Sequential Dosing of Potassium Permanganate for Removal of Microcystin-LR During Pre-Oxidation

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Abstract

Harmful algal blooms are made up of cyanobacteria that can release microcystins (MC's) into drinking water sources, which are toxic to humans. MC's can be removed during drinking water treatment using permanganate pre-oxidation. While permanganate is effective for removing microcystins, it is also non-specific and has the ability to react with dissolved organic matter (DOM) and cyanobacterial cells that exist in the water. Permanganate's reactions with DOM and cells cause competition for MC-LR removal. Additionally, reactions with cells can cause them to lyse and release intracellular MC's into the water. Preliminary work within our group suggests that a sequential dosing technique of permanganate pre-oxidation can reduce competition by DOM. Sequential refers to the additional of fractional doses of permanganate over time as opposed to a single large dose. Kinetic models fitted to observational data of sequential dosing and singular dosing found that competition by DOM decreased with each sequential dose. In the presence of microbial DOM, sequential dosing increased MCLR removal and in the presence of terrestrial DOM sequential dosing decreased removal.

A propidium Iodide (PI) staining assay was developed in order to quantify cell lysis cyanobacterial cells. This assay was validated with the Bioluminescence Assay using *Vibrio fischeri*. When the PI assay was used to quantify cell lysis of *Microcystis* cells, no significant cell lysis was observed using 10 ppm of potassium permanganate. Because of this, sequential dosing was tested using *V. fischeri* cells instead. Sequential dosing of permanganate in the presence of *V. fischeri* cells saw similar amounts of cell lysis when compared to a single dose of permanganate. This suggests that the rate of competition caused by cells does not change between doses of permanganate, unlike in the presence of DOM.

The removal of MCLR by permanganate in the presence of both DOM and *V. fischeri* cells was estimated using a kinetic model. In only the presence of 5 mg/L of microbial DOM and 100 nM of MC, we predict one dose of 12 µM permanganate to remove 92% of MCLR after 40 minutes and two doses of 6 µM to

remove 99% of MCLR after 80 minutes. After adding competition by *V. fischeri* cells (optical density = 0.44), removal by one and two doses of permanganate was predicted to decrease to 50% and 60% total MCLR removal, respectively.

Our simulation suggests that the presence of both cells and DOM have the ability to significantly impact MCLR removal in the worst-case scenario. However, sequential dosing was expected to increase MCLR removal by 10%. This simulation assumed that the cells present would be *V. fischeri*, which is not representative of an actual cyanobacterial bloom. Additional work should be done to more carefully examine the cyanobacterial cells interactions with permanganate.

Introduction

General Introduction

In recent decades, harmful algal blooms have become a growing concern in the Great Lakes and other Ohio waterways. HABS are composed of blue-green algae, or cyanobacteria, that can secrete toxic chemicals and deplete the nutrients and oxygen of a water body in a process called eutrophication. Eutrophication in lakes due to HABs is a result of excessive nutrient loading and favorable growing conditions caused by climate change. Rising water temperatures aid in the increasing severity of annual blooms (Steffan M. B., 2014). This means that the potential for additional cyanobacterial toxins increases as well, which would present a public health risk.

The algal toxins of interest for this paper are Microcystins (MCs), specifically microcystin-LR (MCLR), one of the most well studied MC's. MC's are known hepatotoxins which can cause adverse health effects and potentially increase the risk of liver cancer when inhaled, absorbed through the skin, or ingested via drinking water (He, 2016). Many cities use lakes affected by harmful algal blooms as a source of drinking water therefore, it is important that publicly owned water treatment plants are prepared to treat source water that may contain high levels MCs. Although there is currently no federal maximum contaminant level (MCL) for MCs, the Ohio Environmental Protection Agency has set a health advisory for MCs as 1.6 ppb. In the case of some municipalities, like the Celina Water Treatment Plant (Celina, Ohio), this can be a difficult concentration target to meet. Celina's drinking water source is Grand Lake St. Mary's (GLSM), which can contain levels of MC's greater than 250 ppb during peak bloom conditions in the late summer (OEPA, 2020). In these extreme cases, POTWs will need to reach almost 100 percent removal of MCs, which creates a demand for effective treatment methods for MC removal.

In a standard water treatment plant, cyanobacteria can be treated using coagulation and activated carbon. Coagulation has been shown to remove cyanobacteria cells, but not remove toxins (He, 2016). The presence of these cells and their metabolites can increase the amount of coagulant needed and disinfection byproduct formation, proving to be problematic (Moradinejad, 2019). The toxins can later be removed by powdered activated carbon (PAC) however, PAC can be expensive and although PAC physically removes toxins, they are not broken down into non-toxic byproducts (He, 2016). To effectively remove MCs to reach Ohio advisory levels, there is a need for additional alternative treatment methods.

As a result of the effort to study algal toxin removal, many emerging treatment methods have been developed using physical, biological, and chemical processes. The physical processes include physical oxidation of MCs using ultra-violet (UV) and ultra-sonification (Ding, 2010)and biological treatment of MCs uses microorganisms to degrade MCs into non-harmful byproducts (Massey, 2020). This paper, however, will focus on chemical oxidation of MC. The specific treatment method studied will be chemical pre-oxidation using potassium permanganate ($KMnO_4$).

Chemical pre-oxidation involves adding an oxidant to the treatment plant's intake as to remove any MC's that may exist in the water before even entering the plant. This is done so that the MCs cannot cause issues for the other units further along in the treatment train. Permanganate is often used for pre-oxidation because of its low cost and relative ease of operation. Using permanganate for chemical oxidation will be much less expensive than similar chemical oxidants, specifically ozone. Additionally, the equipment needed for permanganate oxidation is much less complex than the infrastructure needed for a UV or biological treatment facility. These factors make permanganate pre-oxidation a more accessible treatment alternative for smaller plants. Additionally, the Celina plant already uses permanganate in its pre-oxidation to remove MCs.

Although it is useful for the purpose of removing MC's, permanganate is non-specific in that it can react with other species in the water if present. Permanganate is known to have favorable reactions with other organic compounds, including dissolved organic matter that may exist naturally in the water or even the outer membrane of the cyanobacterial cells themselves. If these species co-exist with MC's in the water being treated, their reactions with permanganate may be more favorable than the reaction between permanganate and MC's. If MC oxidation is not the preferred reaction in the system, MC removal by permanganate will be less effective. Therefore, permanganate is susceptible to "competition" posed by other favorable organics in the water.

Permanganate has been shown to provide a multitude of benefits for a water treatment plant, in spite of this competition. However, its ability to control cyanobacterial cells and their toxins must still be carefully assessed (He, 2016). In this paper, the efficacy of permanganate for HABs affected waters will be studied.

Public Health Review

Description of MCLR

The general structures of MCs are important in assessing the toxicity of each compound and what may be the most effective treatment method. MCs are cyclic heptapeptides. The general structure includes a large ring which adds to the stability of the molecule. Attached to the ring and common to all MC variants is the adda moiety. Also on the ring are two positions for variable amino acids. There have been many different combinations of amino acids discovered which has resulted in over 200 different MC variants (Massey, 2020). For MCLR, these two positions are occupied by Leucine (L) and Arginine (R) (Figure 1). MCLR is a variant of MC that can be produced by many different cyanobacterial species, including Microcystis, Pseudanabaena, Oscillatoria, Planktothrix, Nostoc, and Anabaenopsis (He, 2016).

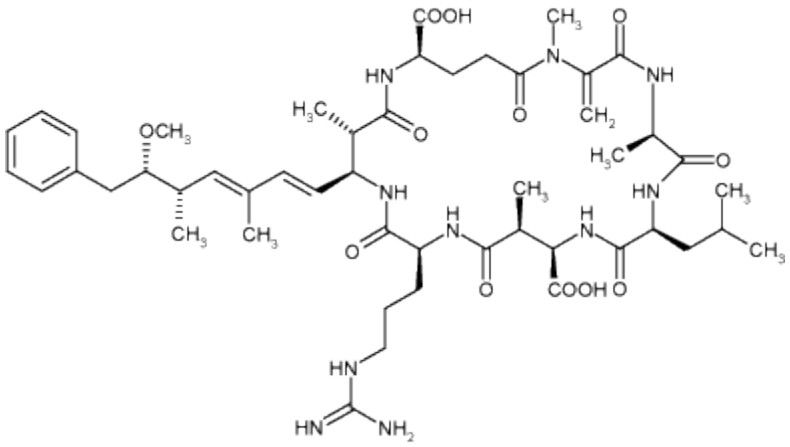

Figure 1: Structure of Microcystin-LR (Enzo Life Sciences, 2021)

Mechanism of Toxicity

There are several pathways of toxicity for MCs, most of which are consequences of initial inhibition of enzymes and oxidation stress. The specific enzymes targeted by MCLR are the protein phosphatases PP1 and PP2A. MCLR's alpha-carboxyl group of the D-glutamic acid and the adda moiety bind near the active site of the enzymes. The complete pathway for the adda's interaction with the enzymes is complex and beyond the scope of this paper, however, it is important to note that the MC's will become significantly less toxic after the adda has been oxidized. The hindrance of the active site inhibits enzymatic activity (Campos, 2010). These enzymes are important to human health because they regulate protein activity through phosphorylation and dephosphorylation. Lack of activity from these enzymes could result in hyperphosphorylation of important proteins, hepatic hemorrhage, DNA damage, and other effects (Massey, 2020). Along with phosphorylation regulation, PP2A is considered to be a tumor suppressor, thus linking the change in its activity to cancer cell development (Campos, 2010). MCLR has also been found to cause adverse health effects in the form of oxidative stress. MCLR can cause oxidative stress through MC mediated reactive oxygen species production (Campos, 2010). The production of ROS in

humans can trigger cellular apoptosis, damage DNA, and enhance cancer cells. The potential lack of PP2A tumor suppression and enhancement of cancer cells makes MCLR a possible carcinogen. Generally, there is no acceptable dose for a carcinogen, but studies have been performed to determine the effects of acute and chronic exposure to MCLR.

