International Journal of Modern Agriculture, Volume 10, No.2, 2021
ISSN: 2305-7246

Potassium Permanganate: a Possible Solution to Cyanotoxin Removal

Juliana Margaux Marston

Founder, Clean Lakes Education and Research, Inc and Independent Researcher, Kinnelon High School, Kinnelon, NJ 07405
marstonjprm@gmail.com
info@cleanlakeseducationandresearch.com

Abstract

Cylindrospermopsin (CYN) along with microcystin-LR and anatoxin-a are common cyanotoxins. The oxidation pathways between the latter two toxins and potassium permanganate (KMnO4) are known and have been shown to be effective. However, the oxidation of cylindrospermopsin via potassium permanganate is largely under-researched. In this paper, possible oxidation pathways between these two reactants are investigated in two categories: pathways related to the oxidation of compounds involved in the biosynthesis of CYN and pathways related to the oxidation of CYN in its final synthesized form. In the former, the oxidation of glycine is the most plausible oxidation pathway and, in the latter, the oxidation of the uracil group of CYN is. In reviewing these pathways in addition to the oxidation of microcystin-LR and anatoxin-a by KMnO4, this oxidant is shown to be the optimal solution to cyanotoxin removal in most cases.

Keywords: Potassium Permanganate, Cyanotoxin, Cynobacteria, KMnO4, Oxidation.

INTRODUCTION

Cyanobacteria, commonly known as blue-green algae, were some of the first aerobic organisms present on Earth; they are responsible for about 99% of the primary production of oxygen through photosynthesis even today [1]. Their innate ability as a keystone and pioneer species to populate relatively inhabitable areas such as volcanic ash and deserts makes them incredibly durable. They are also common in water sources. Unfortunately, cyanobacteria can produce cyanotoxins that contaminate local water bodies; cyanotoxins produced as waste products inside cyanobacteria cells can disperse through cell lysis into surrounding water [2]. Once they accumulate, these biotoxins can stimulate allergen sensitivity, liver and nervous system degradation, and cognitive impairment in humans and domestic animals [3]. This problem has negatively impacted society because such effects can lead to widespread hospitalization and unusable water sources.

The cyanobacteria are important species, even though their biotoxins are dangerous. Therefore, current research aims to find a way to eliminate cyanotoxins from bodies of water without destabilizing cyanobacteria populations. In 2014 a study released by the Environmental Protection Agency analyzed the effects of the most common cyanotoxins, microcystin-LR, anatoxin-a, and cylindrospermopsin. The EPA found that microcystin-LR and cylindrospermopsin primarily affected the liver in animals, causing inflammation and abdominal pain, while anatoxin-a targeted animals' nervous systems, leading to respiratory paralysis and cognitive disability. The EPA also experimented with different

substances to remove these toxins from lake water. Chloramines and chlorine dioxide were ineffective at removing the cyanotoxins, while the most promising solutions were ozone, potassium permanganate, and UV radiation. Ozone was effective at oxidizing extracellular cyanotoxins, and UV radiation easily degraded them. However, both were only effective at impractically high doses. Potassium permanganate was also effective at oxidizing microcystins-LR and anatoxin-a but further research was deemed necessary for understanding its impact on cylindrospermopsin [3]. Thus, out of these options, potassium permanganate appears to have the largest potential as a solution to cyanotoxin removal. Potassium permanganate's theoretical and observed impact on cyanotoxins like microcystins-LR, anatoxin-a, and cylindrospermopsin is widely under-researched. For potassium permanganate to be an optimal solution for the removal of these biotoxins, it must effectively oxidize them. The question remains that, reviewing the molecular structure and reaction between these substances, does this occur? If so, to what extent? Simultaneously, for potassium permanganate to be considered a solution, it will have to remove these cyanotoxins without damaging the existing cyanobacteria populations; Through examining these questions, potassium permanganate will be discussed as the most optimal solution to cyanotoxin removal.

LITERATURE REVIEW

Pre-existing data implies that potassium permanganate effectively oxidizes microcystins-LR. Microcystin-LR is a type of microcystin consisting of multiple peptide residues joined into a 25-membered macrocycle [4]. Looking at its structure, the Ohio branch of the Environmental Protection Agency theorized that microcystin-LR could be oxidized by potassium permanganate, consequently lowering its half-life. In a 2016 study, the organization performed a study to determine this and reported that the half-life of microcystins-LR in the presence of potassium permanganate was 5.2 minutes, lower than its typical half-life of anywhere between four to fourteen days. The Ohio EPA study also investigated the presence of microcystins-LR in water at pH of 7 and a pH of 9, dosed with varying concentrations of KMnO4 (2.5 and 5 mg/L). Most notably, the 2.5 mg/L dose showed a “moderate destruction of cyanotoxins after 90 minutes at a pH of 7” while the 5 mg/L dose showed “significant destruction of cyanotoxins after 90 minutes at a pH 7” [3]. However, at a pH of 9, extracellular cyanotoxins were released but not significantly destroyed after 90 minutes with either dose. This study showed pH is a factor when considering the use of KMnO4, but unless the water is atypically basic, KMnO4 effectively oxidizes microcystins-LR [3]. As the pH of water in nature is actually a bit acidic, approximately a pH of 6 which is below a pH of 7, KMnO4 is normally effective. For this reason, potassium permanganate (KMnO4) successfully oxidizes microcystins-LR.

