmosis (RO) and ultrafiltration (UF) achieved more than 98% removal of cyanobacterial cells and intracellular cyanotoxin [63]. For saline water, reverse osmosis (RO) can be very useful for cyanotoxin removal. Neumann and Weckesser (1998) [64] reported removal of over 95% of MC-LR and MC-RR, subjected to varying initial concentration, ranging from 10 µg/L to 130 µg/L in the presence of 3,000 ppm of sodium chloride. Average retention levels were found to be in the range of 96.7-99.6%. ~~However,~~ RO treatment may not be applicable for the removal of all types of cyanotoxins. Very little to no work has been done to date to study the removal of saxitoxin and cylindrospermopsin (CYN) through RO [7]. It might be due to the persistent nature of these cyanotoxins in an environment which makes them difficult to remove and also the fact that they are difficult to extract from the water bodies as they are not found as prominent as microcystins.

Other membrane processes, such as microfiltration (MF) and ~~ultrafiltration (~~UF~~)~~ are increasingly being used for the small-scale communities as an economical alternative to conventional treatment, such as chlorination. Experimental studies with these types of membranes have shown high removal efficiency (>98%) of *M.aeruginosa* cyanobacteria (whole cells) [51]. Hart et al.~~, (1993)~~ [65] analyzed the effect of microcystin removal through the ~~ultrafiltration (~~UF~~)~~ at the initial concentration ranging between 5 µg/L to 30 µg/L that achieved less than 1 µg/L in the effluent. Nanofiltration (NF) has also been found effective in cyanotoxins removal. Teixeira and Rosa (2006) [66] have found that NF (NFT50 membrane where polypiperazine amide: laid on a

polysulfone microporous and a polyester support) was very effective in the exclusion of microcystins from drinking water. For the initial concentration of 10 µg/L MC-LR (in decanted water) obtained from Tavira water treatment plant (Algarve, Portugal), more than 94% removal was achieved. The process was effective within the range of 4.6-10.2 mg/L as carbon from NOM and pH range of 4.1-7.7. NF membranes also showed promise results on CYN as it removed > 90% by low molecular weight cut-off or "tight" membrane system. Also, other cyanobacterial metabolites including 2-methylisoborneol (MIB) and geosmin (GSM) showed > 75 % removal through NF membrane [67,72]. These results hold promise for the cyanotoxins removal within a DWTP ~~which has the provision for RO~~ using membrane treatment. ~~However~~ Moreover, frequent membrane fouling due to the presence of NOMs and other organic matter, along with high energy footprint make membrane process less versatile for cyanotoxin removal, especially for the community of less population where treatment cost per liter per capita is high. Challenges of higher NOMs (>10.2 mg/L as discussed above) can be linked to reduced efficiency in exclusion of cyanotoxin variants for a longer period of operation. However, it was found that the removal efficiency of MIB and GSM improved with the fouling phenomenon using higher MWCO membrane. This result also seemed applicable to other cyanotoxins viz CYN and MCs where higher MWCO membrane too showed improvement in the removal efficiency due to fouling [68].

Membrane processes also depend on the type of the membrane being used. Lee and Walker (2006) [58] evaluated that cellulose membrane used in ultrafiltration method failed to adsorb MC-LR. Polyethersulfone membrane too failed to adsorb MC-LR, after 60 minutes of operation. Adsorption is the key for membrane processes and dominates most of the rejection for UF membranes. Adsorption effect is linked to the hydrophobic interaction between the membrane surface and the cyanotoxin molecules apart from the hydrogen bonding, porosity, and surface

roughness factors. Polysulfone membranes being a hydrophobic membrane can even adsorb up to > 91 % microcystin-LR molecule while hydrophilic membrane such as cellulose acetate membrane absorbs little to nothing [70]. NF membrane showed electrostatic interaction and a steric hindrance as the primary removal mechanism for the anatoxin-a removal (< 1.3 μg/L) while only steric hindrance as the main mechanism for the MC-LR removal. Also, these membranes showed no specific dependence on the NOMs, flux and performed well at neutral pH.

Apart from such specificity, the complexation of cyanotoxin treatment via membrane processes was also shown to be impacted by the biofilm formation which can reduce the flux rate drastically (from >4 L/h/$m^2$ to less than 1 L/$m^2$/h). This effect in the real application can further get impacted by intact cyanobacterial cells that might accumulate on the membrane surface in a long run. In a gravity-driven membrane, the problem is even more prominent where a significant change in flux rate (> 80%) can be observed after 10 days of operation (Table 2). Overall, the use of NF membrane in the DWTPs may act as an effective barrier to anatoxins, CYN, MCs as well as various cyanobacterial metabolites, such as GSM and MIB as discussed above [67,71].

### 3.4 Miscellaneous methods

Other conventional methods to remove cyanobacterial cells involves the usage of algicides, such as copper sulfate [73]. ~~However,~~ One of the major consequences of using algicide is that it promotes the cell lysis, allowing the release of toxins [74]. Use of other chemicals, such as ferric sulfate can be useful in precipitating out excess phosphorus if the phosphorus level is too high (as a pre-treatment of raw water in DWTP). However, this chemical addition leads to an unnecessary increase in the sludge loading and precipitation of phosphate, which may promote cyanobacterial bloom formation, once introduced to the receiving environment. Moreover, the release of copper ions need to be tackled in the downstream unit operations and their removal needs to be ensured.

~~Hence~~ Also, post-treatment is a must to ensure the economical removal of the cyanotoxins. Use of chemicals, such as potassium permanganate is limited too because they also promote cell lysis leading to cyanotoxin release [75]. Moreover, these chemical methods introduce higher toxicity as they do not destroy the cyanotoxins *per se*. Use of permanganates is highly discouraged owing to the high concentration (sometimes >6 mg/L) required to effectively remove cyanotoxins especially anatoxins and MC-LR. Usage of potassium permanganate is often practiced in a DWTP as a preoxidant in the flocculation mixing tank. MCs have been found to be removed completely at the concentration > 1.5 mg/L [62]. ~~However,~~ If cyanobacterial cells come along the raw water in the DWTP, then care must be taken as high dose than usual can lyse the cells to produce cyanotoxin which might challenge the subsequent operating unit (change in operational parameter, flow rate, etc.), Other cyanotoxins, such as anatoxins and CYN require very high permanganate dosage (> 3 mg/L) as the kinetic rate constant follows a weak second-order rate of < 1 $M^{-1}$ $s^{-1}$, which limits its application in the DWTP or any waterworks [77]. Moreover, the associated problems of cell lysis and frequent dose surveillance becomes difficult to supervise. ~~Hence~~, The plant operators must deal with the degree of cell lysis too that may be expected on a certain day of operation and accordingly might need frequent adjustments in the treatment processes (in form of chlorine dose, ozone dose, etc.). Some other studies related to permanganate and hydrogen peroxide usage are shown in Table 2. The electrochemical method of cyanotoxin removal at the laboratory scale holds promise for their removal in a short time. ~~However,~~ Most of the studies done to date are based on MCs degradation. Hydroxyl radical (generated from oxidation reaction) plays an important role in the overall degradation of microcystins. ~~A recent study by Bakheet et al., (2018) [64] showed c~~ Complete removal of MC-LR (low initial concentration: 2 μg/L) in chloride-free solution was achieved within 30 minutes of electrochemical reaction (electrolysis with a boron-doped diamond)

[78]. Another improved study on boron-doped diamond electrolysis process showed better control over the MCs (high initial concentration: 35 μg/L) achieving 100 % removal within 60 minutes [79]. Use of solid polymer electrolyte not only enhanced the overall removal of MCs but also reduced the terminal voltage (less energy output) and able to generate oxidant molecule in low conductive solution. Even under the high chloride concentration (30 ppm), the system can maintain its efficiency up to 90 %. Electrochemical method also holds promise to perform well under NOMs (can be related to DOCs) as another study ~~by Dubrawski et al., (2018) [66]~~ showed 100 % MC-LR removal (10 μg/L to < 0.1 μg/L) using electrochemical (EC) ferrate at the DOC level of 2 ppm (under natural water as well as pure water) [80]. An advantage in utilizing these EC is that it also acts as a disinfectant and as a coagulant which can be beneficial for the drinking water treatment application. Also, under alkaline pH, electrochemical treatment (graphite electrode assisted by $TiO_2$ nanoparticle) can be put into use for the cyanotoxin treatment (> 90 % removal of MC-LR in < 1h ~~hour~~) [81].