In these studies, the oral LD50 was found to be 5000 ug/kg in mice, but there is not an experimental LD50 found for humans yet. The three leading pathways of human exposure to MCLR are consumption of contaminated food or water, bodily contact, and inhalation. Humans can experience this chronic, low-dose exposure when source water with algal blooms is transported into public drinking water distribution systems. There have also been many documented events of human MC poisoning through drinking water systems affected by HABs. For example, a bloom of Microcystis in a drinking water reservoir contaminated the water supply for a hemodialysis center in Brazil in 1996. The acute exposure to MCs resulted in 131 patient casualties (Massey, 2020). Studies related to chronic, low dose exposure in humans found that the MCLR exposure resulted in alveolar collapse and lung cell apoptosis (Massey 2020). With no federal legislation, there are many communities whose finished drinking water contains MC levels higher than the World Health Organization's (WHO) recommended health advisory of 1 µg/L (ppb, ~ 1 nM). Even with a health advisory of 1 ug/L, some work suggests that chronic exposure events of 1 ug of MCLR can stimulate cancer cell migration (Zhang, 2011). In GLSM, MC levels can reach concentration greater than 100 ppb in the late summer. Constant monitoring of MC in GLSM by the Ohio EPA has consistently reported high concentrations for over a decade (OEPA, 2020). With the constant detection of MC in their source water, the residents of Celina are now at risk of chronic exposure to MCs. However, public exposure to MCLR can be prevented through the use of proper and effective drinking water treatment.

Permanganate pre-oxidation of MCLR

Permanganate has the ability to serve many different roles in a drinking water treatment train. Permanganate is a strong oxidant with highly favorable reactions towards many types of organic compounds. Because of this, permanganate is used in drinking water treatment plants to remove organic contaminants like taste and odor compounds and micropollutants (He, 2016). It has even been shown to selectively react with many antibiotics (Hu, 2010). Permanganate is also effective when used as a disinfectant and can inhibit biological growth (He, 2016). For the purpose of this paper, permanganate's most important function is its ability to remove algal toxins.

The treatment method of interest in which permanganate is used to remove algal toxins is pre-oxidation. Pre-oxidation refers to the addition of oxidants in the source intake of a water treatment plant. The intent is to remove as many algal toxins as possible before reaching the plant. As discussed earlier, MC's can be problematic in other portions of the treatment train, so it is best that they are removed immediately (He, 2016). Additionally, harmful algal blooms can be a season issue for treatment plants, so additional treatment for algal toxins may be more logistically feasible at the beginning of the treatment train.

MCLR Oxidation Performance of other Oxidants

The chemical structure of MCLR is also important in predicting how it may be oxidized by permanganate. MCLR contains 3 alkene groups (double bonds), two of which are on the adda moiety. Permanganate tends to preferentially react with (oxidize) alkenes, and the oxidation of the adda moiety significantly reduces the toxicity of MCLR (Szlag, 2019). This suggests that permanganate reduces MCLR concentrations by oxidizing the alkenes of the adda moiety. The products of this oxidation reaction are an organic product and manganese oxides (MnO_2). Plant operators may be concerned about the

production of manganese oxides because total manganese is regulated by the EPA. The organic by-products of permanganate oxidation are significantly less toxic than MCLR and are no longer of concern. Many treatment plants, however, are hesitant to use permanganate as an oxidant because it is not as well researched compared to traditional oxidants, despite performing similarly with respect to MC removal.

Many studies have been conducted to compare the performance of common pre-oxidation agents. One, conducted by Ding, 2010, compared the ability of several oxidants to remove 6 different MC variants and their ability to inactivate Microcystis aeruginosa cells. Permanganate's performance was compared to that of ozone, free chlorine, chlorine dioxide, and monochloramine. The study found that Microcystis aeruginosa cells were inactivated most effectively in the following order: ozone > permanganate ~= free chlorine > chlorine dioxide > monochloramine. This order also represents the oxidation efficiency of MCs by these oxidants. Although free chlorine and permanganate had similar oxidation rates for total MC's, permanganate removed MCLR, MCRR, MCYR, and MCLA more effectively than free chlorine. For MCLR specifically, oxidation by permanganate was significantly faster than free chlorine. To achieve 100% MCLR removal (20 ug/L MCLR), free chlorine required a concentration times contact time five times greater than that needed by permanganate. The results of this study suggest that permanganate is suitable for pre-oxidation, as it was found to be an effective treatment for both MA inactivation and MCLR removal (Ding, 2010). This study (similar to many others) did not conduct experiments in "natural waters" (or water with DOM). However, DOM will be present in the surface waters being treated, so the interactions between $KMnO_4$ and DOM will need to be examined.

Competition between MCLR and DOM

The presence of DOM in the source water is important because it can influence the performance of pre-oxidation. Pre-oxidation using permanganate is affected by DOM because DOM can compete with MCLR

for permanganate reactions, causing a lower removal efficiency of MCLR removal (Laszakovits J. R., 2019). If some of the permanganate is going to be consumed by DOM during oxidation, then it must be accounted for by increasing the concentration of the permanganate dose. This increase will cause a higher cost for materials needed and produce a higher MnO_2 residual. In order to utilize our pre-oxidation materials most effectively, it is important to understand the competition posed by organic matter.

The reaction mechanisms between permanganate and organic compounds can aid as an explanation as to why DOM causes a significant amount of competition. DOM is a complex mixture of dissolved organic compounds that originates from the breakdown of natural matter like plants and bacterial cells, which will differ for each natural water system. Typically, DOM of natural waters exists along a spectrum of terrestrial (plant-like DOM) to algal (algal cell-like material). The variance in composition leads DOM mixtures to behave differently with respect to permanganate oxidation. For example, some work suggests that permanganate will react preferentially with natural organic matter that has a high molecular weight and is aromatic (Laszakovits J. R., 2020). This suggests that DOM mixtures with a significant portion of high molecular weight and aromatic compounds would likely present more competition than a mixture with a lesser portion of these types of compounds. Although there is a consensus in the literature that DOM can present competition during pre-oxidation with DOM, there is still some contention as to which compounds cause the most competition. In stark contrast to the conclusions drawn in the study by Laszakovits, a study done by Jeong et al, 2017 found that DOM with low molecular weight and low aromaticity were the greatest MCLR oxidation inhibitors (Jeong, 2017). In this paper, I will not examine the exact competition of DOM, but instead treat the mixtures as single entities.

We can model MCLR removal using the rate of the MCLR/permanganate reaction and the DOM/ permanganate reaction, which is the competition reaction (Equation 1). This model, derived in

Laszakovits and MacKay, 2019, can predict MCLR concentration given the conditions of the system i.e., the MCLR/permanganate and DOM/permanganate reaction rates. In this model, k_{obs} represents the observed rate constant in the reaction between DOM and permanganate. The competition rate constant (k_{obs}) can be calculated using observational data of MCLR concentration changes during permanganate pre-oxidation. A high k_{obs} value would indicate that the DOM/permanganate reaction is fast, presenting more competition than a low k_{obs} value. Permanganate oxidation will be most efficient when k_{obs} is closer to zero.

Equation 1

$$[MCLR]_t = [MCLR]_0 e^{-k\left[MnO_4^{-1}\right]_0\left(\frac{e^{-k_{obs}*t}}{-k_{obs}}+\frac{1}{k_{obs}}\right)}$$

Previous preliminary work in our group found that the k_{DOM} of a particular water sample can change as additional doses of permanganate were added. This study found that the competition rate would decrease as additional doses were added, pointing to a possible solution for decreasing competition. So, a sequential dosing technique of permanganate was investigated. Sequential dosing refers to the addition of multiple doses of permanganate over a period of time, as opposed to the traditional method of a single dose of permanganate. In the study, permanganate was added to a solution containing both MCLR and DOM. After the reaction had stopped (the permanganate was consumed), a second, equivalent dose of permanganate was added to the same solution. The MCLR concentration was tracked during both doses. Although both doses of permanganate were equivalent in concentration and therefore should consume the same amount of DOM and MCLR, the second dose removed more MCLR than the first dose. This implies that competition presented by DOM may have decreased between the two doses. The decrease of DOM competition observed shows that sequential dosing could be a potential method to increase removal efficiency of MCLR. The general difference in MCLR removal

between a single large dose and sequential dosing is currently unknown but will be investigated in this paper.

Cell lysis and the release of toxins

Similar to DOM, cyanobacterial cells will also be present in source intake water, so the effect that these cells have on pre-oxidation treatment will also need to be investigated. Using strong oxidants like permanganate or chlorine will be useful in removing MC's but may also attack the cells and cause oxidative stress. The presence of cyanobacterial cells pertinent to pre-oxidative treatment because we want to oxidize extracellular toxins without introducing new, intracellular toxins into the water. When the outer membrane of a microbial cell is compromised, cell lysis occurs. During lysis, the contents of the cell are released into solution. In the case of cyanobacteria, which can contain toxins inside of the cell membrane (intracellular toxins), cell lysis can cause these intracellular toxins to move outside of the cell (now extracellular toxins). The exact chemical pathway in which cell membranes are broken can be different for different oxidants and organisms. When E. coli was exposed to ozone, it was believed that ozone reacted with the lipid bilayer of the outer membrane, lysing the cells (Wert E. C., 2014). And when chlorine reacted with MA cells, the chlorine was shown to have reacted with the amines in the phospholipids of the outer membrane in order to damage cells Ramsier 2011 (Ramseier, 2011).

Currently, literature suggests that permanganate does not cause a significant amount of cell lysis under typical treatment doses (<5 mg/L) but has the ability to do so using higher doses. Qu et al (2015) reported that less than 10% of MA cells lysed after exposure to 1.0 mg/L and 2.0 mg/L of potassium permanganate for 20 minutes (Qu, 2015). Fan et al (2013) reported similar results and that MA cell integrity will not be affected for potassium permanganate doses less than 3 mg/L (Fan J. ,., 2013). However, they did see that the percentage of intact cells was only 74% and almost 0% after 6 hours of exposure to 5 mg/L and 10 mg/L, respectively. Ou et al (2019) also found that doses of permanganate

less than 5 mg/L only caused little cell lysis (Ou, 2012). Doses greater than 5 mg/L did cause significant cell lysis, but only after at least 48 hrs.