The process by which potassium permanganate oxidizes microcystins-LR is relatively simple. Over the last couple of decades, scientists have used mass spectrometry to determine the oxidation reaction pathways of MC-LR (microcystins-LR) regarding KMnO4. Since the Adda (a unique β-amino acid) group is the toxic center of the MCs (microcystins), its oxidation is critical in the detoxification and overall inactivation of microcystins-LR [7]. The oxidation kinetics of this micropollutant by potassium permanganate were found, by researcher Xiaohong Guan and his team at the State Key Lab of Urban Water Resource and Environment (HIT), at the Harbin Institute of Technology in Harbin, China, to be first order concerning both the contaminant and KMnO4 concentrations. Guan also discovered that potassium permanganate oxidizes the heterocyclic aromatics with vinyl moiety in microcystins by adding double bonds [8]. This is an effective process that immediately works to break down microcystins-LR, oxidizing the Adda

group. This effectiveness is also why the half-life of microcystins-LR in the presence of potassium permanganate is so low. The fact that potassium permanganate successfully and quickly oxidizes microcystins-LR makes it a good solution to reducing the cyanotoxin concentrations produced in algal blooms.

In addition, pre-existing data also implies that potassium permanganate effectively oxidizes anatoxin-a. Anatoxin-a is a bicyclic secondary amine [9]. In 2016, the American Water Works Association (AWWA) in collaboration with Utah State University theorized that the cyanotoxin could be oxidized by potassium permanganate due to this structure. In a study they performed, the organizations discovered that, in the presence of potassium permanganate, the half-life of anatoxin-a is 4.8 seconds which is a stark contrast to its normal half-life of two hours [10]. This is due to the effective oxidation process that occurs between anatoxin-a and potassium permanganate; Guan observed that although anatoxin-a underwent a different oxidation reaction than microcystin-LR, both were efficient [8]. Unlike microcystin-LR, anatoxin-a is a type of endocrine-disrupting chemical (EDC). EDCs are defined as any compounds that significantly impact the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones present in the body [11]. The mechanism for the oxidation of phenolic EDCs like anatoxin-a by potassium permanganate is a single electron transfer and aromatic ring cleavage [8]. The process is efficient, immediately working to neutralize EDC cyanotoxins like anatoxin-a. This is why anatoxin-a has such a short half-life when potassium permanganate is present with it.

In comparison to microcystin-LR and anatoxin-a, there is little research surrounding the known impact of potassium permanganate on cylindrospermopsin. Cylindrospermopsin is a cyclic guanidine alkaloid ("Cylindrospermopsin"). Thus, it is a different type of compound than both microcystin-LR and anatoxin-a. While the half-lives of microcystin-LR and anatoxin-a were found to be 5.2 minutes and 4.8 seconds respectively in the presence of potassium permanganate, the AWWA reported that cylindrospermopsin only appeared to have a half-life of 4.2 days when exposed to this oxidant. Though the half-life of cylindrospermopsin with potassium permanganate available is much longer than that of microcystin-LR and anatoxin-a, it should be noted that the average half-life of cylindrospermopsin without oxidants present is anywhere between eleven to fifteen days [10]. Thus the resulting halflife of cylindrospermopsin due to oxidation via potassium permanganate is significantly lower than its normal half-life. Though data on the half-life of cylindrospermopsin is available, the oxidation process present is undetermined [3]. Due to this, the EPA notes that little "conclusive evidence" about the impact of potassium permanganate on cylindrospermopsin is yet available [3]. Additionally, according to the American National Library of Medicine, in a study done by federal researcher Eva Rodríguez, the oxidation process between both compounds appears to be slow, but "more research" on both compounds and the oxidation process between them is needed to understand better how this interaction occur [12]. The common conclusion in these studies that more research on the relationship between these compounds is needed provides a gap in understanding how potassium permanganate oxidizes cyanotoxins.

Finally, it is also important to consider the impact of potassium permanganate on cyanobacteria themselves. The Ohio EPA advises that water systems consider the use of potassium permanganate as it is "less likely to lyse cyanobacteria cells" than other oxidants [3]. Lysing leads to cellular wastes and toxins from inside the cyanobacteria cells spreading into the water, thus contaminating it. When lysing occurs, it also compromises the cyanobacteria cells themselves as their internal organelles are exposed to chemicals and components in the surrounding water. In addition to the EPA's

observations, the Carus Water Corporation reports that the application of permanganate at low doses (e.g. less than 3 mg/L) does not result in significant cell lysis while also allowing the compound to oxidize cyanotoxins [10]. The Carus Water Corporation also recommends that, as a significant amount of toxins will be located within the cell, treatment of algae-impacted water should not contribute to the breaking down of the cell and subsequent release of toxins to protect both these cells' integrity and the quality of the water present; looking at the reports mentioned, potassium permanganate meets this criterion. Therefore, potassium permanganate does not harm cyanobacteria populations through cell lysis or other known means.

Potassium permanganate is an effective oxidant for cyanotoxins like microcystins-LR, and anatoxin-a. Scientists understand the oxidation pathways for these toxins, and studies that investigate said pathways are available. The impact of potassium permanganate on cyanobacteria themselves is also known; the compound minimizes the probability of cell lysis and does not harm the organisms. However, the available research regarding the impact of potassium permanganate on cylindrospermopsin is minimal. Thus, the potential to which potassium permanganate is optimal as an oxidant is undetermined. Looking at existing data, the overall research question of how potassium permanganate impacts cyanotoxins is too broad. Therefore, a more specific research question taking the studies mentioned and gap in current research into account would be: how does potassium permanganate impact the presence of cylindrospermopsin, and, with regards to this as well as existing research on other cyanotoxins and cyanobacteria, is potassium permanganate an optimal solution to cyanotoxin removal?