### 3.5. Physical adsorption methods using activated carbon

Filtration process using powdered activated carbon (PAC) and granular activated carbon (GAC) is based on physical adsorption mechanism and being researched over the last few decades for cyanotoxin removal. The filtration efficacy mainly depends on the filter material being used [82]. Many researchers showed that the filter media affect cyanotoxin degradation [83]. The degradation potential of cyanotoxins also depends on the texture of these materials apart from different bed media used for filtration. For example, Miller and Fallonfield (2001) [82] observed that in case of soil with high sand content (98.5% sand), lower degradation of microcystin has occurred as compared to the clayey soil (16.1% clay content) where soil with maximum organic carbon content (2.9%) was used. Such modifications in the filter media also affect the dose which changes the

contact time necessary for the effective removal of cyanotoxin. ~~Donati et al., (1994) [123]~~ It was reported that with an increasing PAC dose using different filter media (from 25 mg/L in the case of wood-based carbon to 50 mg/L in the case of peat moss-based carbon), MC-LR degradation efficiency was significantly affected (98% removal for former compared to 60% for latter) (Table 3) [84]. ~~Another study by Vlad et al., (2015) [70], where~~ Also, saxitoxin removal was evaluated using PAC, wood, coconut, and coal where PAC achieved 100 % removal as compared to other materials [85]. This indicated that the origin of carbon powder too plays a vital role in cyanotoxin removal. Little to no studies have been reported to date for the CYN removal by PAC. ~~However,~~ From few of the reported studies, it has been found that high dose of PAC is required for CYN removal which also depends on the source from where PAC has been derived. For example, Ho et al.~~, (2008)~~ [86] found, that to remove mere 5 µg/L of CYN, around 25 mg/L of PAC is required at a high contact period (60 minutes) with the difference in efficiency noticed, for PAC obtained from different sources. In fact, the effect of NOMs also played an important role in CYN removal as the adsorption competition among the cyanobacterial metabolites increases with the spike in NOMs concentration. Thus, PAC eventually loses its adsorption efficiency due to pore blockage mechanism which holds good for other cyanotoxins as well [87].

Table 3 shows cyanotoxin removal (especially MC-LR), with initial toxin concentration and PAC dosage. The dose factor becomes a concern in real life scenario, where dissolved organic content varies with time (especially for untreated water received by DWTPs). PAC filter alone can remove cyanotoxins, but often requires high dosage, which challenges the process economics. Moreover, frequent change in cyanotoxin concentration over a month or two may demand periodical check more often. Sometimes, the dose requirement follows an exponential relation with the amount of toxin removed. For example, freeze-dried cyanobacterial toxins were removed up to 90% at the

dose of 20 mg/L of PAC. However, the complete removal required 100-200 mg/L dose of carbon powder making the overall process uneconomical. ~~However,~~ These problems can be overcome by combining one or more process along with PAC adsorption method. For example, alum coagulation in combination with PAC operation showed enhanced cyanotoxin removal [48]. The addition of a lower dose of activated carbon powder (5 mg/L) during coagulation showed an effective removal of some hepatotoxins and more than 50% of the anatoxin-a. Thus, conducting a pre-treatment step for the contaminated water moving into the PAC filter can potentially reduce the higher dose requirement of the activated carbon [57]. Some successful treatment options using PAC has been tabulated under Table 3.

~~However,~~ This may not be always true. In one of the studies, Lee and Walker (2006) [69], where PAC/UF and PAC alone was used to remove MC-LR, it was observed that the adsorption kinetics (1-h cycle) showed by PAC/UF process was lower than the PAC process. Even with a high dose of PAC (10 mg/L), the same trend was observed with an advantage of lower normalized concentration at the end of the adsorption experiment (1% and 2.5% of normalized concentration at 10 mg/L dose as compared to 10.5% and 16% value when PAC dose was 5 mg/L). Additionally, the combined PAC/UF system in the presence of NOMs failed to remove MC-LR to a level below 1µg/L (WHO guideline) [88]. ~~However,~~ The lower initial concentration of MC-LR (in the range of 5.3–7.4 µg/L), showed the final concentration of <1µg/L in the presence of 2.5-5.0 mg/L of NOMs, but at the expense of high PAC dose (15 mg/L). On the other hand, 5 mg/L and 10 mg/L PAC dosage were insufficient in removing MC-LR to a level below 1µg/L. Meanwhile, at 17.1–23.2 g/L MC-$LR_{eq}$, even 15 mg/L of PAC dosage was insufficient for microcystin removal to below 1µg/L.

Also, the kind of membrane (as discussed in the membrane process, section 3.4) to be used for the hybrid process becomes an important factor. For example, using PES (polyethersulphone)-20 kDa membrane used for UF for PAC/UF system, effective MC-LR removal was achieved (85% and 80%, respectively) and were better than PAC adsorption (<75%) [69]. However, when cellulose acetate membrane was used, PAC adsorption alone and PAC/UF showed no major difference for the MC-LR removal. It was demonstrated that the role of PAC in the removal of MC-LR was more responsible than UF, since, only up to 10% of this toxin was removed by UF, indicating that the role of adsorption in MC-LR or other cyanotoxin removal is very important.

Other cyanotoxins, such as saxitoxin removal through sorption were shown to be dependent on the electrostatic and non-electrostatic interactions. These electrostatic interactions were caused due to the range of pH being studied (from 5.7 to 10.2), with a maximum sorption at pH 10.2. At pH 10.2, 1-40 mg/L PAC dose removed >99% of saxitoxin whereas, at pH 5.7, almost no removal (sorption) of saxitoxin was observed for PAC dose between 1-40 mg/L. These observations suggest effective sorption of cyanotoxin molecules under alkaline conditions which are somewhat irrational to be applied in DWTPs. Further, PAC dose varies a lot with the treatment efficiency required. Moreover, the presence of NOMs was shown to decrease the sorption behavior of the PAC for the amount of saxitoxin removed and a hence higher dose of PAC was required to effectively adsorb it [89].

In general, PAC operation is considered cost-effective than GAC, in terms of the capital and operational cost involved [90]. Some studies even suggested that GAC filter is very effective in MC-LR removal [83]. ~~Carlile et al.,(1994) [45]~~ It was even found that GACs could adsorb other cyanotoxins, such as anatoxin (95%; 15 minutes contact time) better than MC-LR (80%-90%) [53]. ~~However,~~ Very few studies are available for other cyanotoxins removal, such as nodularin,

cylindrospermopsin, etc using GACs. Like PACs, removal of cyanotoxins by GACs too depends on the electrostatic repulsion between the cyanotoxin molecule which aids in their overall removal. For example, ~~in a recent study by Silva et al., (2015) [76],~~ in a GAC filter treatment, saxitoxin and decarbomoyl saxitoxin (dc-STX) exhibited cationic nature (between mono-cationic and di-cationic) in their molecular structure due to the presence of the amine group at neutral pH, which further helped these molecules to get removed due to the electrostatic repulsion [91]. However, these electrostatic repulsions might vary among different cyanotoxins based on the size and charges. For example, ~~Wang et al., (2007) [77]~~ it was found that the electrostatic repulsion factor decreased the microcystin removal in a GAC filter column (larger in size as compared to saxitoxin) [92]. In another study, ~~however,~~ an attempt was made to reduce the repulsion by increasing the ionic strength of the solution containing microcystin which resulted in their enhanced removal [93].

Thus, molecule size and hydrophobicity of cyanotoxins also influence the property of GAC to treat them. Higher hydrophobicity of a compound is often associated with high rates of physical adsorption in the filtration process. However, microcystin-LA (MC-LA) molecule, which is smaller and more hydrophobic than MC-LR showed relatively lower removal rate on GAC filter (both spiked at the initial concentration of 10 µg/L) [94]. Thus, among variants of the same cyanotoxin, removal efficiency can vary due to the varied nature of the molecular structure. Also, NOMs interference has been a concern for activated carbon filtration process too (as discussed above). With adsorption being the principal mechanism, the problem of early breakthrough arises due to a decrease in the adsorption over the time [53]. Moreover, the problem of plugging due to high organic content reduces the filterability of the bed and hence affects the overall efficacy of the filter.

Adsorption isotherm and thermodynamics studies can further help to achieve the effective cyanotoxin treatment in the DWTPs. Adsorption isotherm indicated that the adsorption capacity of MC-LR depends on the materials. For instance, wood-based carbon showed more adsorption capacity (280 μg/mg of carbon) as compared to the coal-based adsorbants (70 μg/mg of material), while coconut-based carbon showed adsorption of mere 20 μg/mg of carbon material [84]. MC-LR adsorption is an entropy-driven process where the influence of the solvent comes into play. Like other organics, MC-LR too adsorption is thermodynamically more favored under the negative entropy system [95]. MC-LR showed Freundlich adsorption isotherm where adsorption Freundlich capacity ($k_f$) showed a significant difference between the virgin carbon ($k_f$ = 50) and the competitive one ($k_f$ = 13) with NOM presence tested condition [96]. Thus, DWTP operator needs to be extra attentive in simulating the necessary operating conditions to tackle the NOMs level and other important parameters which affect the adsorption behavior of the compound over the material in general (such as, pH and temperature change).

On another note, the role of biological activity accompanied by the adsorption has shown enhancement in the cyanotoxin removal. Most of the researchers have shown an increase in cyanotoxin removal due to the inclusion of biological activity over GAC media. Sand media with bacterial activity too were shown to enhance the cyanotoxins removal. However, not much has been reported to date on the sand media filtration for the cyanotoxin removal. ~~However~~ Biological activity over the sand media has shown promise to effectively degrade cyanotoxins with filtration rate close to a rapid sand filtration system (4-10 m/h) [97]. The filtration unit forms the primary treatment step in any DWTPs where no chemical dose or high energy involvement is demanded. ~~Hence,~~ In the near future, the sustainable solution for the natural degradation of cyanotoxin can be

achieved by modification in the adsorption processes using GAC, PAC or sand as an effective filtration media.

Oxidation process, especially photocatalysis and ozonation has proved to be quite effective, quick and achieved an almost toxic-free solution for the removal of different cyanotoxins. Certain reaction mechanisms have been portrayed in the next section especially for these two oxidation processes. This upcoming section will give a more detailed idea about their process with discussions mainly related to the primary reaction mechanisms involved.