Permanganate's ability to lyse cyanobacterial cells can be compared to that of other oxidants to determine if it is suitable for pre-oxidation treatment. There are existing studies comparing the effects that compare these oxidants with respect to cell lysis. A study by Wert et al. In 2013 tested the degree to which MA cells would lyse after exposure to ozone, free chlorine, chlorine dioxide, chloramine. The study defined general cell damage as a loss of chlorophyll-a. Cell lysis was determined using flow cytometry (FCM). After lysis, the cells have fragmented into particles that are too small to be read on the FCM, and the particle counts by the instrument would report a concentration change in "alive" cells. All of the oxidants tested were shown to cause some degree of general cell damage, but the MA cells were most susceptible to lysing after exposure to ozone and chloramine. Although this study did not examine permanganate, it is still important to recognize that these common water treatment plant oxidants have the ability to damage cells, so any oxidant used during pre-oxidation could potentially release toxins through cell lysis (Wert E. C., 2013).

The study by Ding et al (2010) discussed earlier in the paper also examined the effects of their suite of oxidants on MA cell lysis. This study did not measure cell viability concentrations of damage directly, but defined cell lysis as an increase of MCLR concentration. Oxidants were dosed into a solution with both cells and MCLR and the concentration of MCLR was measured throughout the reaction. Free chlorine, ozone, and permanganate did not show any buildup of MCLR. This does not mean that cells were not lysis because the cells were successfully inactivated, but it is possible that any additional MCLR that was released was also oxidized. Chlorine dioxide, however, did see cause a buildup of MCLR. This was because the rate of MCLR oxidation was not as fast as the rate of M-LR release by the cells (Ding, 2010). Based on these findings, some oxidants can be strong enough or dosed in such a high amount that they may cause cell lysis and simultaneously oxidize the now extracellular toxins. Because permanganate did

not cause an increase in MCLR after exposure to some doses, this “over-dosing” is a possible treatment method.

A study by Wert, 2014 reported similar results. In this study, MCLR concentrations were measured after MA cells were exposed to ozone, free chlorine, chlorine dioxide, and chloramine at different concentrations. Oxidant doses lower than 0.63 mg/L experienced a positive change in MCLR concentration after oxidation. Oxidant doses (except for chloramine) greater than 0.63 mg/L saw no increase in MCLR, suggesting that any released MCLR was subsequently oxidized. The fact that lower doses of oxidant showed a positive increase in MCLR, and higher doses showed no MCLR buildup suggests that there is some threshold where the rate of MCLR oxidation exceeds the rate of intracellular toxin release (Wert E. C., 2014). Although this “over-dosing” technique eliminates the issue of MCLR release by cells, the excessive use of oxidants can potentially be a waste of resources and produce higher residuals in the case of permanganate, where total manganese is regulated. To determine how to more efficiently utilize our resources, we can further investigate the occurrence of cell lysis on the basis of each oxidant’s reaction with the cell membrane.

A study by Ramseier 2011 examined the membrane damage of aquatic cell cultures native to Zurich, Switzerland after exposure to ozone, chlorine dioxide, chloramine, chlorine, ferrate, and permanganate. This study measured cell damage using a Sytox Green (SGI)/ Propidium Iodide (PI) staining method coupled with FCM. The staining method uses fluorescence to differentiate between live and dead cells, where live, undamaged cells will be SGI positive and PI negative. PI is a membrane impermeable molecule and a nucleic acid specific stain. Cells will only be stained with PI when their membranes are compromised enough to allow PI to enter the cell. Among the oxidants tested, chlorine was able to damage membranes at the lowest CT values and permanganate and chloramine required the highest CT values to damage cells (on the order of 100 mg*min/L). Ramseier suggested that the reaction of amine moieties plays an important role in membrane damage (Ramseier, 2011). Based on this study,

permanganate may be a more suitable candidate for pre-oxidation because it required the highest CT in order to lyse cells, which was likely due the difference in reaction mechanisms of cell damage when compared to chlorine-related oxidants.

Measuring Cell Lysis

An important distinction to make between the previously discussed studies was the way in which cell lysis was quantified. In this paper, I will also discuss results based on how cells have been lysed. Because cell lysis is difficult to directly measure, it is important to choose a cell lysis measurement method that can accurately convey the results of a specific experiment. One method used to quantify cell lysis is by directly measuring the contaminate of interest, MCLR. The central reason that we are concerned with cell lysis is due to the potential release of cyanotoxins, so measuring MCLR directly responds to that concern. However, MCLR measurement without any other measures of cell lysis leaves much to speculation. Measurements of MCLR cannot differentiate between toxins intentionally released by the cell and those unintentionally released through a broken membrane. Additionally, as observed in Ding 2010 and Wert 2014, the rate of MCLR oxidation can exceed the rate of MCLR release, which makes it nearly impossible to determine how much MCLR was released by higher doses of oxidants.

A similar method to measuring MCLR concentration is the measurement of dissolved organic carbon (DOC) before and after oxidation. In this method, cells that have been damaged by oxidation will release their intracellular organic matter (including their toxins), and the release of their organic components will show a positive change in DOC content (Wert E. C., 2014). In addition to being non-specific to cyanotoxins, my experiments will require a high concentration of background DOM. This background content may make it difficult to measure small changes in DOC due to cells.

Cell lysis can also be interpreted through cell viability assays. The main concern with viability assays is that it can be difficult for them to differentiate between inactivated or “dead” cells with no membrane

damage and cells that not dead but have a somewhat damaged membrane. A study by Moradinejad, 2019 highlights the discrepancy between MA cell viability and cell lysis. After oxidation by permanganate and others, cells were examined using flow cytometry (FCM), Scanning Electron Microscopy (SEM), and liquid chromatography with organic carbon and nitrogen detection (LC-OCD-OND). After permanganate oxidation, the total counts of cells did not change much, implying that no cell death/lysis had occurred, however, FCM of the same samples found that viability was actually much lower (between 15-60% viability) (Moradinejad, 2019). Information taken from these assays suggests that cells were inactivated and partially but not completely fragmented.

For my experiments, I have chosen to use a PI staining assay for its potential to measure additive cell lysis. As mentioned earlier, PI is a membrane impermeable molecule, and will fluoresce when bound to nucleic acids. If a cell's membrane has become compromised enough to the point that its DNA can be released from the cell, the PI will bind to this extracellular DNA. In this way, PI signal can be used as proxy for cell lysis where the fluorescence of a sample should positively correlate to the number of cells lysed (Stiefel, 2015). I have chosen PI staining as opposed to FCM because I do not need to examine the degree to which cells are damaged or their viability. This method will determine if cells are damaged "enough" to be stained with PI.

Limitations of previous studies

Permanganate tends to be missing among the oxidants tested in MCLR removal and cell viability studies. In the studies that do include permanganate, it tends to perform similarly too or better than chlorine in terms of MCLR oxidation. Several studies have shown that permanganate has the ability to lyse cells at certain doses, but typically causes less lysis than chlorine. The difference in cell lysis may be attributed to the difference in reaction mechanisms between the two. Permanganate's MCLR oxidation

performance and its lower rates of cell lysis (when compared to chlorine) suggest that it is a strong candidate for pre-oxidation.

Other environmental factors can influence oxidant performance other than the specific oxidant species chosen. The morphology of cyanobacterial colonies and antibiotics pollution was shown to inhibit cell damage and increase MCLR release, respectively but this is far beyond the scope of this paper (Fan J. R., 2016) (Liu, 2020). However, in the case of permanganate, DOM has been demonstrated to inhibit MCLR removal. This DOM will scavenge permanganate and can potentially impact the interactions between cells and permanganate. There is a gap in literature with respect to how the presence of DOM and cells can affect MCLR oxidation and subsequent toxin release. Because both DOM and cyanobacterial cells will be present during pre-oxidation, there is a need to develop a treatment method that can mitigate the issues associated with both DOM competition and cell lysis.

Overview of Thesis

In this paper, I will further examine sequential dosing and its effects on MCLR removal. Sequential dosing has been suggested to decrease competition caused by DOM between doses. It may be possible that this decrease in DOM competition will positively impact MCLR removal. Competition for permanganate reactions has been shown to differ between DOM compositions, so it was important to include different DOM isolates in our analysis. Sequential dosing has not been tested in the presence of cyanobacterial cells so its effects on cell lysis are unknown. Across all oxidants in the studies discussed earlier in this paper, bacterial cell lysis tends to positively correlate with oxidant dose, so that low oxidant doses typically cause less cell damage than larger doses. Sequential dosing involves the addition of multiple “small” doses of permanganate over a longer period of time as opposed to one large dose. Spreading the permanganate dose over time may prove to be a “gentler” treatment method for cells and result in less call damage. There is also a lack of data related to MCLR removal in the presence of

DOM and cyanobacterial cells. If sequential dosing of permanganate is used in this scenario, the decrease in competition DOM with each sequential dose may also impact the rate at which cells are lysed. I hypothesize that sequential dosing od permanganate will increase removal MCLR when compared to a single dose in the presence of both DOM and cyanobacterial cells.

To test this hypothesis, I will need to establish rate constants for the reactions that permanganate has with MCLR, DOM, and cyanobacterial cells. First, I determined the second order rate constant between MCLR and permanganate and compare that to other rate constants reported in the literature. Then, observational data of MCLR removal was collected using sequential dosing in the presence of DOM. Sequential dosing was compared to an equivalent single dose of permanganate. The observational data was fitted to an equation that can determine the rate constant of DOM competition for that dose.

To determine the rate at which permanganate will react with cyanobacterial cells, I developed a biological assay using propidium iodide (PI). The PI assay will show how cells lyse with respect to time and this lysis rate will be used as a proxy for the permanganate/cell reaction. Out of the interest of time, observational data of MCLR removal in the presence of DOM and cells could not be collected. However, I will conclude this paper by building a model to predict how MCLR may be removed in this scenario using the aforementioned rate constants.

Research significance

If sequential dosing is shown to improve MCLR removal in the presence of DOM and cyanobacterial cells, sequential dosing can serve as an accessible treatment method for drinking water treatment plants that experience seasonal or year-round harmful algal blooms. Additionally, sequential dosing can be easily implemented at plants that already use permanganate for pre-oxidation purposes. Sequential dosing may be the easiest to implement at water treatment plants that source water from the Great Lakes and transport the water miles inland. Two dosing points could be installed in between the plant

and the lake so that the plant would not have to manually increase the retention time to accommodate sequential doses. Most importantly, if successful, sequential dosing will improve MCLR removal while using the same amount of permanganate resources.