METHODOLOGY

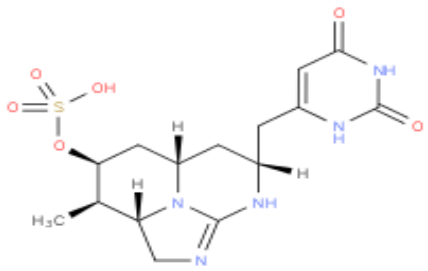
To determine the effectiveness of potassium permanganate as an oxidant for cyanotoxins, most experiments mentioned look at the impacts of the substance on the half-lives of microcystins-LR and anatoxin-a; the half-life of each respective toxin when potassium permanganate is present helps to show the rate and extent to which the oxidation of these cyanotoxins occurs. Hypothetical analyses of the oxidation reactions, chemical structures, and environmental factors present are also discussed to further examine how potassium permanganate oxidizes these biotoxins. The culmination of this evidence in a factor analysis methodology allows researchers to determine the effectiveness of potassium permanganate and understand why it is able to oxidize various cyanotoxins. This paper will follow this same methodology to analyze both the potassium permanganate and cylindrospermopsin compounds and the overall effectiveness of potassium permanganate as an oxidant for this specific cyanotoxin. Therefore, a structural analysis of both cylindrospermopsin and potassium permanganate is a requirement to answer the first part of the specified research question. In addition, hypothetical oxidation reactions between both compounds and theories proposed by researchers will also contribute to discovering the impact of this oxidant on cylindrospermopsin. Finally examining this hypothetical and analytical evidence and the known data for the impact of potassium permanganate on cylindrospermopsin will start to demonstrate whether it is an optimal oxidant for cyanotoxin removal. The last portion of the question will be further determined by examining additional research on the effectiveness of potassium permanganate as an oxidant for cyanotoxins like microcystins-LR and anatoxin-a. This will show the overall effectiveness of potassium permanganate as an oxidant in relation to multiple cyanotoxins.

FINDINGS

As mentioned in the Literature Review, Cylindrospermopsin (CYN) is a cyclic guanidine alkaloid; specifically, it is an alkaloid consisting of a tricyclic guanidine coupled with hydroxymethyluracil. Scientists have identified this cyanotoxin as a zwitterionic, highly water-soluble molecule [13]. This cyanotoxin acts as an inhibitor of protein synthesis, correlating to an observed detachment of ribosomes from membranes of the rough endoplasmic reticulum and recent studies suggest CYN, with a potentially reactive guanidine, may also act through covalent binding and breakage of DNA in cells [14]. There are two known structural variants: deoxycylindrospermopsin and 7-epicylindrospermopsin. The former appears to correlate to less protein inhibition by ribosome detachment than the latter, making it slightly less toxic-though the mechanism by which this occurs is inconclusive [13]. These zwitterion variants consist of a negatively chargeable sulfate group, a positively chargeable tricyclic guanidine portion and a biologically active uracil group. The tricyclic guanidine portion consists of a tricyclic guanidino moiety linked via a hydroxylated bridging carbon (C7) to uracil; the uracil moiety is required for toxicity [30]. However, there are still a few structural differences between these analogs.

Deoxycylindrospermopsin (deoxyCYN) is classified as a form of triazaacenaphthylene [15]; A triazaacenaphthylene is a cyclic protein with the formula C9H5N3 [5]. It is a structural derivative of sulfuric acid; this cylindrospermopsin analog contains a sulfur oxoacid consisting of two oxo and two hydroxy groups joined covalently to a central sulfur atom. DeoxyCYN is different from 7-epicylindrospermopsin, as the latter has a hydroxyl group attached to the carbon adjacent to its uracil ring. Considering that deoxyCYN lacks this hydroxyl group (replaced by –H) at the "uracil bridge," the decreased polarity and water-solubility resulting from this is a possible theory regarding its lower observed toxicity. However, the implications of this structural difference are minimal; it is not likely that the difference between deoxyCYN and 7-epicylindrospermopsin would make the former significantly lipophilic, and the apparent decrease in polarity is rather slight [14]. The structural difference between these two CYN derivatives is clear. However, whether or not this variation accounts for the slight difference in toxicity is still debated. The structure of deoxycylindrospermopsin is given in the model below:

Figure 1: *Deoxycylindrospermopsin*



Source: [16]

7-epicylindrospermopsin, like deoxyCYN, is classified as a triazaacenaphthylene. It is a cyanotoxin produced by several species of freshwater cyanobacteria, such as *Aphanizomenon ovalisporum* [16]. Discovering its structure has been a much more tedious process than its counterpart deoxyCYN. As mentioned, its main difference from deoxycylindrospermopsin is its hydroxyl group attached to the carbon adjacent to its uracil ring [14]. There are a variety of proposed structures of this toxin. The initial assignment of the relative stereochemistry of cylindrospermopsin (structure 1, Figure 2) with particular regard to the C-7 hydroxyl group was made on the basis that the uracil D ring existed as an unusual enol tautomer intramolecularly hydrogen-bonded to a nitrogen terminus of the guanidine moiety as shown in structure 6 (structure 6, Figure 3) [17]. Structure 6 appears to be a derivative of structure 7, an important conclusion noting that 7-epicylindrospermopsin is derived from cylindrospermopsin; this evidence strengthens the credibility of these theoretical structures by illustrating the connection between the parent toxin (CYN) and derivative toxin (7- epicylindrospermopsin). However, upon stereoselective total synthesis, it became clear that the stereochemical nature of the C-7 hydroxy group was reversed, nullifying the theory that these alkaloids existed as uracil tautomers and also establishing with certainty that cylindrospermopsin was accurately represented by the first proposed structure (structure 1, Figure 1) and it's epimer, 7-epicylindrospermopsin, by structure 2 (structure 2, Figure 1) [17]. The presence of the C-7 hydroxyl group may enable 7-epicylindrospermopsinto to act as a more efficient protein inhibitor than deoxyCYN, though no conclusive theory has prevailed. The structure of 7-epicylindrospermopsin is given below:

Figure 2: *Proposed 7-epicylindrospermopsin and CYN Structures*

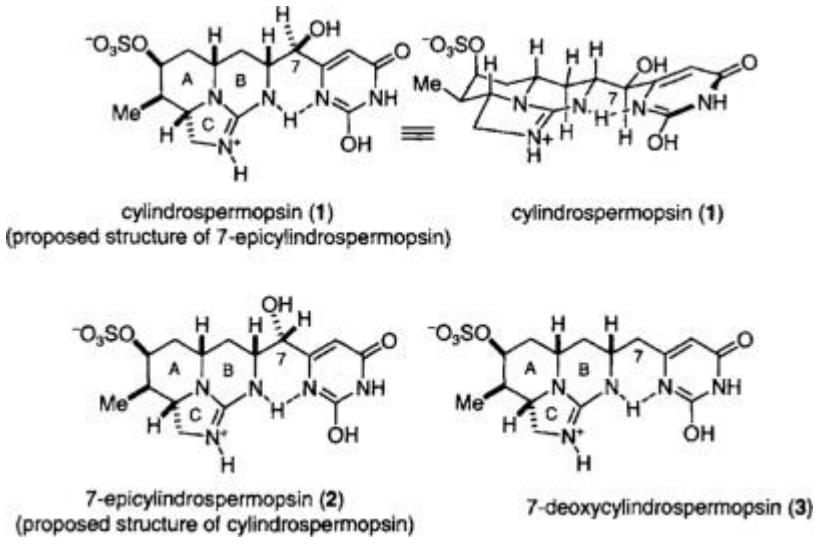
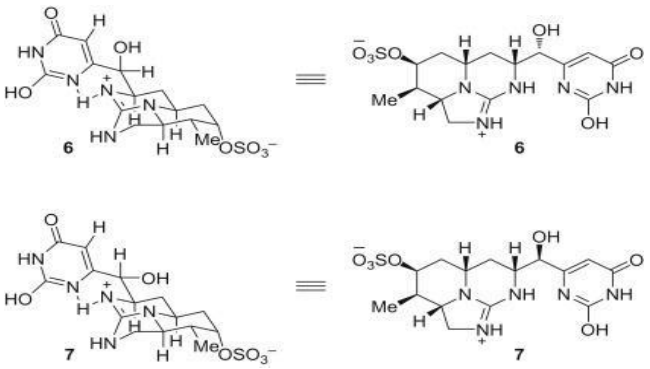
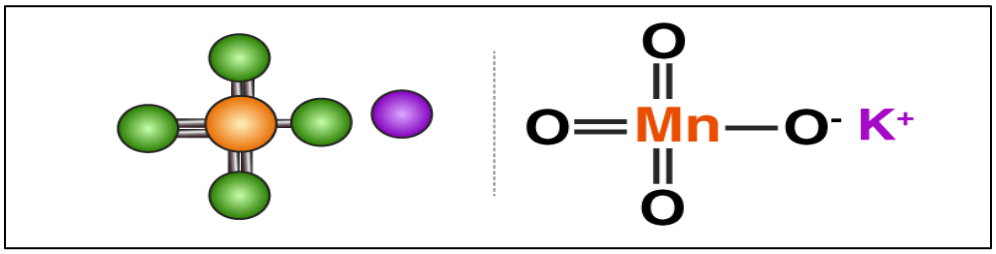


Figure 3: *Disproved 7-epicylindrospermopsin Structures*



Potassium permanganate (KMnO4) is an ionic compound that is widely known and utilized as an oxidant. Its structure is also known and universally accepted; unlike both cylindrospermopsin variations, it is relatively simple. It is an ionic compound composed of the potassium cation (K+) and the permanganate anion (MnO4-), in which the manganese atom is attached to four oxygen atoms through three double bonds and one single bond. The manganese metal is in the +7 oxidation state in this salt [18]. The structure is depicted below (See, Fig. 4):

Figure 4: *Structure of Potassium Permanganate*



Its structure, though simple, allows KMnO4 to act as an oxidant; in fact, potassium permanganate is one of the safest and most effective oxidants available, leaving practically no scope for harmful by-product formation Its strength as an oxidant primarily relates to how it contains manganese (Mn) in its highest oxidation state (+7) as cations become more electronegative as the oxidation states of their atoms increase; the permanganate anion in potassium permanganate reacts rapidly with other compounds in an oxidation reaction because of this [19]. KMnO4, being a very strong oxidizing agent, can react with a variety of groups effectively removing byproducts and even toxins.

Treatment processes to reduce CYN in drinking water through oxidation are based on two approaches: oxidizing cell-bound CYN by physical removal/inhibition of its creation in cells and reducing dissolved CYN outside of cells. Both methods allow for this toxin to be broken down in bodies of water, improving water quality and lowering its harmful effects on other organisms. Studying the different points at which an oxidation reaction could prevent the full formation of CYN or reduce its toxicity after it has been formed by cells and dissolved in nearby water, in comparison to whether or not potassium permanganate could be used in these pathways will allow for the potential oxidation reactions occurring between cylindrospermopsin and KMnO4 to be examined.

The structures of cylindrospermopsin, illustrated above by its main two structural variations, deoxycylindrospermopsin and 7-epicylindrospermopsin, have specific proposed oxidation reactions. Looking at the overall synthesis of this cyanotoxin, feeding experiments on a type of cyanobacteria that produce CYN (*C. raciborskii)* have demonstrated that the amidination of glycine is a likely first step in the biosynthesis of CYN. After this step, the polyketide chain of CYN is biosynthesized using the product guanidinoacetate and five units of acetate [20]. Although the whole biochemical pathway for CYN synthesis has not been totally clarified, these two steps are theorized to be the most likely initial phase of CYN creation. A process halting these steps would effectively halt the formation of CYN in cyanobacteria cells; if an oxidation pathway were to halt the amidination of glycine or break down either of these later products (guanidinoacetate or acetate), cylindrospermopsin would not be able to fully form. This theoretical oxidation would break down CYN as it forms though it would not degrade the final form of CYN.