## 4. Reaction pathway/mechanism of oxidation processes

### 4.1 Photocatalytic process

Use of VIS photocatalysis could potentially be the renewable, sustainable and emerging technology for the drinking water treatment which accounts for over 40% of the solar energy. The mechanism of cyanotoxin removal is underdetermined yet and further studies are required in this field [98]. Contrary to UV-A technique of photocatalysis, VIS degradation of cyanotoxin degradation (MC-LR or CYN), is mainly governed by $O_2^{\cdot -}$ and $HO_2^{\cdot}$ reactive oxygen species (ROS), unlike UV-A where ROS are $HO^{\cdot}$ and $^1O_2$. Figure 1 shows the degradation pathway for CYN and anatoxin molecule due to ozonation and UV/$H_2O_2$ process where more hydroxylation could be possible (for CYN and anatoxins molecule) due to the generation of more $OH^{\cdot}$ radicals.

This further helps in breaking the C-C bond to effectively mineralize the by-products fragment. Under visible light irradiation, CYN showed effective degradation followed by several intermediates where hydroxyl radical played a major role. Moreover, the formation of inorganic ions such as $NO_2$, $NO_3$, $SO_4^{2-}$ and $NH_4^+$ proved the mineralization of CYN in the reaction pathway (Figure 1). Under UV-A and solar light irradiation, the product intermediates were similar

highlighting the importance of hydroxyl radical for the CYN degradation. It was also proved that CYN gets demineralized effectively under photocatalysis action by utilizing the solar light [36]. Hence, the above mechanism can become a potential reaction pathway that can be applied in the future to achieve sustainable treatment of other cyanotoxins as well. However, change in environmental conditions can alter the degradation pathway. Under basic environment, more carbonate ions form which suppress the formation of sulfate radical-transformed products and sulfate individually too. ~~However~~Also, it was found that these carbonate ions selectively responded to the CYN degradation with higher specificity than the hydroxyl ions. Also, since carbonate ions are electrophilic in nature (which could also be the reason for high specificity), ~~hence~~ they attack the nitrogen-containing phenols and organics too [99]. However, this unique mechanism pathway can eventually turn out to be toxic in nature as carbonate ions in large number have low reaction rate specificity with the uracil moiety. ~~Hence~~ Thus, $TiO_2$ photocatalytic degradation under high pH should be discouraged for the drinking water treatment or otherwise inclusion of neutralization tanks can be promoted, but at the expense of higher capital cost and difficulty in processing downstream unit operation.

Similar to CYN, hydroxyl radical is the primary reason for the ring opening in complex MC-LR compound. The diene bond, methoxy group of Adda, Arg amino acids, MeAsp Leu, Mdha Ala, Arg-MeAsp peptide bonds, ~~etc~~ were found to be the sites prone to photocatalytic degradation and initiation of the MC-LR oxidation [100]. These intermediate products represent the hydroxyl substitution with the addition of unsaturated bonds in the MC-LR structure. Such an addition in MC-LR side chain is caused due to attacks of $O_2^{\cdot-}$ and $H_2O_2$ radical at varying position on the aromatic ring (conjugated double bond of Adda side chain/Mdha double bond). ~~However,~~ This mechanism of MC-LR degradation hinders some aspects of drinking water treatment and thus

~~hence~~ commercialization is difficult to achieve. It has been found that the doped $TiO_2$ nanomaterial often releases into the aqueous solution during water treatment by following this mechanism of MC-LR degradation. Optimum dopant concentration is needed as otherwise causes a decrease in the degradation rate too (as excess dopant causes electron-hole pair and low dose is insufficient for degradation). In addition to this, the by-products formation too affects the degradation potential of these nanomaterials and in the severe case leads to the deactivation of an overall process. Hence, a novel degradation mechanism is needed to be proposed in the near future which can provide the physical as well as the chemical stability of the doping compound by understanding the pathway involving the by-products formation.

Major challenges posed by photocatalytic degradation of cyanotoxin includes NOMs presence, pH change, and presence of other oxidants (as discussed in Figure 2). Figure 2 (E) depicts the advantage of doping metal to the photocatalytic metal oxide (for example $TiO_2$). It reduces the recombinant rate of electron and hole thereby enhancing the photocatalytic activity. Kumar et al.~~,~~ ~~(2017)~~ [101], has discussed enhancing the photocatalytic activity in general (no cyanotoxin related) where the idea of isolating the redox site which helped in reducing the recombinant rate, was evaluated. Use of heterocoupling of two metal oxides was used for achieving the same. Thus, in future, to compensate for and enhance for the loss of cyanotoxin efficiency due to the above-mentioned challenges can be effectively overcome using heterocoupling methods (such as ZnO-$TiO_2$, $TiO_2$-$WO_3$, and ZnO-$WO_3$). Also, the charge carrier mechanism as discussed by Sushma et al.~~, (2017)~~ [102] holds immense prospects for the micropollutant removal. Proper charge carrier mechanism can improve the visible light threshold (carbon acts as sensitizer: N-2p orbitals hybridize with O-2p level). Also, other species, such as sulfate exhibits synergism and promotes pollutant (here cyanotoxins as the possibility) adsorption by trapping CB electrons to inhibit charge

carrier recombination. This way an effective mineralization of cyanotoxin molecule can also be achieved [86]. Scheme 1 (A) shows the schematic representation for the photocatalytic treatment of cyanotoxins from simple to most advanced version studied so far, depicting its importance, significance, limitations, and challenges to be tackled in future.

Not many studies have been reported on the degradation mechanisms related to photocatalytic treatment for other cyanotoxins. These cyanotoxins can be quite specific in their reaction pathway and may require modification of the catalysts in action. For example, saxitoxins were shown to undergo selective removal through hybrid photocatalysts by the introduction of molecular recognition sites on the $TiO_2$ surface [104]. This process showed enhanced saxitoxin removal as compared to the bare $TiO_2$ surface application. However, the adsorption on active sites carried out through the functional ionic compounds (formed by immobilizing on sensor plates) can be affected by the presence of other competitive ions and NOMs.

Till date, photocatalysis for the removal of various cyanotoxins has been performed at four different levels: 1) simple photocatalysis (un-doped); 2) metal-doped or compound photocatalysis; 3) solar light source photocatalysis and; 4) UV/LED assisted photocatalysis. Scheme 1 (A) shows highlight for the above-mentioned photocatalytic mode of operation for the degradation of cyanotoxin molecule. Overall, in general, photocatalysis works best at acidic pH which may demand neutralization step after the cyanotoxin treatment in a DWTPs. Solar light can prove to be an efficient as well as sustainable approach in future. The persistence cyanotoxins, such as anatoxin and saxitoxins are still poorly studied and their removal needs further research work. Nearly 95 % of the study so far is performed at laboratory scale (< 300 mL reactor) and thus their scale-up remains a major challenge in future, if it must find its place in a DWTP.

### 4.2 Ozonation and peroxide process

The ozone molecule attacks double bond in the uracil moiety of CYN through the Criegee mechanism (Figure 1) [105]. This is followed by a series of different ring-opening molecules that are transformed and generated. Ozone molecule can also attack the tertiary amine in the tricyclic guanidine moiety through oxygen and electron transfer mechanism (proven by the formation of hydroxylamines and nitrones). Reaction mechanism also proved that $OH^{\bullet}$ is, not the only major radical responsible for the CYN degradation. This was proved by quenching the hydroxyl radical through tert-butyl alcohol which showed no effect later on the degradation rate [89]. Other studies too proved that hydroxylation appeared to be the primary reaction pathway carried out by hydroxyl radical in UV/$H_2O_2$ process. Secondary alcohol metabolites and its oxidation were also considered to be an important reaction mechanism in CYN degradation (as discussed earlier in section 3). This reaction mechanism (involving transformation and cleavage of the uracil moiety) and hydroxymethyl bridge oxidation results in the reduced toxicity of CYN overall [107]. Extended reaction further eliminated the sulfate group and the destruction of tricyclic guanidine ring via hydroxyl radical-AOP (Figure 1).

On another note, UV-C or $H_2O_2$ alone were found to be insufficient for the anatoxin removal. ~~However,~~ In contrast, combined UV-C/$H_2O_2$ showed effective hydroxyl radical generation which guided the anatoxin-a molecule to undergo 60% reduction in TOC, followed by 45% conversion of carbon into acetate and almost complete mineralization of nitrogen portion into $NH_4^+$, $NO_2^-$ and $NO_3^-$ ions [108] (Figure 1). This proposed reaction mechanism could possibly support the toxic-free treatment of anatoxins present in the drinking water sources. Also, with the proposed degradation mechanism, the process is quite slow (420 minutes) and require high energy input. The higher requirement of $H_2O_2$ makes the oxidation processes uneconomical as already discussed in earlier sections. For example, ~~a study by Afzal et al., (2010) [92]~~ it was shown that anatoxin-a

and hydroxyl radical had a second-order reaction ($(5.2 \pm 0.3) \times 10^9$ $M^{-1}s^{-1}$) achieving more than 80% anatoxin removal [109]. However, the high UV dose of 1285 $mJ/cm^2$ was required to degrade anatoxin-a (>85% and <50% of removal at higher concentration of anatoxin-a: 0.6 mg/L and 1.8 mg/L). Increase in the $H_2O_2$ concentration (from 30mg/L to 40 mg/L) led to more UV light absorption which led to more $OH^{\cdot}$ radical generation enhancing further degradation of anatoxin-a. However, higher $H_2O_2$ concentration led to the scavenging effect on the OH radical. Nevertheless, such high concentration can be detrimental to the water quality, if not regulated by another treatment unit in a DWTP. Thus ~~Hence~~, an alternative solution or degradation pathway is needed as the process is not only uneconomical but becomes unfeasible at times too.