Methodology

Permanganate – MCLR rate constant

Procedure

The second order rate constant for the MCLR/permanganate reaction was determined using a pseudo-first order kinetics method. A pH 7 buffer was made using solid monosodium phosphate to achieve a concentration of 5 mM of phosphate and adjusted using 1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH). For each kinetic run, a 100 nM solution of microcystin-LR was made in pH 7 buffer. Then, potassium permanganate (between 1 µM and 3 µM) was added to the solution based on the desired half-life. Half-lives were estimated using the second order rate constant of 272 1/min as reported by Laszakovits and Mackay, 2019. As the reaction proceeded, 2 mL of sample were removed from the buffer-MCLR-permanganate solution every 6 minutes over the course of two half-lives. After the sample was pulled, it was quenched with 0.08 mM thiosulfate quencher to achieve a working concentration at least 10 times greater than the concentration of permanganate. Each quenched sample was filtered with 0.2 um nylon filters and discharged into an LC vial for analysis. The samples were then analyzed using ultra-high pressure liquid chromatography (UPLC) on Waters Aquity Class H UPLC. The following calibration curve was used to calculate MCLR concentrations at given timepoints (Figure 2).

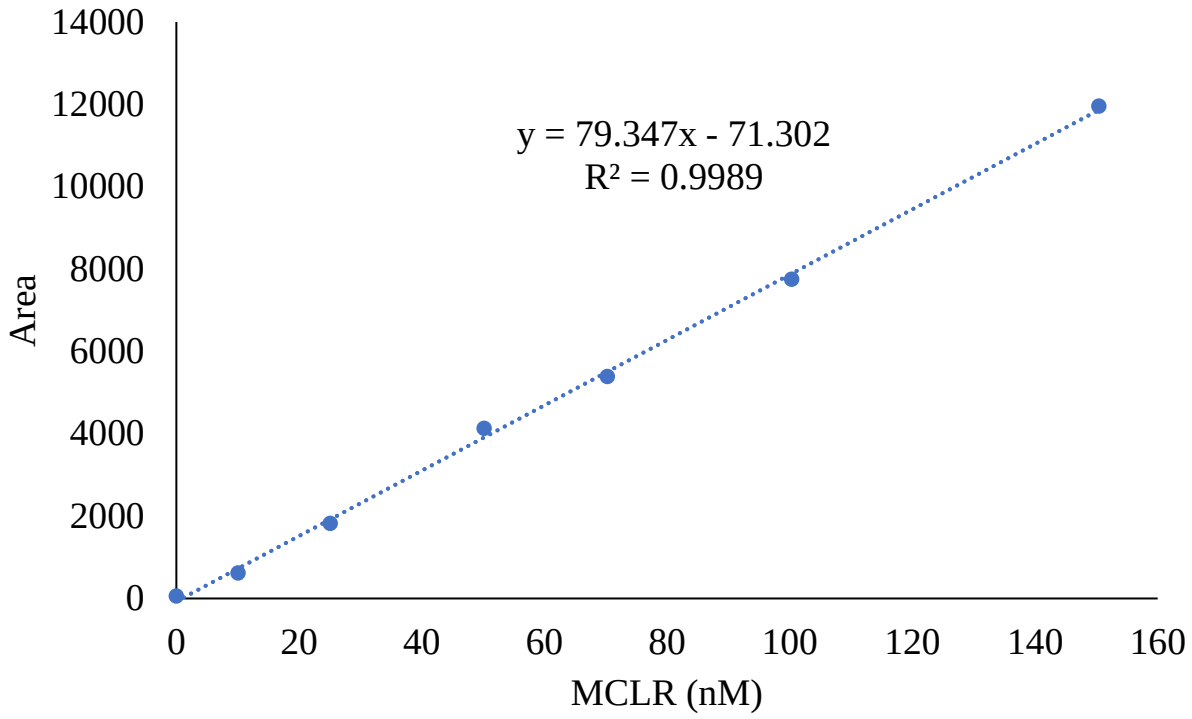

Figure 2: UPLC calibration curve to determine MCLR concentration

Data Analysis

The second order rate equation for the reaction between permanganate and MCLR is shown in Equation 2, where k is the second order rate constant.

Equation 2

$$\frac{d[MCLR]}{dt} = -k[KMnO_4][MCLR]$$

Because the concentration of permanganate is in excess (at least 10 time greater than [MCLR]), the decay of MCLR can be converted into a pseudo-first order rate equation. In Equation 3, k_{obs} is the observed first order rate constant of MCLR degradations.

Equation 3

$$\frac{d[MCLR]}{dt} = -k_{obs}[MCLR]$$

The linear form of the first order rate equation along with a linear regression was then used to determine the observed first order rate constant (Equation 4).

Equation 4

$$[MCLR]_t = -k_{obs}(t) + [MCLR]_0$$

Removal of MCLR by Permanganate in the presence of DOM

Procedure

The competition rate constant of the DOM/permanganate using an experimental procedure similar to that used to determine the MCLR/permanganate second order rate constant. Organic matter solutions were made in the same pH 7, 5 mM phosphate buffer. Using either Suwannee River Fulvic Acid (terrestrial) or Grand Lake St. Mary's (microbial) DOM isolates were added to the buffer to achieve 5 mg-C/L. 100 nM microcystin-LR (MCLR) was added to the buffer/DOM solution. 5 mM potassium permanganate stock solution was added to 40 mL of the buffer/DOM/MCLR solution to achieve working concentrations of either 2, 3, 4, 6, or 12 µM of permanganate.

For each dose added in the same kinetic run, 7 timepoints were taken and each timepoint was either 6 or 7 minutes apart (depending on the dose). At each timepoint, a 2 mL sample of the permanganate/MCLR/buffer/DOM solution was pulled and quenched with 0.08 mM thiosulfate solution. Each quenched sample was discharged into an LC vial for analysis. Each separate dose of permanganate was allowed to react in solution for at least 40 minutes. The samples were analyzed using UPLC on a Waters Aquity Class H UPLC and MCLR concentrations were calculated using the calibration curve in **Error! Reference source not found.**.

Data analysis

Equation 5 determines the concertation of MCLR at a given time using the second order permanganate/ MCLR rate constant, k, the observed first order permanganate/DOM rate constant, k_{DOM}, the initial concentration of MCLR, and the initial concentration of permanganate (Laszakovits J. R., 2019). Experimental data of MCLR degradation from each separate dose of permanganate was fitted to this equation to estimate k_{DOM} using the MATLAB fit tool.

Equation 5

$$[MCLR]_t = [MCLR]_0 e^{-k[MnO_4^{-1}]_0\left(\frac{e^{-k_{DOM}*t}}{-k_{DOM}}+\frac{1}{k_{DOM}}\right)}$$

Bioluminescence Assay and Propidium Iodide controls

Procedure

The propidium iodide cell lysis assay was validated using a bioluminescence assay. The bioluminescence assay (BLIA) was used with *Vibrio fischeri* according to the methods described Abbas et al, 2018 (Abbas,

2018). *V. fischeri* cultures were obtained from Dr. Natalie Hull at Ohio State University. A dose-response curve for luminescence of cells with respect to permanganate or copper sulfate exposure was created. Samples were analyzed in 96-well luminescence plates. Cell cultures of *V. fischeri* were prepared for the PI assay using the same experimental procedure as the BLIA. After cells were exposed to either copper sulfate or permanganate for at least 30 minutes in 96-well fluorescence plates, 1.5 mM of PI stock solution was added to each well to achieve a working concentration of 15 µM of propidium iodide. Samples were allowed to stand for at least 25 minutes for full formation of DNA-PI complex. The fluorescence of the samples was read with excitation of 490 nm and an excitation of 645 +/- 40 nm on a BioTek Synergy HTX multi-mode plate reader. The subsequent fluorescence - response curve was then compared to the luminescence response curve to validate the propidium iodide method.

Data Analysis

Equation 6 was used to determine the percentage of cells alive after 30 minutes of exposure to either copper sulfate or potassium permanganate where L_i is the luminescence corresponding to some concentration of oxidant, i, and L_0 is the luminescence of the negative control, when 100% of cells should be alive.

Equation 6

$$\%\ Alive_{BLIA} = \frac{L_i}{L_0}$$

The percentage of cells alive as determined by the PI assay is shown in Equation 7. In this equation, PI_i is the PI fluorescence signal corresponding to some concentration of oxidant, i, $PI_{100\%}$ is the PI signal corresponding to the highest oxidant dose (when 100% of the cells are dead), and PI_0 is the PI signal of the negative control.

Equation 7

$$\%\ Alive_{PI} = \frac{PI_{100\%} - PI_i}{PI_{100\%} - PI_0}$$

Sequential dosing – cells

Procedure

The propidium iodide assay was used to track the degree of cell lysis with respect to time during sequential dosing of permanganate. A cell preparation procedure similar to the one described in BLIA was used to prepare cells. Starter cultures of *Microcystis aeruginosa* were obtained from Nicholas Dugan, US Environmental Protection Agency, Water Infrastructure Division, Cincinnati OH. Either *V. fischeri* or *Microcystis* cells were diluted from their pure cultures and resuspended in 1X phosphate buffer solution (PBS). Then, 20 mL of prepared cell solution (OD 600 = approximately 0.44) was dosed with 5 mM permanganate stock solution to achieve working concentrations between 12.6 µM (2 ppm) and 63 µM (10 ppm). Additional permanganate was added to the same reaction solution in the case of sequential doses. Approximately 10 time points were taken during every 40-minute dose as the reaction proceeded. At the specified time point, the solution was plated in 96-well plated and quenched with 2.6 mM Thiosulfate to achieve a working concentration of 433uM. After all samples were quenched, 1.5 mM of PI stock solution was added to each well to achieve a working concentration of 15 µM of propidium iodide. Samples were allowed to stand for at least 25 minutes for full formation of DNA-PI complex. The fluorescence was then read on the BioTek Synergy HTX multi-mode plate reader using the 485/20 excitation filter and 645/40 excitation filter. The positive control for these experiments was solution of cells (OD600 = 0.44) that was exposed to 83 µM of permanganate for 30 minutes, then was quenched with 2.6 mM of Thiosulfate to achieve a working concentration of 433 µM. Based on results

from the BLIA, the positive control will represent 100% of the cells lysed. 15 µM of PI was also added to the positive control and allowed to stand for at least 25 minutes before its fluorescence was read. The background fluorescence was determined using a negative control containing only PBS and 15 µM of PI.