Later on in the biosynthesis of CYN (and its variants) nonribosomal peptide synthetases, polyketide synthases, and tailoring enzymes are all utilized. The genes related to these proteins are organized in a large gene cluster (cyrA-O, ca. 44 kbp) that is known from several species (C. raciborskii, Chrysosporum ovalisporum, Aphanizomenon sp., Oscillatoria sp., Raphidiopsis curvata). The regulation of gene transcription and CYN production is thought to be coupled with the cells' nitrogen metabolism. The presence of the biosynthesis cluster in the genome of a particular strain is a strong indicator of CYN production, i.e., CYN is synthesized constitutively by toxigenic strains [30]. If an oxidation process interferes with the creation or function of these proteins, this would similarly prevent the full formation of CYN or break it down as it forms within the cell.

As mentioned, both cylindrospermopsin variants (deoxycylindrospermopsin and 7-epicylindrospermopsin) and CYN itself have a few structural components in common; all three consist of a negatively chargeable sulfate group, a positively chargeable tricyclic guanidine portion, and a biologically active uracil group which is the main cause of their toxicity [21; 30]. The degradation of this uracil group through an oxidation pathway could deactivate the cyanotoxins; breaking the hydrogen bonds within the uracil group, for example, would oxidize any of the three structural versions of CYN. In addition, with the negatively chargeable sulfate group and positively chargeable tricyclic guanidine portion, if an oxidation pathway were to release an intermediate component that reacted with the charged particles and altered their oxidation levels within the CYN structure, this could also effectively detoxify the CYN cyanotoxin and its analogs. Regarding the structural differences between deoxycylindrospermopsin and 7-epicylindrospermopsin, there are also other possible oxidation pathways respective to each analog. As discussed, 7-epicylindrospermopsin has a hydroxyl group attached to the carbon near its uracil ring while deoxyCYN only has a Hydrogen atom. As the uracil ring is the main toxic component of cylindrospermopsin that enables it to inhibit protein synthesis, degrading this and nearby components like the given H or OH groups respective to the variants could potentially limit toxicity. If an oxidation pathway were to contain an intermediate or compound with the potential to interact with and/or alter the oxidation state of the hydroxyl group from 7-epicylindrospermopsin, or H atom in deoxyCYN, the relative toxicity of the entire compound may shift.

Reviewing both the structural analyses and hypothetical oxidation pathways presented, the effect of potassium permanganate on cylindrospermopsin as an oxidant must be determined by whether KMnO4 follows one or more of the possible reactions presented. The first pathway presented involves the oxidation of glycine, guanidinoacetate, or

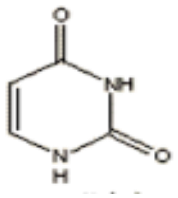
acetate, reactants in the first step of CYN biosynthesis. This theoretical oxidation would break down intermediate forms of CYN, though it would not necessarily break down the final form of CYN. Potassium permanganate oxidizes glycine and other α-amino acids through a successive one-electron transfer mechanism of inner-sphere nature [22]; the oxidation of glycine by permanganate ions (a product of aqueous KMnO4) in aqueous phosphate buffers is autocatalyzed by the soluble form of colloidal manganese dioxide formed as a reaction product. Both the noncatalytic and the catalytic reaction pathways are first-order regarding permanganate while the noncatalytic pathway is also first-order in glycine [23]. In oxidizing and effectively breaking down glycine, potassium permanganate halts the amidination of glycine. Though the extent of this pathway is unknown, it is a possible manner by which potassium permanganate oxidizes cylindrospermopsin, albeit through preventing the full formation of CYN from the beginning. This one condition acts as a limitation; the observed research in the literature review shows an oxidation reaction of some sort occurring between potassium permanganate and cylindrospermopsin after cylindrospermopsin has been formed. Additionally, considering that CYN forms in cyanobacteria cells and potassium permanganate only works to oxidize it once it is out of the cell, this pathway is unlikely though possible. Perhaps, on the condition that cells were injected with KMnO4, so that it could directly interfere with the process of CYN formation, this pathway could be a potential oxidation reaction-though between potassium permanganate and a reactant used to form CYN rather than a reaction between potassium permanganate and CYN directly.

As mentioned, compounds found in later steps of the biosynthesis of CYN (and its variants) include non-ribosomal peptide synthetases, polyketide synthases, and tailoring enzymes; thus, if potassium permanganate, through an oxidation process, interferes with the creation or function of these proteins, this would similarly prevent the full formation of CYN or break it down as it forms. There is no substantial evidence that potassium permanganate oxidizes or is involved in an oxidation reaction that impacts non-ribosomal peptide synthetases or polyketide synthases. However, there is potential interference between potassium permanganate and the function of tailoring enzymes used in biosynthesis of CYN; one type of tailoring enzymes, encoded by the cyrJ gene of cyanobacteria, catalyzes sulfation, the replacement of one of the hydrogens on a benzene ring by the sulfonic acid group, -SO3H. The sulfation reaction produces an alkylbenzene group that becomes incorporated in the structure of cylindrospermopsin. Though alkyl groups are usually fairly resistant to oxidation, when they are attached to a benzene ring, they are easily oxidized by an alkaline solution of potassium manganate (VII) (potassium permanganate) [24]. Thus, potassium permanganate can prevent the formation of CYN by oxidizing the alkylbenzene component later incorporated into the toxin, created by the sulfation reaction catalyzed by these tailoring enzymes. Though the extent of this pathway is still unknown, it is a possible manner by which potassium permanganate oxidizes cylindrospermopsin, albeit through preventing the full formation of CYN from the beginning. Similar to the previously discussed pathway, this one condition acts as a limitation again; the observed research in the literature review shows an oxidation reaction of some sort occurring between potassium permanganate and cylindrospermopsin after cylindrospermopsin has been formed. Additionally, considering that CYN forms in cyanobacteria cells and potassium permanganate only works to oxidize it once it is out of the cell, this pathway is unlikely though possible. Perhaps, on the condition that cells were injected with KMnO4, so that it could directly interfere with the process of CYN formation, this pathway could be a potential oxidation

reaction-though between potassium permanganate and a reactant used to form CYN rather than a reaction between potassium permanganate and CYN directly.