Moreover, the second order reaction rate showed a decrease of over 56% when experiments were conducted with natural water instead of synthetic water. These reaction rates can decrease further if the effect of NOMs comes into play. ~~However,~~ In one of the studies, an interesting observation was made where UV-C photolytic process under NOMs showed a positive effect on anatoxin-a degradation via photosensitization effect, unlike normal UV/$H_2O_2$ process where more OH radical is demanded to counteract the effect of NOM. However, no specific degradation mechanism was laid out for the above observation [110].

For other cyanotoxins such as anatoxin-a, an increase in the oxidant reagents and anatoxin-a degradation followed a direct relationship, mainly guided by the hydroxyl radical. It was found that the mixture of one or two oxidants, apart from $H_2O_2$ enhances the degradation rate of anatoxins [111]. However, the reaction mechanism cannot be proposed as to whether degradation is effectively due to hydroxyl radical or due to a range of oxidants under input. But, the effectiveness of hydroxyl radical is enhanced using other oxidants such as $O_3$, $Fe^{2+}$, $O_3/H_2O_2$ and Fe(II)/$H_2O_2$.

For example, ~~Tak et al., (2018) [91]~~ it was found that ozone alone (2 mg/L) degraded 68% of anatoxin while ozone with $H_2O_2$ and Fe (II) degraded 100% and 85% respectively [108]. The overall mechanism works in a way that hydrogen peroxide in aqueous solution dissociates into $HO_2$- which reacts with the ozone molecule providing a chain of reactions to produce more hydroxyl ions. Likewise, $Fe^{2+}$ increase the number of hydroxyl radicals formed through a reduction reaction of ozone with an iron molecule (photo-Fenton reaction). Iron ions and $H_2O_2$ combination not only helps effective degradation of cyanotoxins but also promise to reduce the simulation activating endocrine disrupting chemicals which modulate the estrogenic activity. Liu et al.~~, (2018)~~ [112] studied the reduced estrogenicity of the treated CYN ($p < 0.05$) where the intermediate products oxidized by $Fe^{III}$-B*/$H_2O_2$ shown effective catalytic oxidative degradation of CYN molecule where most intermediates found with destroyed ring evidence. ~~However, O~~ther cyanotoxins (anatoxin) showed insignificant ($p > 0.05$) change in the estrogenicity mainly attributed to the sustained ring structure as it provides toxic stability and is more susceptible towards the estrogen receptors. The proposed mechanism by Chang et al.~~, (2015)~~ [113] showed that the combination of UV and ozone process can degrade the MC-LR compound at more than three reactive sites followed by modification/destruction of the Adda moiety (which is essential for the expression of toxicity) in all the formed intermediates. These four reactive sites were conjugated double bond of Adda chain, double bonds of Mdha and two acid-free groups of MeAsp and Glu part. ~~However,~~ The contribution of $OH^{\cdot}$ and ozone were not differentiated which were collectively held responsible for the oxidation of Adda and Mdha along with the isomerization, hydroxylation and oxidative cleavage of the parent MC-LR compound. Meanwhile, complete oxidation of most of the formed intermediates happened with combined UV/Ozone treatment which lacked in the individual processes. This further ensures the removal of Adda side chain

(dominantly formed during degradation pathway) which will ensure safe and toxin-free drinking water treatment.

Hydroxyl radical is the key for anatoxin-a removal too. However, it was observed that ~~an interesting study by Onstad et al., (2007) [97] showed that the~~ reaction of hydroxyl radical and the ozone molecule depends on the second order kinetic constant. Among anatoxin-a, CYN and MC-LR it was anatoxin-a which showed highest second order kinetic constant indicating overall higher reactivity [114]. The only reactive sites available for ANTX is the double bond holding the functional group, which is more susceptible to breaking, at low pH with ozone molecule. ~~However,~~ In contrast, under alkaline conditions, neutral amine dominates. The protonated amine interaction with ozone is not impacted much at pH over 7 and thus, ozone-ANTX could possibly have a variant mechanism for ANTX degradation at pH above 7. Scheme 1 (B) shows the schematic representation for the ozonation treatment of cyanotoxins from simple to most advanced version studied so far, depicting its importance, significance, limitations, and challenges to be tackled in future. Till date, primarily, three kinds of ozone operation have been dealt for the efficient removal of various cyanotoxins. These processes are only ozone application, ozone treatment in combination with UV and ozone in a combination of peroxides. For the solo ozone treatment of cyanotoxin, it requires more energy and dose input as compared to the other hybrid ozonation technique (assisted with UV and peroxides). Overall, ozone assisted by peroxides seems promising in future to efficiently degrade cyanotoxins in a DWTPs. One of the major advantages of this technique is that it has been shown to maintain its effectiveness even at high NOMs presence (> 4 ppm). Although the toxic metabolites have been found very low in concentration by this technique, still further research is needed to confirm for the other cyanotoxin variants as well, especially that formed by anatoxins and saxitoxins.

### 4.3 Chlorination process

Chlorination activates the MC-LR molecule (chloro-MC-LR molecule formation) through substitution and addition reaction mechanism for effective MC-LR degradation. Also, combining the UV irradiation method with conventional chlorination process activates the MC-LR molecule where the active radical formation is held responsible for simultaneous photo-degradation and photo-detoxification in a more effective manner. ~~Zhang et al., (2016) [98]~~ It was found that reduction in the toxicity level of the degraded sample through the application of combined chlorination/UV process ~~showed~~ possess comparatively less toxicity than chlorine process alone [115]. This proposed mechanism of dual objective (photo-degradation and photo-detoxification) can prove to be effective in removing other cyanotoxins as well from drinking water sources. A general perspective explaining the better degradation efficiency through the intrusion of UV might be attributed to the generation of common reactive oxygen species (ROS) and reactive chlorine species (RCS) such as HO•, Cl•, $Cl_2^{\bullet-}$, and $ClO^{\bullet}$. It was found that the highest contributor of MC-LR degradation was due to $OH^{\cdot}$ followed by RCS (> 25 %) and UV (< 10 %) [116].

~~Duan et al., (2018) [99] showed~~ A reduction in the by-products toxicity and enhancement by over 30 % in MC-LR removal was achieved by incorporating the UV treatment along with chlorination [116]. Such hybrid treatment method proceeds with the substitution mechanism where chlorine molecule attacks on the benzene ring of adda molecule (in MC-LR) followed by hydroxylation at the same position (addition of OH group). These hydroxylation reactions were hypothesized to occurs at multiple places (m/z = 1045.5, 1029.5, 1047.5) and especially found to be more susceptible to the adda portion of the MC-LR molecule. Such reaction mechanism delivered partial/full elimination of adda portion (m/z = 835.4) from the hydroxylated molecule and might be the reason for lower toxicity of the degraded molecule. Although chlorination treatment forms

hydroxylated-MCs product due to the nucleophilic substitution reaction, still it might be believed that the formation rate of these hydroxylated products (linked to reduced toxicity) is accelerated using UV/chlorination treatment. ~~Hence,~~ Hybridized oxidation methods not only have potential to eliminate the toxicity level of the final solution but also promises to reduce the treatment period which is equally important for the drinking water plant (operational) perspective.

A different perspective of reducing the microcystin toxicity apart from hydroxylation is the ability of MCs to form ketone by-products (keto-MCs) [117]. A DWTP which employs permanganate addition before chlorination (for disinfection), might change the requirement of chlorine dose demanded in excess to remove the cyanotoxins (or MCs). Use of excess permanganate can set a different platform for the chlorination mechanism for its interaction with the cyanotoxin molecule. Permanganate forms keto-MCs and hence will present the reduced toxic by-product/s to deal with chlorine molecule thereafter. ~~However,~~ It is still not known about the impact it will have on the chlorine dose requirement as it is not proven anywhere whether keto-MCs is less toxic or hydroxyl-MCs and vice-versa. ~~However~~ Also, combining permanganate (pre-treatment of raw water) and disinfection (in form of chlorine) has a wide scope and promise in a DWTP due to dual-mechanism as discussed. CYN degradation through chlorination is mainly accompanied by three mechanisms: addition, substitution and oxidation reaction. However, chlorine addition is expected to undergo a slow oxidation process to access the double bond in the CYN molecule. ~~Senogles et al., (2000) [101]~~ It has been proposed that either oxidation or substitution follows the main mechanism of CYN degradation [118]. There has been no study reported for the hybrid chlorination treatment related to the CYN degradation. However, the similar way of substitution reaction as shown by MCs can be expected where the unsaturated bond of uracil moiety can be hypothesized to undergo the chlorine substitution reaction with reduced toxicity anticipated. There has not been much

documentation and investigation about the behavior of anatoxin behavior with the chlorine molecule.