Data Analysis

A slightly modified version of Equation 6 was used to determine the percentage of cells lyses after exposure to permanganate with respect to time. In Equation 8, $PI_{100\%}$ is the PI signal of the positive control, which represents a reading where 100% of the cells have lysed, PI_0 is the PI signal at time zero where 0% of the cells should have lysed, and PI_t is the PI signal at time t.

Equation 8

$$\%\ Alive_{PI} = \frac{PI_{100\%} - PI_t}{PI_{100\%} - PI_0}$$

The degradation of permanganate was estimated using the cell lysis curve from Equation 8. At some point during exposure to permanganate, the reaction appeared to stop when the differences in PI signal readings became statistically insignificant. This was typically around 14 minutes. I assumed that at this point, all of the permanganate had been consumed. It was also assumed that the permanganate degradation was proportional to the total percentage of cells removed. Equation 9 estimates the permanganate concentration at time t using this relationship where t_{end} is the time where the reaction between permanganate and cells has ended, PA_0 is the percentage of cells alive at time zero, $P_{t,end}$ is the percentage of cells alive at t_{end}, and PA_t is the percentage of cells alive at time t.

Equation 9

$$[KMnO_4]_t = \left(\frac{PA_t - PA_{t_{end}}}{PA_0 - PA_{t_{end}}}\right) * [KMnO_4]_0$$

Results and Discussion

KMnO4/MCLR rate constant

We have determined the second order rate constant of the permanganate/MCLR reaction to be 282 1/M*sec (+/- 39 1/M*sec) (Figure 3). This is generally consistent with other reported rate constants. Laszakovits and MacKay 2019 reported a rate constant of 272 1/sec*M (+/- 23). Rodriguez et al, 2007 reported a rate constant of 357.2 +/- 17.5 1/Ms.

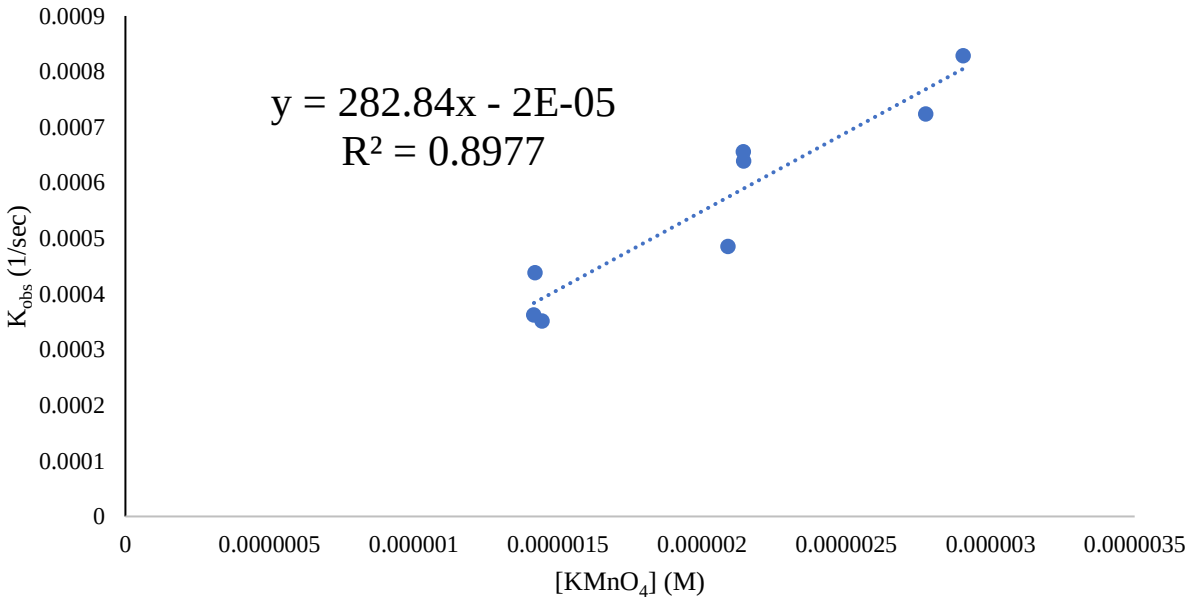

Figure 3: Second order rate constant for reaction between permanganate and MCLR determined between relationship between observed rate constants and permanganate dose

MCLR/ Permanganate kinetics in presence of DOM

Sequential dosing versus a singular dose of permanganate was tested in 5 mg/L DOC of either SRFA or GLSM DOM isolates (Figure 4 a-f.).

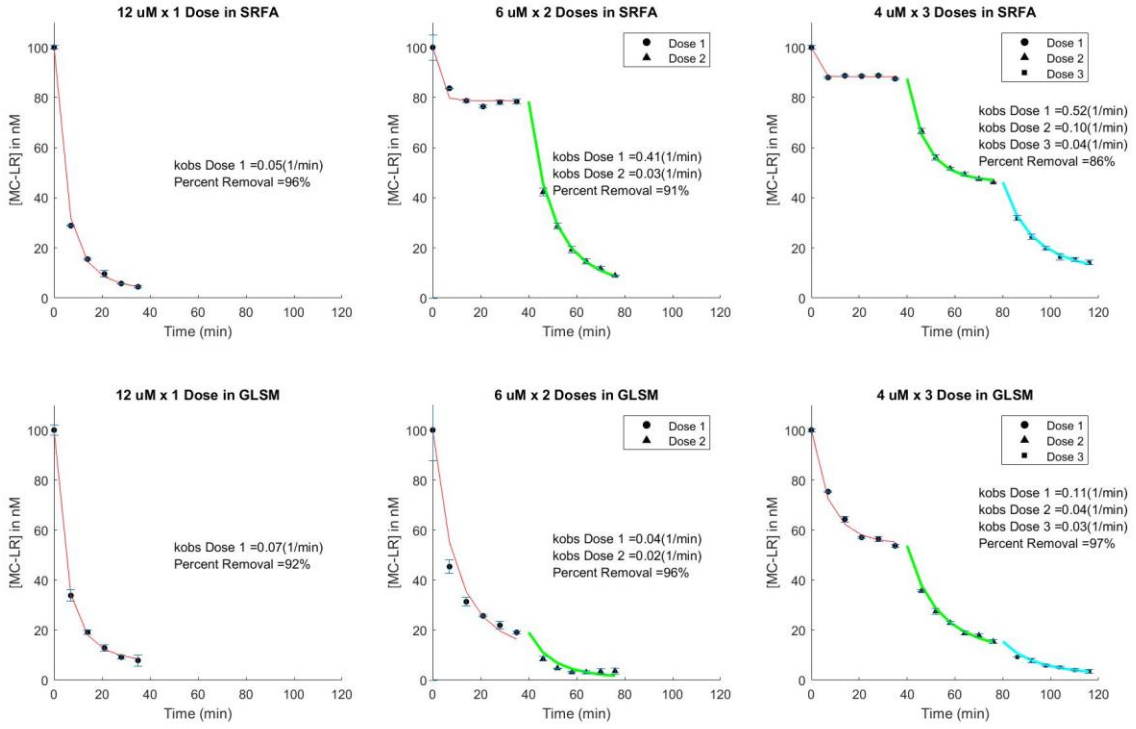

Figure 4: A through C, sequential dosing of 12 µM of permanganate total separated into either 1, 2, or 3 does in 5 mg/L DOC SRFA phosphate buffer (pH 8). E through F, sequential dosing of 12 µM of permanganate total separated into either 1, 2, or 3 does in 5 mg/L DOC GLSM phosphate buffer (pH 8). On each individual plot are the k_{obs} (k_{DOM}) values for each dose (in 1/min). Solid lines on each plot are the theoretical values for MCLR degradation used in Equation 5.

SRFA

For those experiments conducted in the presence of SRFA isolate, the rate of DOM competition decreased with each sequential dose. For two doses of 6 µM, k_{DOM} decreased from 0.41 to 0.03 1/min

and for three doses of 4 µM, k_{DOM} decreased from 0.52 to 0.10 to 0.04 1/min. However, this decrease in competition did not lead to an improvement in MCLR removal as we had anticipated. Between one dose of 12 µM, two doses of 6 µM, and three doses of 4 µM, MCLR removal increased from 96% to 91% and 86%, respectively. This may be attributed to the negative correlation between the concentration of the first dose and the observed competition rate. The observed k_{DOM} of one dose of 12 µM was 0.05 1/min, while the first dose of 6 µM experienced a competition rate almost 10 times greater. The initial competition rate also increased between 6 µM and 4 µM, but only from 0.41 to 0.52 1/min. For both of the dosing methods that used sequential dosing, the subsequent doses showed a competition rate similar to the competition rate experienced by the first dose of a single dose. For instance, the competition rate of the second dose of 6 µM is approximately 8% of the initial competition rate and the second dose of 4 µM is approximately 19% of the initial competition rate. Between the second and third doses of 4 µM, the competition rate decreased by 60%. This is still a substantial decrease in competition rate, but not as great as the difference between the first and second doses. This suggests that the initial competition from DOM is the biggest "hurdle" when using pre-oxidation in the presence of SRFA DOM.

GLSM

Similar to those experiments conducted in SRFA buffer, the competition rate in the presence of GLSM isolate buffer also decreased with each sequential dose. Between two doses of 6 µM, the rate of competition decreased from 0.04 to 0.02 1/min, and between three doses of 4 µM, the rate of competition decreased from 0.11 to 0.04 to 0.03 1/min. Unlike the SRFA, MCLR removal increased with the number of doses used from 92% to 96% to 97% with one, two, and three doses, respectively. There was also not any trend in the initial competition rate for the first dose of each experiment. However, for those sequential dosing experiments, the initial k_{DOM} was much lower in the presence of GLSM than in the presence of SRFA.

This is likely due to the differences in composition between the two DOM isolates. Permanganate has been shown to preferentially react with aromatic DOM compounds, which are positively correlated to both SUVA254 and electron donating capacity (Laszakovits J. R., 2020). GLSM and SRFA DOM were measured to have SUVA254 values of 1.23 and 4.83, respectively. SRFA's higher SUVA254 value suggests that its increased competition stems from a higher aromaticity than GLSM. GLSM isolate is derived from a lake that often experiences intense algal blooms and is considered to be more microbial cell related DOM and SRFA is more terrestrial or plant DOM. Because of this, we can assume that the DOM present during an algal bloom will behave similarly to the GLSM isolate.