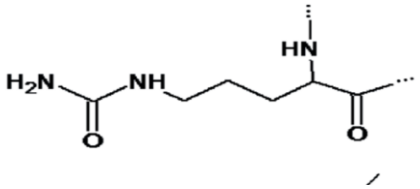
Related to the final structure of Cylindrospermopsin, other possible oxidation pathways discussed are degradation of the uracil group, negatively chargeable sulfate group, and/or positively chargeable tricyclic guanidine portion in CYN and its variants. There is no conclusive evidence or theoretical research that supports the oxidation of the sulfate group or tricyclic guanidine components of CYN. However, there may be a possible oxidation pathway between potassium permanganate and the uracil group of CYN. [6] found that, in an aqueous solution of tetraethylammonium chloride, potassium permanganate oxidizes free uracil nucleotide bases. The free nucleotide bases then become ureido residues due to this [25]. Unfortunately, the capability of this oxidant to significantly impact a uracil group attached to another molecule is inconclusive; though the theory that potassium permanganate oxidizes CYN through oxidizing its uracil group is possible given this experimental evidence, no substantial proof that this oxidation occurs when uracil is bound to other complex molecules has been recorded.

Figure 5: *Free Uracil Group*



Source: [26]

Figure 6: *Ureido Group (from a citrullin residue)*



The final possible oxidation pathway that would lead to the degradation of CYN relates to the structural difference between the toxin's variants, deoxyCYN and 7-epicylindrospermopsin; while 7-epicylindrospermopsin has a hydroxyl group attached to the carbon near its uracil ring, deoxyCYN only has a hydrogen atom. As the uracil ring is the main toxic component of cylindrospermopsin that enables it to inhibit protein synthesis, degrading this and nearby components like the given H or OH groups respective to the analogs could potentially limit toxicity. With most aromatic and alkaloid hydrocarbon compounds, the ability of potassium permanganate to abstract a hydrogen atom via oxidation is rationalized based on the strong O-H bond formed through the addition of an H atom to permanganate/KMnO4 [27]. Though deoxyCYN, the variant with the H atom, is classified as a type of alkaloid compound, no conclusive theoretical or experimental evidence exists to justify the presence of this particular pathway. A similar issue exists with the possible OH group oxidation in 7-epicylindrospermopsin; KMnO4 has been observed to oxidize the OH groups of different hydrocarbon derivatives such as primary alcohols [28]. However, there is no

substantial evidence to prove that this same type of oxidation pathway occurs between potassium permanganate and other hydrocarbons like alkaloids, 7-epicylindrospermopsin included. Thus, though these pathways may be possible, there is no evidence as of yet to prove that they are the manner by which KMnO4 oxidizes the two variants of CYN.

DISCUSSION

CYN (figure 2) is a cyclic guanidine alkaloid with two structural variants, deoxyCYN (figure 1) and 7-epicylindrospermopsin (figure 2). Potassium permanganate (figure 4) is an ionic compound. Looking at the structure of CYN and its variants, the possible oxidation pathways of this cyanotoxin can be separated into two categories: pathways related to the oxidation of intermediate forms, reactants, and compounds involved in the biosynthesis of CYN and pathways related to the oxidation of CYN in its final synthesized form. In the former, the reactions examined in this paper were the oxidation of glycine (intermediate of the first step of biosynthesis), and the oxidation of non-ribosomal peptide synthetases, polyketide synthases, and/or tailoring enzymes (proteins involved in later steps of biosynthesis). In the latter, the pathways examined were the oxidation of the uracil group, negatively chargeable sulfate group, and/or positively chargeable tricyclic guanidine portion of the final form of CYN and its variants. Specific to both structural variants, the possible oxidation of the hydroxyl group in 7-epicylindrospermopsin and hydrogen atom of deoxyCYN (both attached to the carbon near its uracil ring) were the final investigated hypothetical pathways.

Though no evidence supporting the oxidation of either peptide or polyketide synthases via potassium permanganate is currently available, the other two pathways are much more promising. Glycine is directly oxidized by potassium permanganate, which halts the amidination of glycine and thus prevents the formation of CYN. KMnO4 also interferes with the function of tailoring enzymes; these enzymes are not directly oxidized, but the alkylbenzene component that they create in the biosynthesis of CYN through a sulfation reaction (catalyzed by these tailoring enzymes) is. In halting the formation of an intermediate form of CYN, potassium permanganate stops the creation of this toxin by interfering with the function of tailoring enzymes. The first mentioned pathway, the oxidation of glycine, is the most promising in this category given the direct relationship observed between the oxidant, KMnO4, and reactant, glycine. Though there is merit to the theory of CYN oxidation through interference with the tailoring enzymes, the relationship is much less direct; the oxidation pathway occurs between KMnO4 and alkylbenzene compounds, a product of sulfation which is catalyzed by tailoring enzymes, rather than the oxidation pathway directly relating to the enzymes themselves. Still, there is a limitation to this overall category; these pathways prevent the full formation of CYN initially, while the observed research in the literature review shows an oxidation reaction of some sort occurring between potassium permanganate and cylindrospermopsin post-synthesis. The American Water Works Association reported that cylindrospermopsin appeared to have a half-life of 4.2 days when directly exposed to this oxidant in bodies of water, contrasting to a normal half-life of eleven to fifteen days without KMnO4 [10]. This proves the existence of an oxidation reaction occurring between the final form of CYN and potassium permanganate, but does not yield conclusive experimental evidence that a reaction occurs between KMnO4 and the intermediate compounds involved in the biosynthesis CYN. Additionally, considering that CYN forms in cyanobacteria cells and potassium permanganate normally works to oxidize it once it is out of the cell, this pathway is unlikely; there would have to be a manner by which cells were injected with KMnO4, so that it could directly interfere with the process of CYN formation. There is also no guarantee that this would not result in the lysing of cyanobacteria cells, which destroys