Scheme 1 (C) shows the schematic representation for the chlorination treatment of cyanotoxins from simple to most advanced version studied so far, depicting its importance, significance, limitations, and challenges to be tackled in future. Till date, three types of chlorination technique have been dealt with effectively for the treatment of various cyanotoxin as discussed in scheme 1 (C). It includes only chlorination, chlorine dioxide/chloramine/hypochlorite, and chlorination assisted with UV. Only chlorination required a high dose of chlorine and is inefficient in dealing with other cyanotoxins such as anatoxin. While UV assisted chlorination not only showed enhancement in the MCs removal but also reduced the toxicity level which places this technique right in front when compared to other techniques (chloramine, chlorine dioxide, etc) for the drinking water purpose. Years of further research proved better degradation kinetics than other older technique. Overall, chlorination assisted by UV is promising to be employed in a DWTPs in form of UV photoreactors. Future work needs to be done on other cyanotoxins as well to justify this hybrid technique before it runs full-fledged in a DWTPs.

## 5. Conclusion

Physico-chemical treatment methods, such as photocatalysis, ozonation, chlorination~~,~~ and membrane processes ~~etc.~~ have been widely practiced in a drinking water treatment plant (DWTP) for the raw water treatment. Of which, chlorination and ozonation are used as the routine treatment steps. These unit operations have also been successful to some extent in handling different cyanotoxins. However, either or more of the mentioned fallouts such as a) high energy consumption and operating costs, b) poorly understood kinetics, and most importantly, c) the production of harmful and toxic by-products, challenges and limits the commercial applications of

these technologies. With routine dose and treatment criteria, cyanotoxin removal is partially achieved in most of the cases. Thus, to enhance the treatment efficiency, high energy and chemical dose become the only options available. And in doing so, the excess/residual dose breaches the recommended guideline values for the drinking water treatment. However, modification in these physicochemical technologies can be guided through the change in reaction pathway or mechanism to enhance the overall water treatment efficiency (dealing with foreign substances or environmental conditions such as NOMs, change in pH, etc.) and make them sustainable and rational to meet the guidelines and effectively handle the cyanotoxins. Recent and advanced modification in the treatment process of photocatalysis, ozonation, and chlorination has ensured the removal of some persistence cyanotoxins including saxitoxin, anatoxin and cylindrospermopsin apart from just the removal of microcystins. ~~However,~~ On the other hand, certain promising treatment techniques, such as electrochemical process and photocatalysis may become a costly affair owing to a large amount of water treatment and the experimental monitoring and control needed. Finally, it is imperative for the old or existing plants to consider cyanotoxin as a serious threat to the public safety ensuring their health. An appropriate and more suitable treatment technologies need to be retrofitted which must also be less energy intensive, economical, with lower operational constraints (based on the type of source water to be handled) and easy in operation.

**Conflict of interest**

None.

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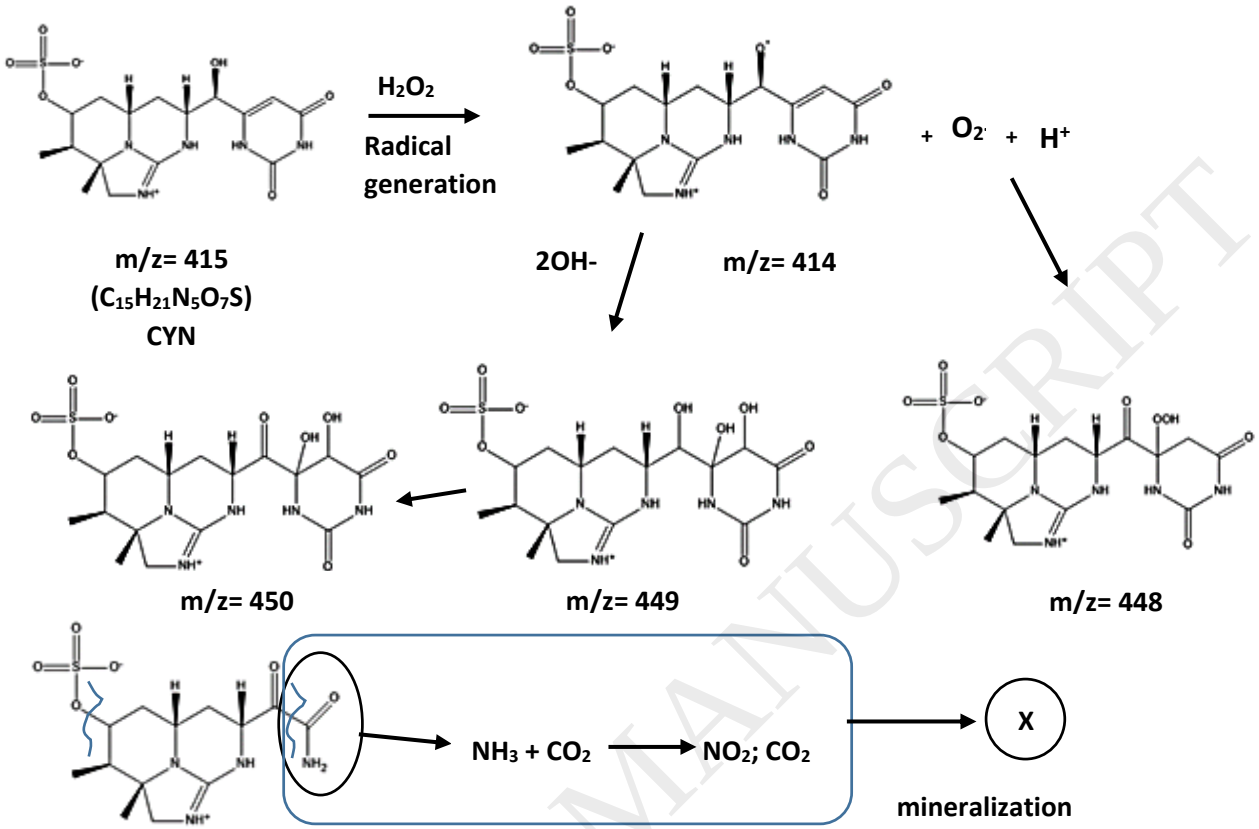
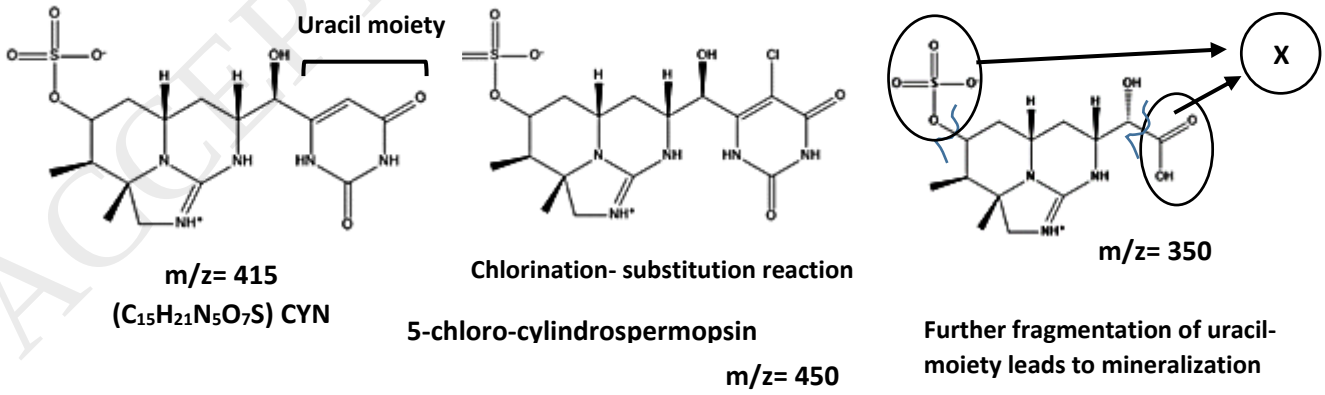
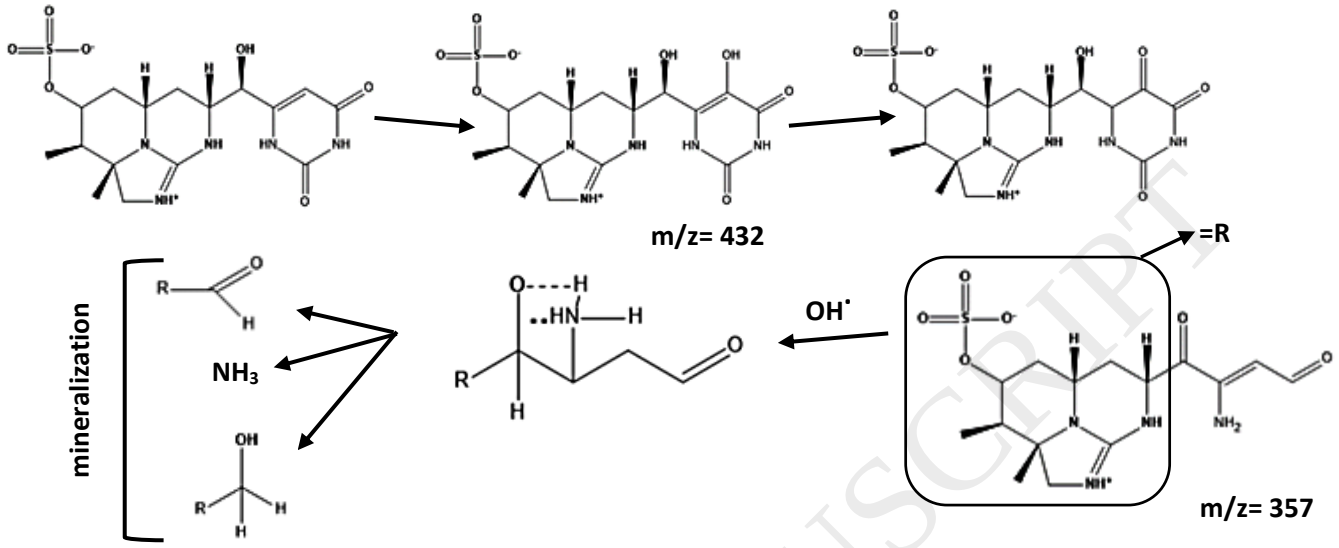