BLIA/Propidium iodide verification

The dose-response curves of the bioluminescence assay (BLIA) using both copper sulfate and potassium permanganate are shown in Figure 5. For copper sulfate, samples had near zero luminescence readings by 31 µM and for permanganate, samples had near zero values with approximately 100 µM. A luminescence reading of zero would indicate that all of the cells are at least non-viable. Based on the dose response curve, permanganate was able to damage all of the cells, although it required much higher concentrations than copper sulfate to do so.

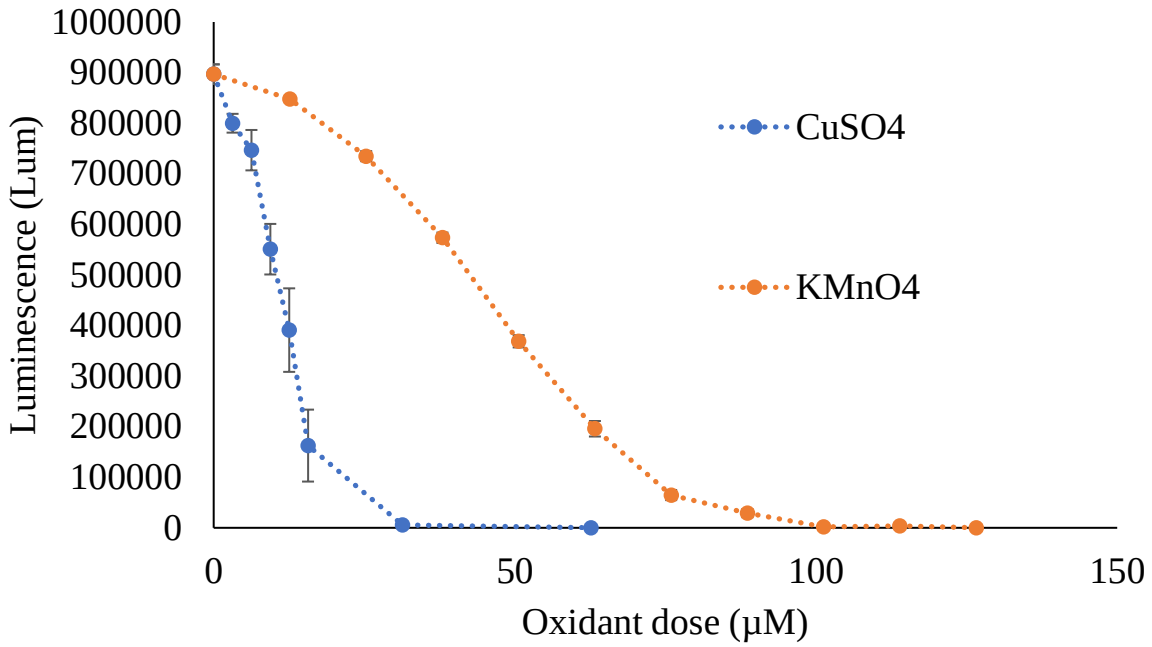

Figure 5: BLIA Dose-response curve for Vibrio fischeri cells after exposure to either 0-63 µM of copper sulfate or 0-126 µM of permanganate for 30 minutes

With only the BLIA dose response curve, it is difficult to determine how the cells have become non-viable. They may have been fully lysed or just significantly damaged. The propidium iodide dose response curve suggests that cells are lysing at least to some extent (Figure 6).

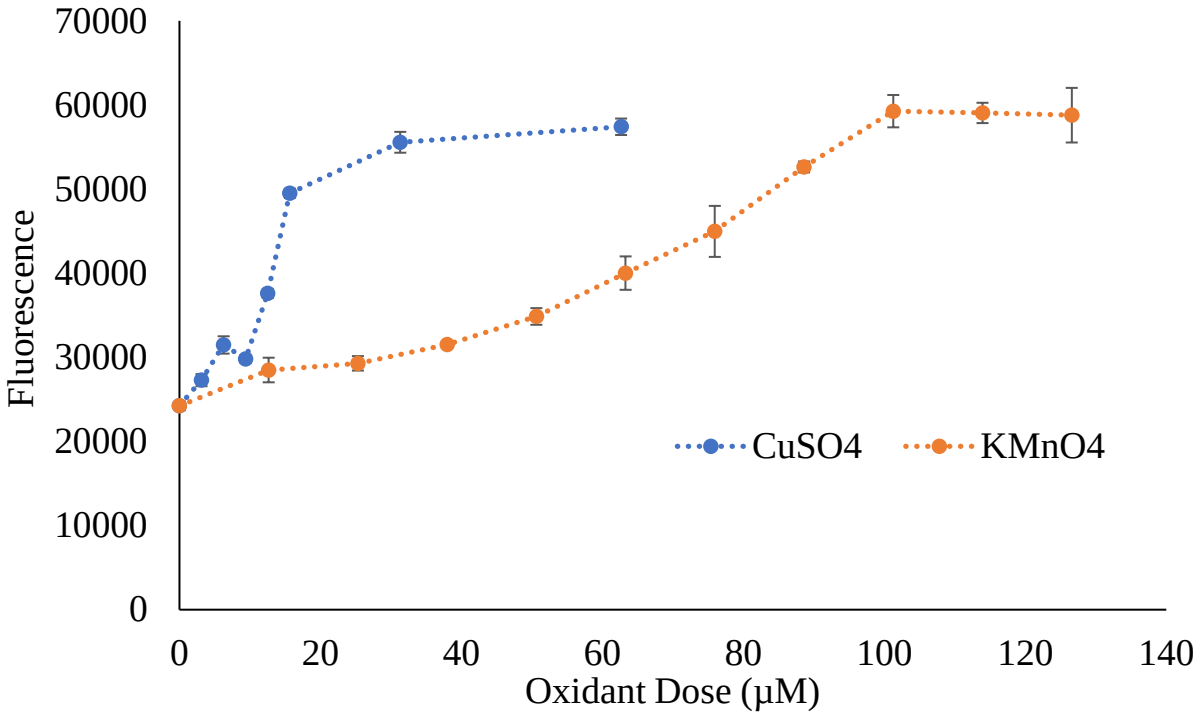

Figure 6: Propidium Iodide Dose-response curve for Vibrio fischeri cells after exposure to either 0-63 µM of copper sulfate or 0-126 µM of permanganate for 30 minutes

Using the PI assay, the highest fluorescence values occurred using the highest dose of either oxidant, which is also when the lowest luminescence readings occurred. So, at these points, the cells are all no longer viable (according to the BLIA) and each oxidant has reached its maximum PI signal. Again, with only these two assays, it is impossible to know if 100% of the cells have lysed, but the general agreeance between the maximum scalar fluorescence values achieved by both copper sulfate and permanganate suggests that this is the maximum amount of cell lysis that can occur. For the remainder of this study, we will assume that the maximum lysis is the same as 100% of the cells lysing.

Using both assays, their readings have been normalized to represent cell viability curves (Figure 7 and Figure 8). For both copper sulfate and permanganate, the viability curves generated with the BLIA and PI assay agree fairly well. The similarities between the assays suggest that cells with membranes that are

compromised enough to have been dyed by PI are also non-viable cells. The agreement between these two assays means that the PI assay can be used to track cell lysis of bacterial cells that do not also bio-luminesce (I.e., cyanobacteria).

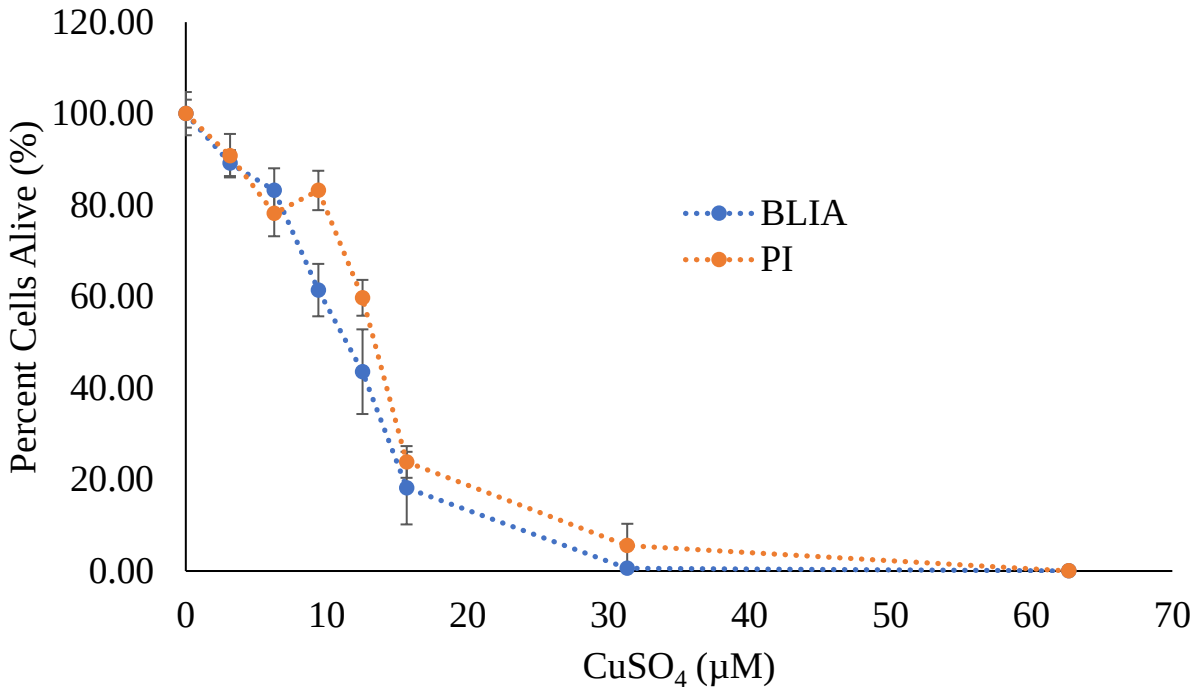

Figure 7: Percentages of cells alive after exposure to 0-63 µM of copper sulfate for 30 minutes as determined by both BLIA and PI Assays