them [3]. Thus, there is a lack of experimental data to justify the existence of these pathways despite their theoretical merit and actions taken to make these pathways possible have the potential to harm the cells. These factors limit the possible optimization of KMnO4 as an oxidant for CYN specifically, however, that does not mean that an oxidation reaction between the reactants and/or intermediates of CYN and KMnO4 is impossible: the oxidation of glycine [23] and degradation of the product of the tailoring enzymes [29] via KMnO4 are both proven phenomena that exist. More experimental data confirming their prevalence in the creation of the CYN compound is needed to definitively prove that they are a means by which KMnO4 oxidizes CYN, but they are still solid theories until otherwise proven. Thus, this category of pathway could be a potential oxidation reaction-though between potassium permanganate and a reactant or intermediate used to form CYN rather than a reaction between potassium permanganate and CYN directly. For most of the pathways regarding the oxidation of the final form of CYN, there was little conclusive evidence proving the existence, or in contrast the impossibility, of them. At the moment, there is no conclusive experimental or theoretical research that supports the oxidation of the sulfate group or tricyclic guanidine components of CYN. Potassium permanganate has been found to oxidize free uracil nucleotide bases, resulting in ureido residues, but whether or not KMnO4 can oxidize uracil groups attached to other molecules (as in CYN) is currently unknown. With the structural deviations of the 7-epicylindrospermopsin and deoxyCYN variants, it may be possible for KMnO4 to oxidize the hydroxyl group of the former and H atom of the latter. Potassium permanganate has been shown to oxidize H atoms and hydroxyl groups from similar hydrocarbon derivatives; however, the fact remains that no conclusive theoretical or experimental evidence exists to justify the presence of either of these particular pathways in the CYN variants. In light of this, the most promising theory of the oxidation of CYN by potassium permanganate is the oxidation of the uracil group; KMnO4 can oxidize free uracil groups, which have the same composition as the attached ones in CYN and its variants. This is more solid evidence compared to the oxidation of OH or H considering that potassium permanganate has been shown to oxidize these molecules in similar but still separate types of molecules. In any case, more conclusive data is needed for any of the pathways in this category to be proven to exist. Theoretical merit exists regarding these pathways as discussed, but without further experimental evidence justifying the existence of any of them, no reaction can be proven to be how KMnO4 oxidizes CYN. This is an important limitation because if the pathway itself is unknown, this means that the efficiency and optimization of this oxidant (KMnO4) cannot yet be determined. Still, that does not make any of the pathways discussed impossible-it only means that no definitive conclusion can be made regarding exactly how KMnO4 oxidizes the final form of CYN at this point.

CONCLUSION

The deep analysis of all studies under review highlighted that potassium permanganate is still likely the most optimal solution to removing select cyanotoxins but it is not applicable in all situations. This conclusion can be reached through an analysis of the oxidation pathways of common cyanotoxins such as microcystins-LR and anatoxin-a by KMnO4, and research showing that this oxidant has no negative effects on the cyanobacteria cells themselves, both of which are as seen in the sources discussed in the literature review.

This research has discussed multiple possible pathways by which this oxidant could hypothetically oxidize and thus degrade cylindrospermopsin. However, there is little conclusive evidence to definitively prove which pathway occurs,

and thus, the extent to which this oxidation is effective is unknown. In light of this, potassium permanganate is the most optimal solution to the removal of select cyanotoxins, like microcystins-LR and anatoxin-a, however, the lack of conclusive theoretical and experimental evidence surrounding the oxidation of cylindrospermopsin via KMnO4 does not make it the most optimal one in every case - as seen through the inconclusive oxidation pathway of cylindrospermopsin with regards to this oxidant. In the future, experimental studies looking at each possible oxidation pathway discussed in this paper regarding CYN to see whether or not an oxidation reaction occurs, and its extent will yield more conclusive results.

REFERENCES

1. Shapley (2010) Cyanobacteria and the Formation of Oxygen.
   Retrieved from:
   http://butane.chem.uiuc.edu/pshapley/environmental/l30/1.html#:~:text=Currently, nearly 99% of primary,the oxygen on the Earth.
2. Learn about Cyanobacteria and Cyanotoxins. (2020, September 28).
   Retrieved from:
   http://www.epa.gov/cyanohabs/learn-about-cyanobacteria-and-cyanotoxins
3. Ohio: Ohio Environmental Protection Agency, American Water Works Association Ohio Section Technology Committee.
   doi:https://epa.ohio.gov/Portals/28/documents/hab/Algal%20Toxin%20Treatment%20White%20Paper%20FINAL%20November%202016.pdf
4. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 445434, Microcystin-LR.
   Retrieved May 17, 2021 from:
   https://pubchem.ncbi.nlm.nih.gov/compound/Microcystin-LR.
5. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 54058133, Triazaacenaphthylene.
   Retrieved May 17, 2021 from:
   https://pubchem.ncbi.nlm.nih.gov/compound/Triazaacenaphthylene.
6. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 42628600, 7-Epi-cylindrospermopsin.
   Retrieved May 17, 2021 from:
   https://pubchem.ncbi.nlm.nih.gov/compound/7-Epi-cylindrospermopsin.
7. Szlag, D. C., Spies, B., Szlag, R. G., & Westrick, J. A. (2019, May 28). Permanganate Oxidation of Microcystin-LA: Kinetics, Quantification, and Implications for Effective Drinking Water Treatment.
   Retrieved from: http://www.hindawi.com/journals/jt/2019/3231473/
8. Guan, X., He, D., Ma, J., & Chen, G. (2010). *Application of permanganate in the oxidation of micropollutants: A mini review* (Rep.). Frontiers of Environmental Science & Engineering in China. doi:https://link.springer.com/article/10.1007/s11783-010-0252-8