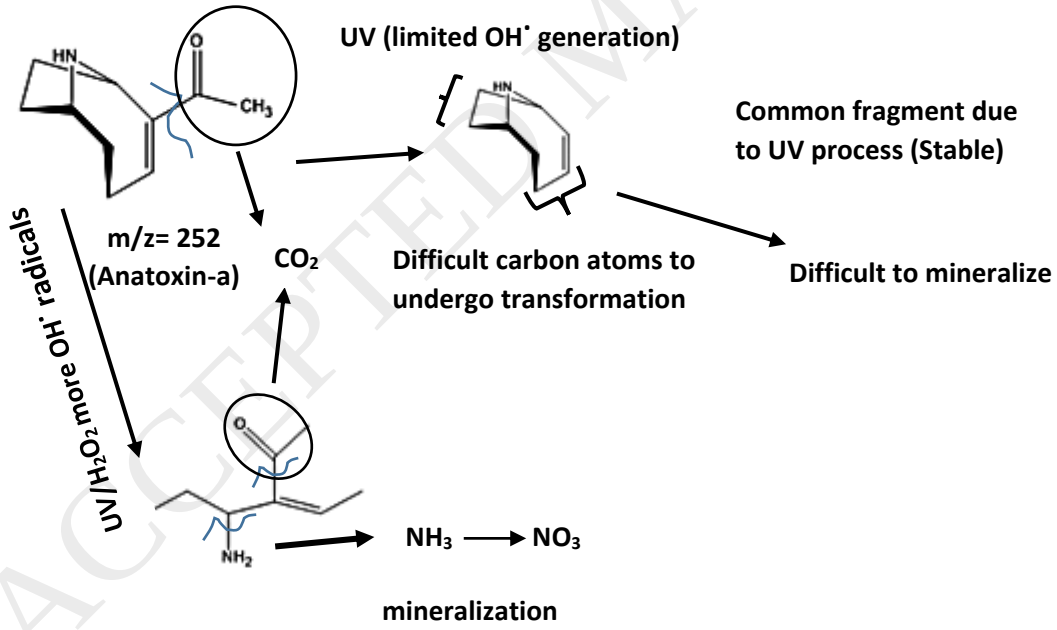

**Photocatalytic process (Cylindrospermopsin: change in structure)**




**Chlorination process (Cylindrospermopsin: change in structure)**

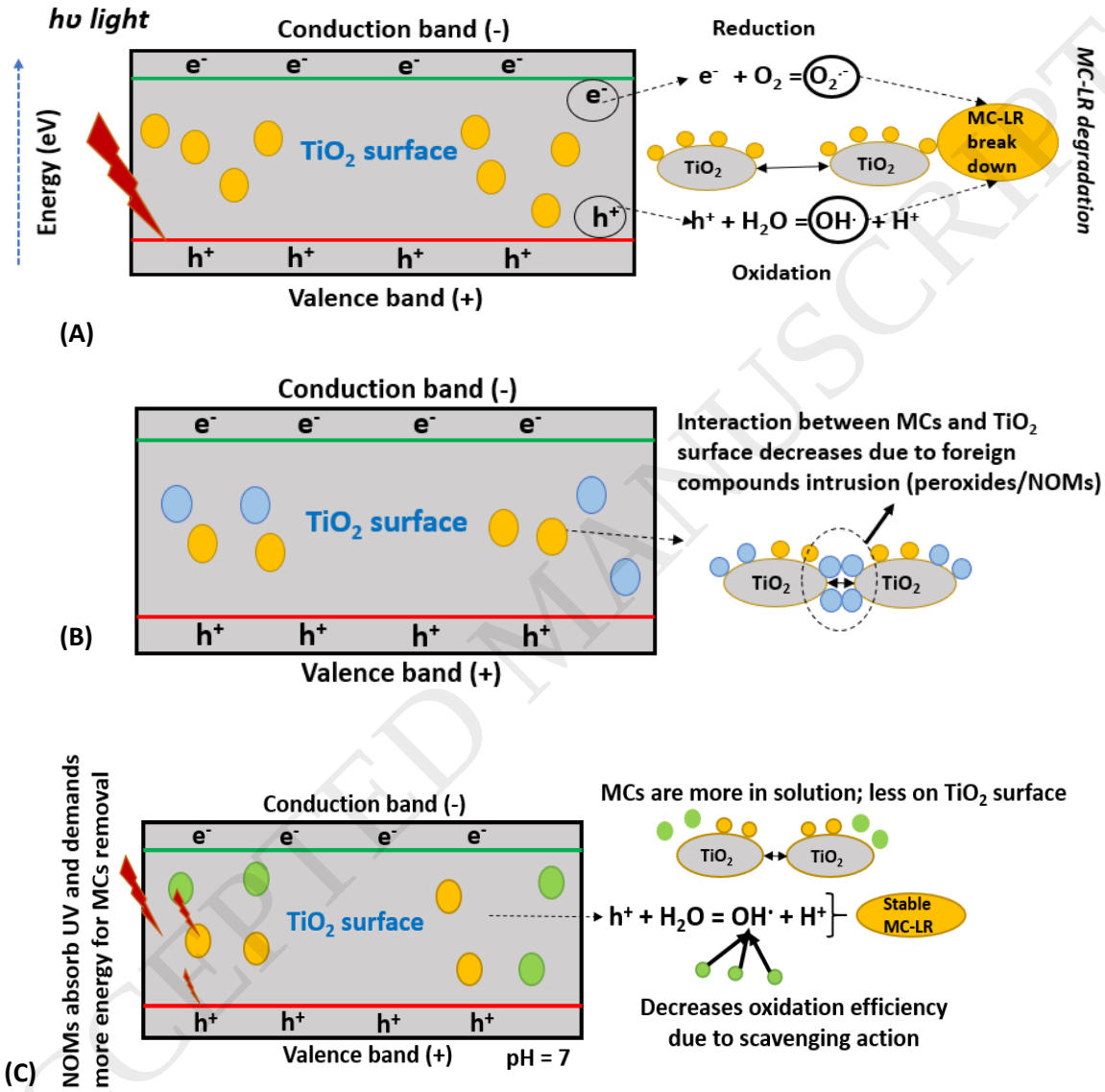


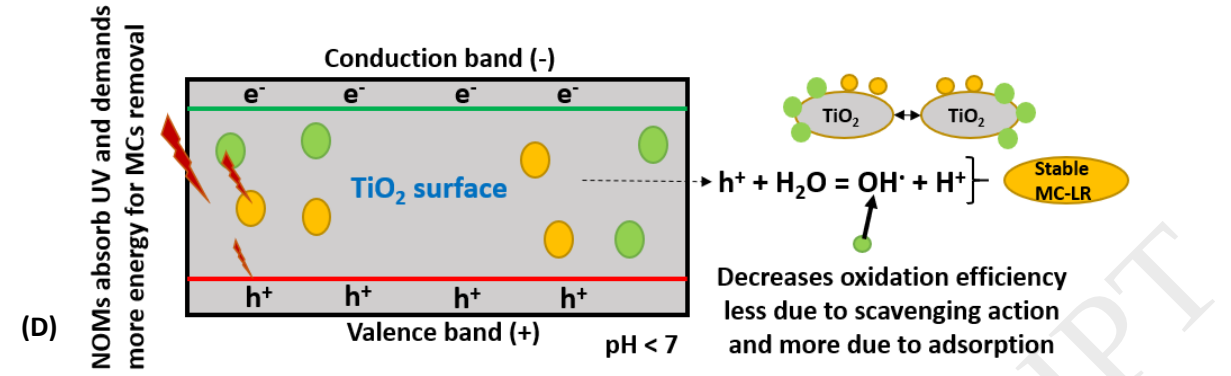

**Ozonation process (Cylindrospermopsin: change in structure)**



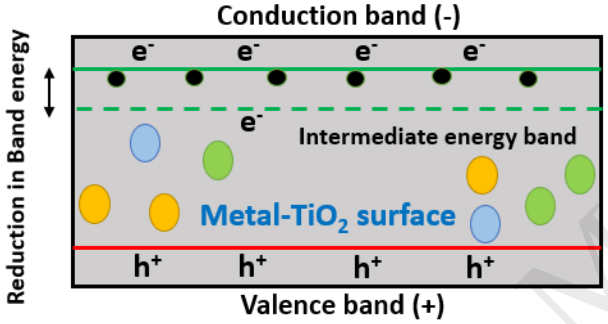

**Photocatalytic/ozonation process (Anatoxin: change in structure)**

**Figure 1: Different degradation pathway for cylindrospermopsin via photocatalysis, ozonation and chlorination process and via oxidation process for the anatoxin showing scope for mineralization (adapted and modified from Fotiou et al., 2015; Antoniou et al., 2008; Banker et al., 2001)**