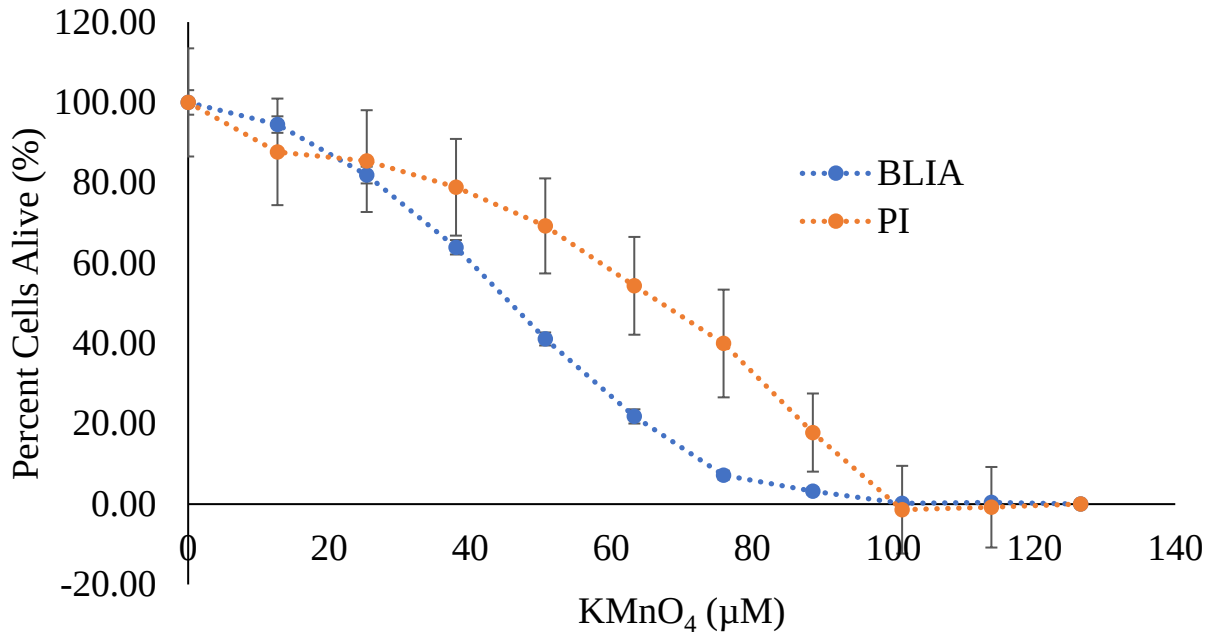

Figure 8: Percentages of cells alive after exposure to 0-126 µM of potassium permanganate for 30 minutes as determined by both BLIA and PI Assays

PI Assay with *Microcystis* Cells

The PI cell lysis assay was then tested using *Microcystis aeruginosa* (MA) cells. The positive control in this assay was a sample of MA cells that have went through three freeze-thaw cycles, which is thought to completely lyse MA cells (USEPA, 2021). As shown in Figure 9, samples were exposed to 63 µM (10 ppm) of permanganate and no significant cell lysis was observed. Additionally, when the same experiment was conducted using *Vibrio fischeri*, the signal of the *V. fischeri* positive control was approximately 48,000 and the MA positive control was approximately 21,000 while both negative controls (only non-lysed cells and PI) had similar values of approximately 20,000 when read in the same microplate.

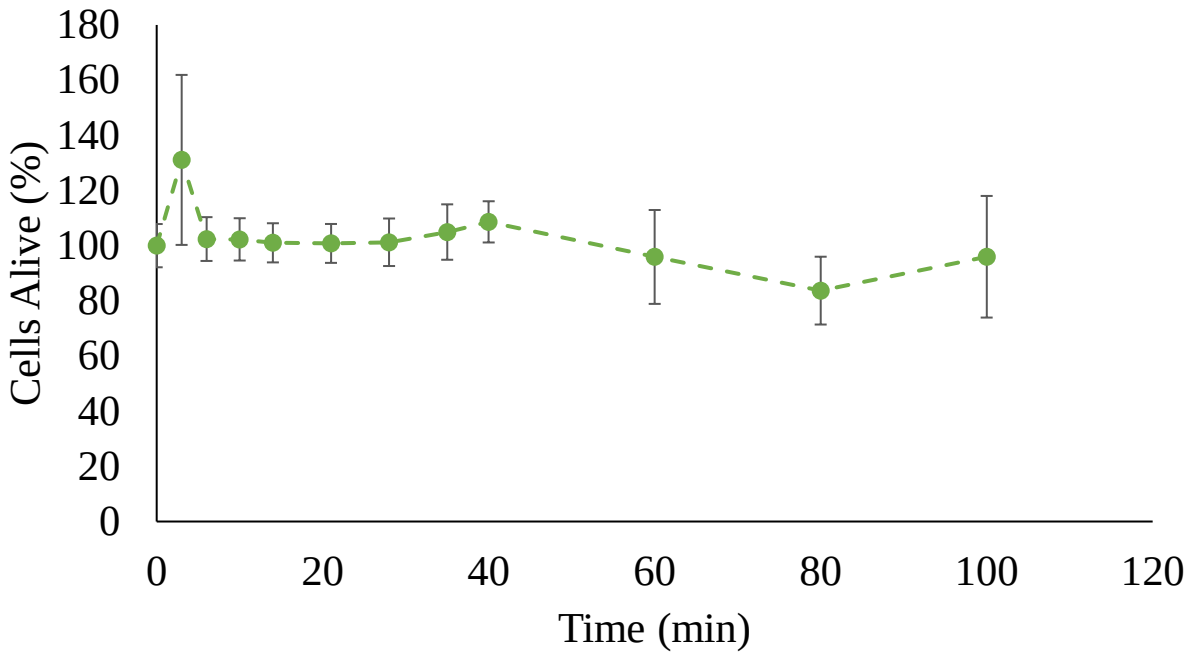

Figure 9: Percentage of Microcystis cells alive versus time after exposure to 63 µM (10 ppm) of potassium permanganate

The difference in PI signal between the two organisms can be a result of many reasons. *V. fischeri* may contain more genetic material for PI to stain compared to MA cells. The low PI signal of the positive control supports suggests that at this concentration of MA cells, a fully lysed sample does not contain much genetic material to be observed. Concentrations of the cells were determined using optical density, so the cell-count-to-optical-density relationship may also be different between the two.

The low PI signals may also suggest that MA cells are somewhat resistant to permanganate in terms of cell lysis. Our results agree with findings in the literature. Many studies have found that significant cell lysis of MA cells did not occur for doses of permanganate less than 5 mg/L. Additionally, much longer contact times were needed to observe significant lysis (>6 hours). Therefore, typical treatment doses of permanganate (< 3 mg/L) should not be expected to cause a significant amount of cell lysis. Additional work should be done using the PI assay to confirm that significant MA cell lysis does not occur during the first hour of treatment.

Sequential dosing with Cells

Sequential dosing of 5 ppm as potassium permanganate was tested in 1 X PBS with *V. fischeri* (OD600 = 0.44). Between one dose of 5 ppm and two doses of 2.5 ppm, we did not observe any significant difference in cell death (Figure 10). In both cases, approximately 45% of cells were viable after oxidation.

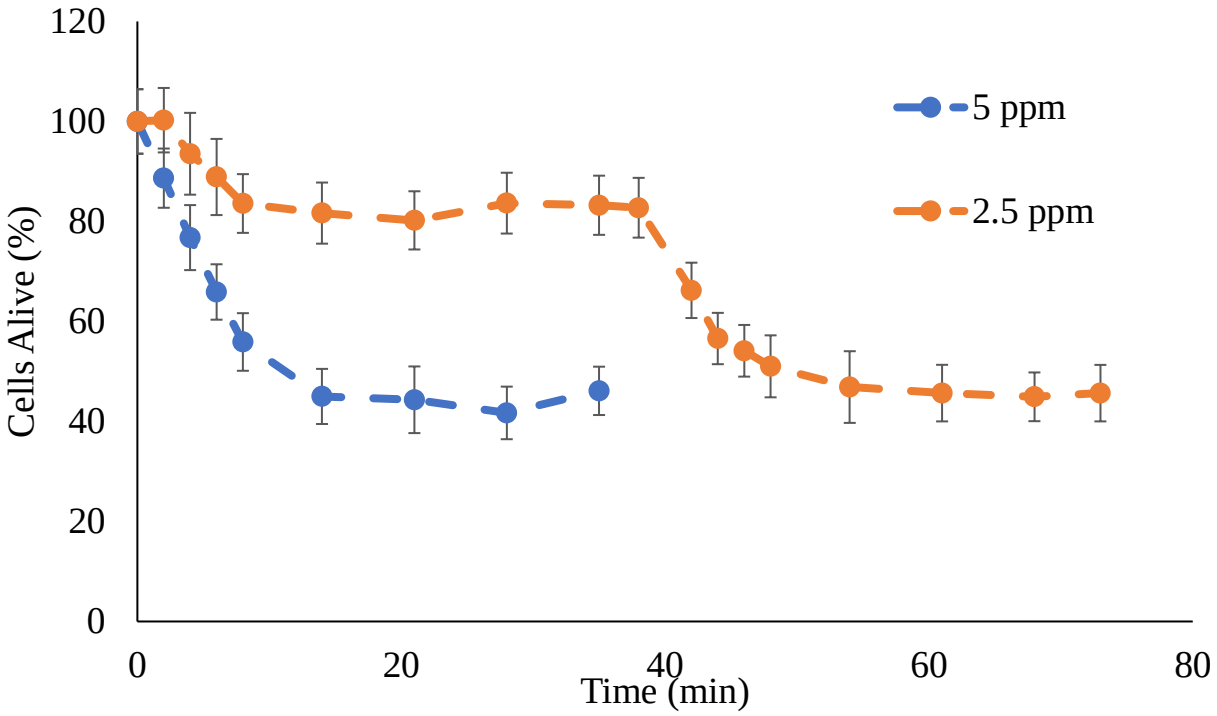

Figure 10: Percentage of cells alive versus time after exposure to either one dose of 5 ppm potassium permanganate (31.5 µM) or two doses of 2.5 ppm potassium permanganate (15.75 µM)

Our results indicate that cell lysis caused by one dose will be similar to that caused by sequential dosing. The similarity between the performance of a singular dose and sequential doses of permanganate suggests that the reaction rate between sequential doses is not changing enough to affect the number of cells lysed. Although we did not observe any significant effects with cell lysis rate immediately after oxidation, there may be long term effects on the cells during the remainder of the treatment train.