9. Toxinology. (n.d.). Anatoxin.
Retrieved from:
http://toxinology.nilu.no/Researchareas/Cyanobacterialtoxins/Factsheets/Anatoxina.aspx#:~:text=Anatoxin-a is a bicyclic,have been isolated and described *White Paper On Cyanotoxin Treatment* (Working paper). (2016).
10. C. (2016). *Permanganate: An Initial Barrier for Extracellular Cyanotoxins* (Rep.). Carus Europe. doi: www.carusllc.com/resources/content/4/8/7/documents/cyanobacteria-tech-brief.pdf
11. Diamanti-Kandarakis, E., Bourguignon, J., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., . . . Gore, A. C. (2009, June). Endocrine-disrupting chemicals: An Endocrine Society scientific statement.
Retrieved from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2726844/.
12. Rodríguez., JL;, R. E. (2007). Kinetics of the oxidation of cylindrospermopsin and anatoxin-a with chlorine, monochloramine and permanganate.
Retrieved from: http://pubmed.ncbi.nlm.nih.gov/17353030/.
13. Cylindrospermopsin. (2013).
Retrieved from:
http://www.sciencedirect.com/topics/neuroscience/cylindrospermopsin#:~:text=Cylindrospermopsin is an alkaloid consisting,et al., 1992).
14. Berry, J. P., Gibbs, P. D., Schmale, M. C., & Saker, M. L. (2009, February). Toxicity of cylindrospermopsin, and other apparent metabolites from Cylindrospermopsis raciborskii and Aphanizomenon ovalisporum, to the zebrafish (Danio rerio) embryo.
Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3513286/
15. Elixir Core Data Resource (2015, September 29). ChEBI.
Retrieved from:
https:/www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:88050#:~:text=CHEBI:88050deoxycylindrospermopsin&text=CYN is a polycyclic uracil,modify DNA and/or RNA.
16. Elixir Core Data Resource (2015, September 29). ChEBI.
Retrieved from https://www.ebi.ac.uk/chebi/searchId.do?chebiId=88049
17. Amidinotransferases. (2011). Retrieved from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/amidinotransferases
18. SoftSchools.com, Potassium Permanganate Formula - Potassium Permanganate Uses, Properties, Structure and Formula. (n.d.).
Retrieved from:
https://www.softschools.com/formulas/chemistry/potassium_permanganate_uses_properties_structure_formula/280/#:~:text=It is an ionic compound,has an orthorhombic crystal structure.
19. Rahman, I. (2013, October 05). Potassium permanganate, potassium dichromate – one of the excellent t...
Retrieved from:
https://www.slideshare.net/RabbyIstik/potassium-permanganate-potassium-dichromate-one-of-the-

excellent-tools-of-analysis

20. Jiang, Y., Xiao, P., Yu, G., Sano, T., Pan, Q., & Li, R. (2012, April 01). Molecular Basis and Phylogenetic Implications of Deoxycylindrospermopsin Biosynthesis in the Cyanobacterium Raphidiopsis curvata. Retrieved from: https://aem.asm.org/content/78/7/2256
21. Libretexts. (2020, September 13). Oxidation of Organic Molecules by KMnO4. Retrieved from: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Reactions/Oxidation_and_Reduction_Reactions/Oxidation_of_Organic_Molecules_by_KMnO4
22. Hassan, R. M. (1990, June 22). (Publication). Retrieved from: https://cdnsciencepub.com/doi/pdf/10.1139/v91-292
23. Perez-Benito, J. F., Mata-Perez, F., & Brillas, E. (1987, January 27). *Permanganate oxidation of glycine: Kinetics, catalytic effects, and mechanisms* (Publication). Retrieved https://cdnsciencepub.com/doi/pdf/10.1139/v87389#:~:text=The%20oxidation%20of%20glycine%20by,formed%20as%20a%20reaction%20product.&text=Additions%20of%20potassium%20chloride%20to,kinetic%20effect%20on%20the%20reaction.
24. Libretexts. (2020, September 13). Other Reactions of Benzene and Methylbenzene. Retrieved from: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Arenes/Reactivity_of_Arenes/Other_Reactions_of_Benzene_and_Methylbenzene
25. Bui CT;, Cotton RG (2002). Comparative study of permanganate oxidation reactions of nucleotide bases by spectroscopy. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/12020137/
26. The Biology Project, The University of Arizona (2003, January). Retrieved from: http://www.biology.arizona.edu/biochemistry/problem_sets/large_molecules/06t.html
27. JM Mayer;, KA Gardner (1995). Understanding C-H bond oxidations: H. and H- transfer in the oxidation of toluene by permanganate. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/7569922/
28. Wiley, The Oxidation of Alcohols. (2017, May 2) Retrieved from: https://www.chemistryviews.org/details/ezine/10517511/The_Oxidation_of_Alcohols.html
29. Admin. “KMnO4 (Potassium Permanganate) - Structure, Preparation, Properties, Uses.” BYJUS, BYJU'S, 7 June 2021, byjus.com/chemistry/kmno4/.
30. Humpage, Andrew. “Cyanobacterial Toxins: Cylindrospermopsin.” *World Health Organization*, Nov.2019,www.who.int/water_sanitation_health/waterquality/guidelines/chemicals/cylindrospermopsin-gdwq-bd-for-review-20191122.pdf.