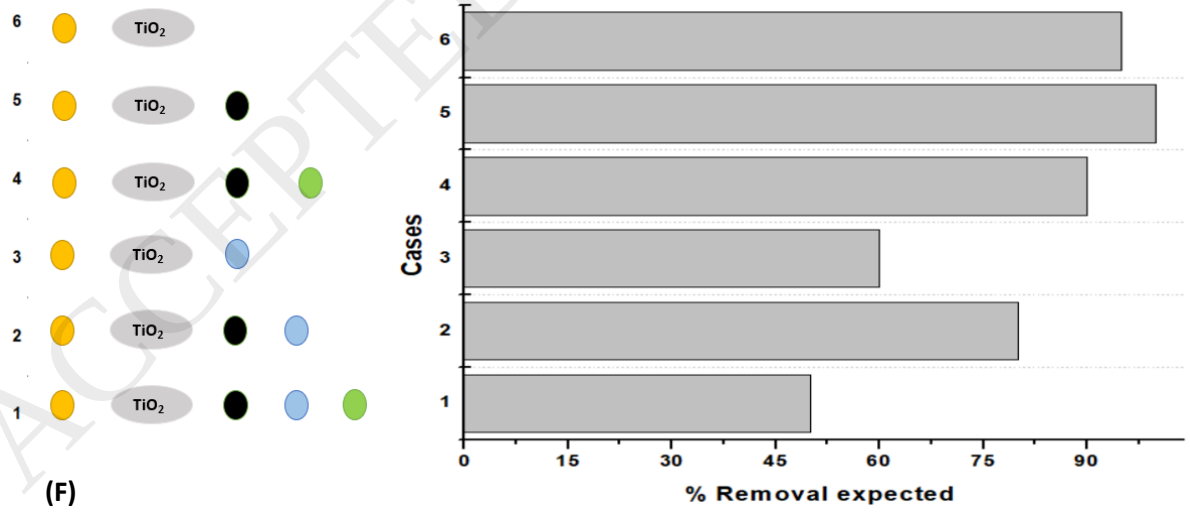



(D)

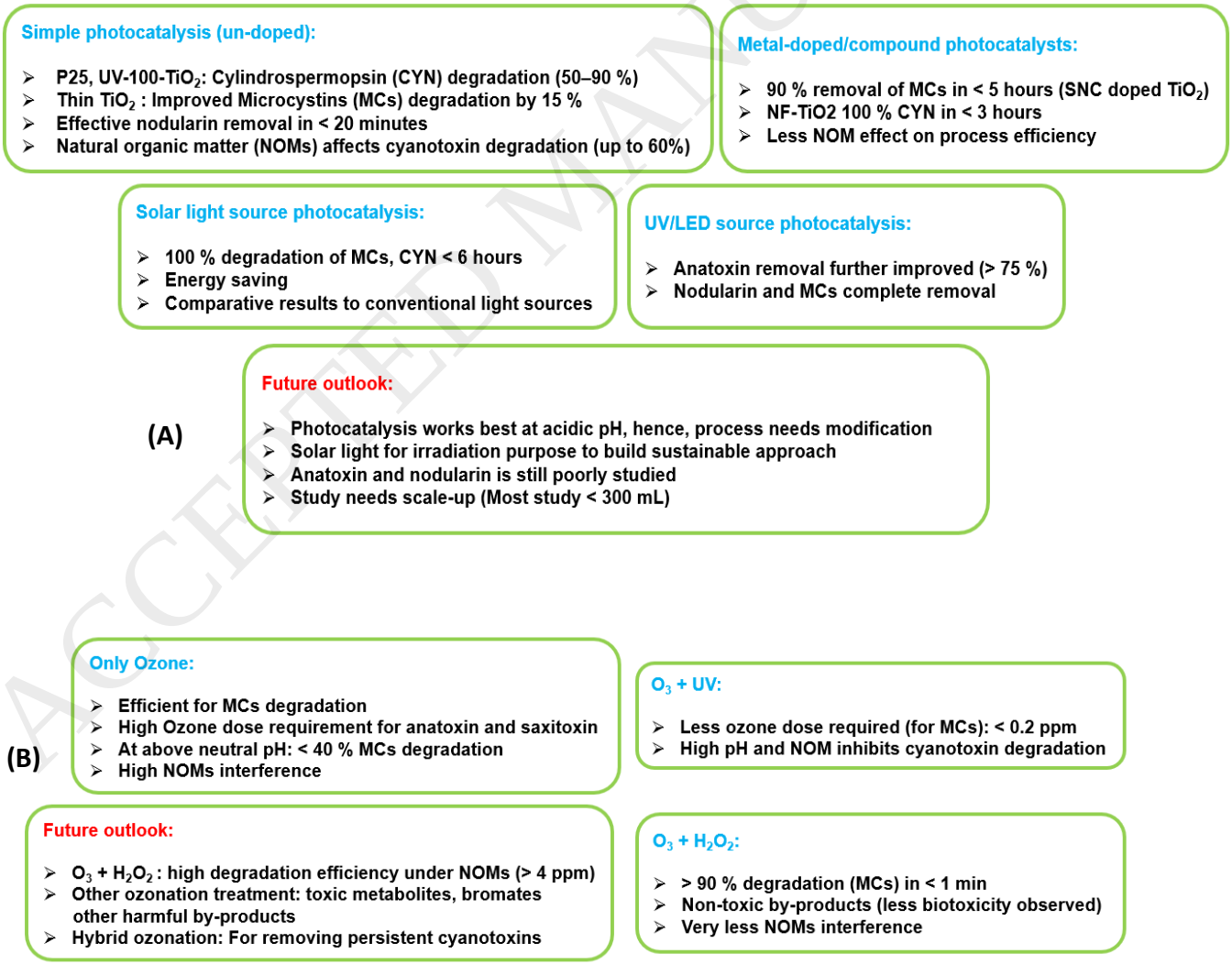

- Decreases pairs` ($e^-/h^+$) recombination rate
- Intermediate energy level formation supports photocatalytic activity
- Interparticle distance and average size reduction: more catalytic activity
- Band energy decreases

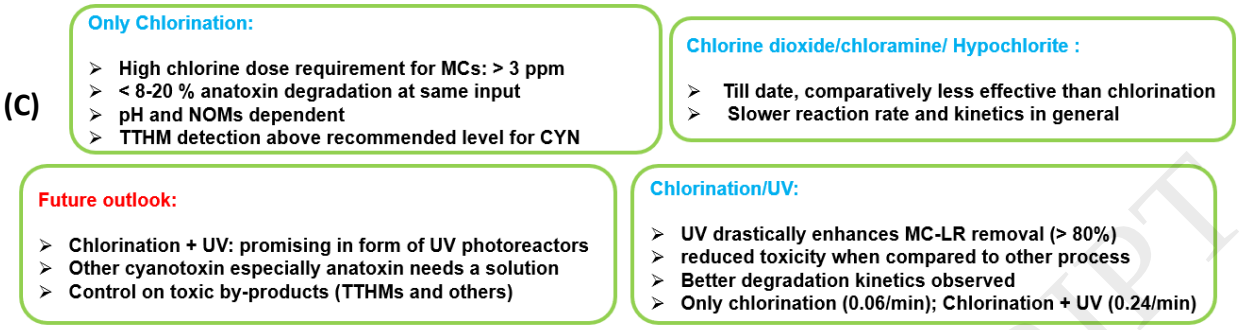
(E)




(F)

**Figure 2:** (A) A photocatalytic surface ($TiO_2$) with Microcystin-LR (MC-LR) molecule showing effective interaction; (B) Oxidants ($H_2O_2$) with $TiO_2$ and MC-LR molecule; (C) NOM interaction with $TiO_2$ and MC-LR at pH=7; (D) NOM interaction with TiO2 and MC-LR at pH < 7; (E) Metal-doped $TiO_2$ interaction with MC-LR, oxidants and NOMs (explained more in text); (F) Overall comparative degradation efficiency analysis for various cases.






**Scheme 1:** Future outlook and discussing challenges based on simple to advanced process (How far have we come?) for (A) Photocatalysis (Senogles et al., (2001)[132], Liu et al., (2005)[128] Antoniou et al., (2009)[127], Pelaez et al., (2012)[25], El-Sheikh et al., (2014)[24], Pinho et al., (2015)[27]) (B) Ozonation (Von-Gunten et al., (2003)[35], Lu et al., (2018)[38], Afzal et al., (2010) [92], Guzman-Guillen et al., (2012)[90], Verma et al., (2015)[93]) and (C) Chlorination (Rodriguez et al., (2007)[130], Pinkernel and Gunten (2001)[44], Zhang et al., (2016)[129], Duan et al., (2018)[99]). (elaborative description in the supplementary file)

**Table 1: Groups of cyanotoxin, cyanobacterial genera and their geographical distribution**

| Cyanotoxin class | Cyanotoxins | LD50 ($\mu g\ kg^{-1}$) [12] | Years to reach $LD_{50}$ value in human body ** | Genera | Biological characteristics | Geographical distribution | References |
|---|---|---|---|---|---|---|---|
| Cyclic peptides | Microcystin | 25-60 | 16 years | Anabaena, Microcystis, Nostoc, Hapalosiphon, Planktothrix(Oscillatoria) | *Microcystis aeruginosa:*<br><br>Unicellular colonial cyanobacteria<br><br>Can grow under dark anaerobic conditions | Australia, Canada, China, Denmark, Finland, France, Germany, Japan, Portugal, South Africa, UK, USA. (Almost worldwide) | [119,120] |
| | Nodularin | 60 | 38 years | Nodularia | *Nodularia sp.*<br><br>Filamentous and tolerant to UV radiation.<br><br>Potential to grow in salty or brackish waters | USA, Canada, Oceania | [121] |
| Alkaloids | Anatoxin-a | 200-250 | 128 years | Planktothrix, Anabaena, Aphanizomenon | *Anabaena sp.*<br><br>heterocyst-forming, filamentous and nitrogenase producer | Finland, Japan, Ireland, Germany, Canada, Denmark | [122] |
| | Anatoxin-a(S) | 20 | 12 years | Anabaena | | | |
| | Aplysiatoxin | | | Lyngbya, Schizothrix, Planktothrix (Oscillatoria) | | NA | |
| | Cylindrospermopsin | 300 | 191 years | Cylindrospermopsis,Aphanizomenon, Umezakia | Aphanizomenon sp.<br><br>Heterocyst-forming produces biologically-useful nitrogen | Israel, Australia, Hungary, Japan | [123,124] |
| | Lyngbyatoxin-a | | | Lyngbya | | NA | |
| | Saxitoxin | 10 | 6 years | Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis | | Australia, USA, Brazil | [125] |

**Table 2: Various conventional and alternative treatment methods for cyanotoxins removal**

| Methods | | Type of cyanotoxins | Results | Shortcomings | Reactor volume | References |
|---|---|---|---|---|---|---|
| **Photocatalysis** | (TiO2/ZnO) | Microcystin-LR | 100% removal in 5 minutes | ---------- | 250 mL | [126] |
| | NF-TiO2 | Microcystin-LR, -RR, -LA, -YR and CYN | 100% removal in 2 hours | Presence of NOM reduced rate of degradation; Overall, the process is pH dependent | 10 mL | [127] |
| | UV-C LEDs/UV-C LED/H2O2 | Anatoxin-a | Removal decreased from 97% to 77% by addition of $H_2O_2$ and for 97% to 72% when DOC was at 1.4 mg/L (lake water) | Presence of NOMs decreased the efficiency significantly (by around 20%); scale up is not studied | 5 mL | [110] |
| | UV LED/$TiO_2$ Using Photospheres™ | Nodularin and microcystin variants | All cyanotoxin removed in less than 6 minutes (nodularin being least adsorbed due to darkness) | Further research is necessary to crosscheck result in the more complex matrix, amino group affects results. | 3 mL | [128] |
| | $TiO_2$ | Microcystin-LR and Cylindrospermopsin (CYN) | MC-LR achieved significant removal based on certain conditions; CYN adsorption on $TiO_2$ nanoparticles did not occur even at pH of 7 | Requires high solar exposure time to achieve high degradation; Formation of reaction intermediates is difficult to evaluate in order to assess the energy dose required for the formation of non-toxic compounds. | 20 L | [35] |
| | **UV/$TiO_2$/$HiO_2$** | MC-LR 100% | 100% removal within 60 min at pH 3.5 | Requires upscale evaluation | 100 mL | [129] |

| Methods | | Type of cyanotoxins | Results | Shortcomings | Reactor volume | References |
|---|---|---|---|---|---|---|
| | NF-TiO2 PF-TiO2 and S-TiO2 | CYN (6-HOMU), model compound for CYN | 100% (2hr),100% (4hr) and 80% (4hr) removal in NF-TiO2, PF-TiO2 and S-TiO2 respectively | | 100 mL | [130] |
| | Graphene Oxide- $TiO_2$ (doped) Medium: Water | Microcystin-LR | 97% removal | Process was highly pH dependent | 20 mL | [131] |
| **Membrane techniques** | RO Membrane | Microcystin-LR and microcystin - RR | >95% retention rate achieved for salt and tap water | Retention of toxic particles need safe disposal; membrane fouling | Flow rate: 250 L/h | [64] |
| | NF membrane (Two flat-sheet nanofiltration membranes, NF-270 and NF-90) | Saxitoxin and congeners | <20% removal of neoSTX, dcSTX and STX from NF-270 and 100 % from NF-90 | Specificity is required for the removal of the main toxin and congeners | Flux (NF 270): 250 L/m2/h | [132] |
| | Gravity-driven membrane (GDM) | microcystins | 100% in 10 days of biofilm growth over the membrane | Biofilm increased from day 1 to day 10 and flux rate decreased from >4 L m−2 h−1 to around 1 L m−2 h−1 | mean flux of 4.7 L m−2 h−1 | [133] |
| **Chlorination** | | Microcystin-LR | Can be ineffective process sometimes; generally >95% removal | pH dependent; production of harmful by-product | 300 mL | [34] |
| | | NOD | Effective in removal (almost 95%) A concentration of 10 mg/1 free chlorine effectively removed 95% of nodularin (initial concentration 440/~g/l) with a chlorine residual of 2 mg/l after 5 min | pH-dependant, mechanism of degradation not known | 50 mL | [54] |
| | | CYN | Complete CYN | pH Dependant, one new | <15 ml (stock solution: 60 | [61] |

| Methods | Type of cyanotoxins | Results | Shortcomings | Reactor volume | References |
|---|---|---|---|---|---|
| | | degradation in <20 min with 3 by-products formation (each being less toxic than parent: cell viability 30-35% better than a parent) | unidentified by-product which could be toxic, more inline cells are necessary for cytotoxicity experiment | µM and final solution: 20 µM) | |
| | Saxitoxins | >99.1 % removal at higher pH (8) | pH dependent kinetics behaviour (oxidation being more effective at pH values over 6.5) | 250 mL | [134] |
| | Anatoxin | <10% removal was achieved (poor oxidation) | pH-dependent and high chlorine dose required than normal; high chlorine dose require hence high TTHM formation | 5 mL | [41] |
| **Ozonation** | Microcystis bloom toxins | 100% removal mostly | But by-product characterization found difficult | 1.5 L | [48] |
| | Saxitoxin and their by-products | O3 dose (continuous)removed 31% of GTX-5, and 77% of STX, batch O3 treatment removed 86% of STX. Batched O3 in combination with H2O2 destroyed 63% STX and 46% GTX-5 | Highly dependent on the mode of experiment<br><br>Also, some unidentified peaks were deciphered which could be toxic | 50 mL | [135] |
| | CYN | CYN IC50 at 24 incubations (MTT assay) 64.1 µM; ozonation products of CYN shows no measurable cytotoxicity to human cells (HepG2 cells). | More than 32 by-products formed which requires further study | ND; ozone doses (0−62.5 µM) Initial CYN: 20.0 µM | [105] |
| | Microcystin-LR | 95% removal in just 30 mins | Little known about any by- | 100 mL | [59] |

| Methods | Type of cyanotoxins | Results | Shortcomings | Reactor volume | References |
|---|---|---|---|---|---|
| **Permanganate (Potassium)** | | | products, their character, and nature | | |
| | Anatoxin and MC-LR | Both are removed at higher concentration (>90%) | At lower concentration (2-10 µg/L), high permanganate is required (>6mg/L) which is unacceptable owing to guidelines | <50 mL | [136] |
| **Hydrogen peroxide** | Microcystins | Ineffective in removal (to as low as 17%) | Reaction kinetics are quite unfavourable for microcystin removal | 100 mL | [137] |
| | CYN (through UV/H2O2, UV/S2O82−, and UV/HSO5) | Presence of metal ions in tap water enhanced degradation (almost 100%); In general: UV/PS > UV/PMS > UV/H2O2 is the order for relative removal of CYN | Depends on metal ion concentration and NOM present in raw water | <50 mL | [138] |

**Table 3: Different cyanotoxin (microcystin, anatoxin, and saxitoxin) removal using powdered activated carbon**

| No. | Cyanotoxins removed | PAC/GAC concentration | Initial concentration of toxin | Removal efficiency | References |
|---|---|---|---|---|---|
| 1 | Freeze dried cyanobacterial material (PAC) | 20 mg/L | 15 µg/L | 90 % | [48] |
| 2 | microcystin-LR (PAC) | >20 mg/L | 40 µg/L | 85% | [65] |
| 3 | microcystin-LR (PAC) | 25 mg/L[4] | 50 µg/L | 98% | [84] |
| 4 | microcystin-LR (PAC) | 50 mg/L[5] | 50 µg/L | 60% | [84] |
| 5 | microcystin-LR (PAC) | 12 mg/L | 50 µg/L | 95% | [140] |
| 6 | microcystin-LR (PAC) | 30 mg/L | 0.5 µg/L | 82% | [96] |
| 7 | microcystin-LR (PAC) | 100 mg/L | 22 µg/L | 86.4% | [83] |
| 8 | microcystin-LR (GAC) | 100 mg/L | 9 to 47 µg/L | 100% | |
| 9 | Anatoxin-a (GAC) | 10 mg/L; 30 mg/L | <10 µg/L | 60-90%; 50-90% | [139] |
| 10 | Anatoxin-a (PAC) | 50 mg/L | 100 µg/L | 100% | [85] |
| 11 | decarbomoyl saxitoxin (dc-STX); STX (GAC) | 3 mg/10 mL | 10.5 and 60.4 | >90% | [91] |
| 12 | Saxitoxin (PAC) | 1-90 mg/L | 25 µg/L | 100% at pH 10.2 and almost no removal at pH 5.7 | [89] |

[4] Wood based carbon powder

[5] Peat moss based carbon powder