Cells competition rate constant

Using fundamental rate equations, the observed reaction rate between permanganate and *V. fischeri* cells was determined for each dose. Equation 10 is the second order reaction rate between the cells and permanganate.

Equation 10

$$\frac{d[KMnO_4]}{dt} = -k[Cells][KMnO_4]$$

After using doses permanganate upwards of 63 µM (10 ppm KMnO4, which are unrealistically high in a typical treatment setting), permanganate would not cause 100% cell lysis. So, there was not a scenario in which the cells were the limiting reactant in this reaction. Because of this, we can assume that the concentration of cells is in excess, and this reaction can be considered pseudo-first order (Equation 11).

Equation 11

$$\frac{d[KMnO_4]}{dt} = -k_{cells}[KMnO_4]$$

Equation 11 is the pseudo first order rate of decay of permanganate, where k_{cells} is the observed first order rate constant. Using the cell viability data shown in Figure 10, the permanganate degradation of each dose was estimated based on when the reaction "stopped." The reaction was determined to have stopped after the observed cell viability became consecutively statistically insignificant, or

concentrations showed no further change with time. This was typically around 14 minutes after exposure to permanganate. At this point, we assumed that all of the permanganate was consumed.

Then, k_{cells} was determined for each dose using the linearized first order rate equation (Equation 12). In this equation, $[KMnO_4]_0$ is the initial concentration of permanganate and $[KMnO_4]_t$ is the estimated remaining concentration of permanganate at time t. $[KMnO_4]_t$ was estimated by assuming that all permanganate had been consumed approximately 14 minutes after the addition of each dose and the consumption of permanganate was proportional to the total number of cells degraded. A linear regression was then used to determine k_{cells}.

Equation 12

$$\ln[KMnO_4]_t = -k_{cells} * t + \ln[KMnO_4]_0$$

Figure 11 shows the k_{cells} for a dose of 5 ppm of permanganate in potassium permanganate. For this dose, k_{cells} was determined to be 0.199 1/min +/- 0.023 1/min. For the first and second doses of 2.5 ppm, their k_{cells} values were 0.273 1/min (+/- 0.68 1/min) and 0.265 1/min (+/- 0.018 1/min), respectively. Although there was a slight decrease in the k_{cells} between the two doses, this decrease is only approximately 3%. This aids the suggestion that the competition posed by the cells is not dramatically changing in between doses.

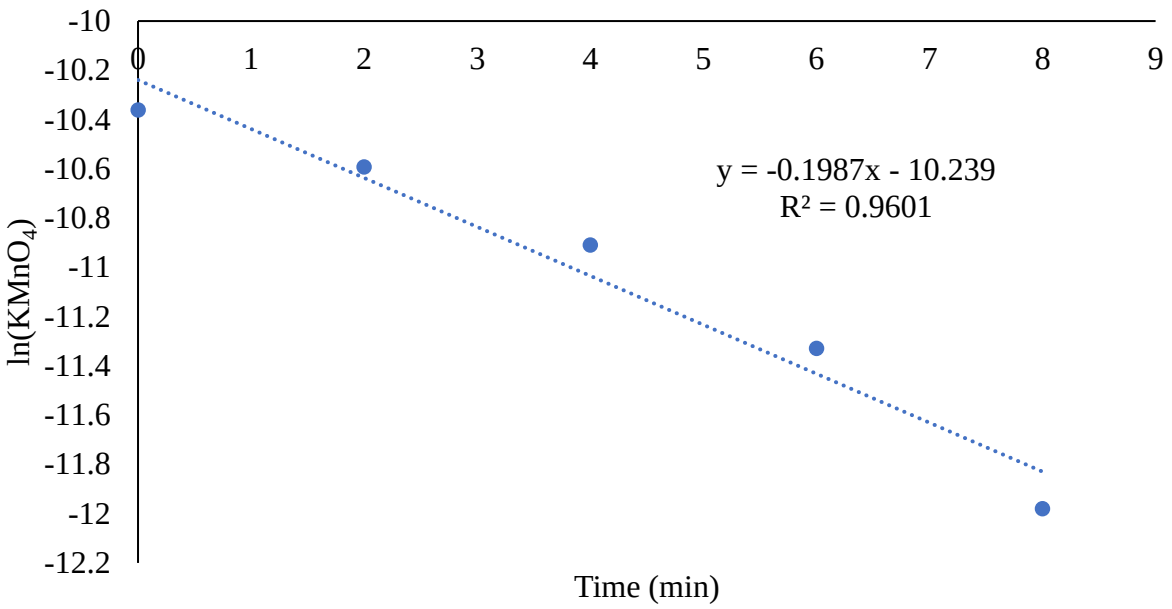

Figure 11: Natural log of estimated potassium permanganate remaining (μM) versus time to determine the observed rate constant between permanganate and V. fischeri cells

Full Simulation

In order to simulate MCLR removal in the presence of DOM and cells, the remaining permanganate must be estimated while accounting for consumption by MCLR, DOM, and the cells. The rate of MCLR decay is shown on Equation 2. The rate of permanganate degradations can be seen in Equation 13. In this model, the rates of permanganate consumption by all three species are additive.

Equation 13

$$\frac{d[MnO_4^-]}{dt} = -k[MCLR][MnO_4^-] - k_{DOM}[MnO_4^-] - k_{cells}[MnO_4^-]$$

Both rates of MCLR decay and permanganate decay can be expanded into a numerical solution made up of two coupled differential equations. Equation 14 and Equation 15 determine the concentrations of MCLR and permanganate at each time step based on the conditions of the previous time step.

Equation 14

$$[MCLR]_{t+1} = [MCLR]_t + (-k[KMnO_4]_t[MCLR]_t) * dt$$

Equation 15

$$[KMnO_4]_{t+1} = [KMnO_4]_t + (-k[KMnO_4]_t[MCLR]_t - k_{DOM}[KMnO_4]_t - k_{cells}[KMnO_4]_t) * dt$$

This model aims to simulate a "worst case" scenario, with a high content of both MCLR and cells, simulating an intense bloom. The initial conditions include 100 nM of MCLR and 5 mg/L DOC of GLSM DOM isolate. The organism being simulated in this model is *V. fischeri*, where the solution should have a starting optical density of approximately 0.44. *V. fischeri* was simulated as opposed to *Microcystis* because our lysis data related to MA cells was inconclusive when compared to data collected using *V. fischeri* cell, which indicated lysis after exposure to permanganate. Figure 12 demonstrates the expected MCLR degradation using one dose of 12 µM and two doses of 6 µM of permanganate in the presence of DOM and in the presence of both DOM and *V. fischeri* cells.

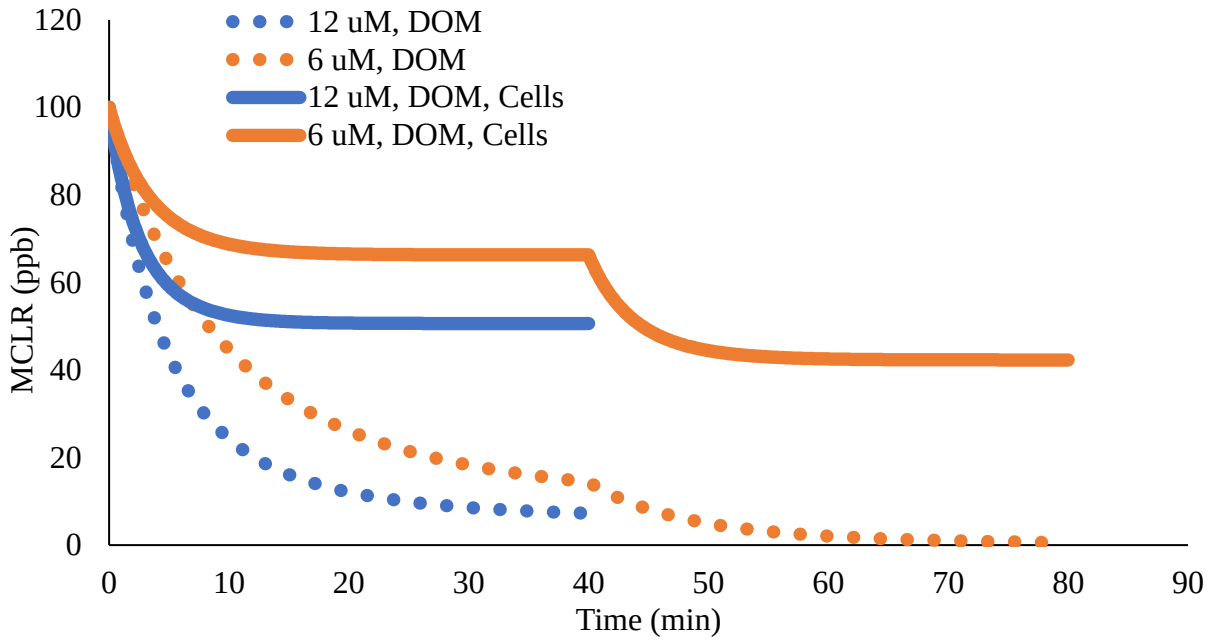

Figure 12: Expected MCLR removal by one dose of 12 µM and two doses of 6 µM of permanganate. Dotted lines are the expected removal curves in the presence of DOM (5 mg/l DOC of GLSM isolate). Solid lines are the represented removal curves of MCLR in the presence of both DOM and V. fischeri cells (OD600 = 0.44)

The MCLR and permanganate second order rate constant used was 272 1/sec (Laszakovits J. R., 2019). The k_{DOM} values used in this simulation were pulled from experimental results, so the k_{DOM} of the one dose of 12 µM was 0.07 1/min and the k_{DOM} values for the first and second doses of 6 µM were 0.04 and 0.02 1/min, respectively. The k_{cells} values for each dose were estimated by using a linear regression for the relationship between the concentration of permanganate and the experimental k_{cells}, like those determine in Figure 13.Using this, the k_{cells} value of one dose of 12 µM was estimated to be 0.22 1/min, and the k_{cells} value of both doses of 6 µM was estimated to be 0.20 1/min, assuming that the competition rate of the cells does not change between doses.

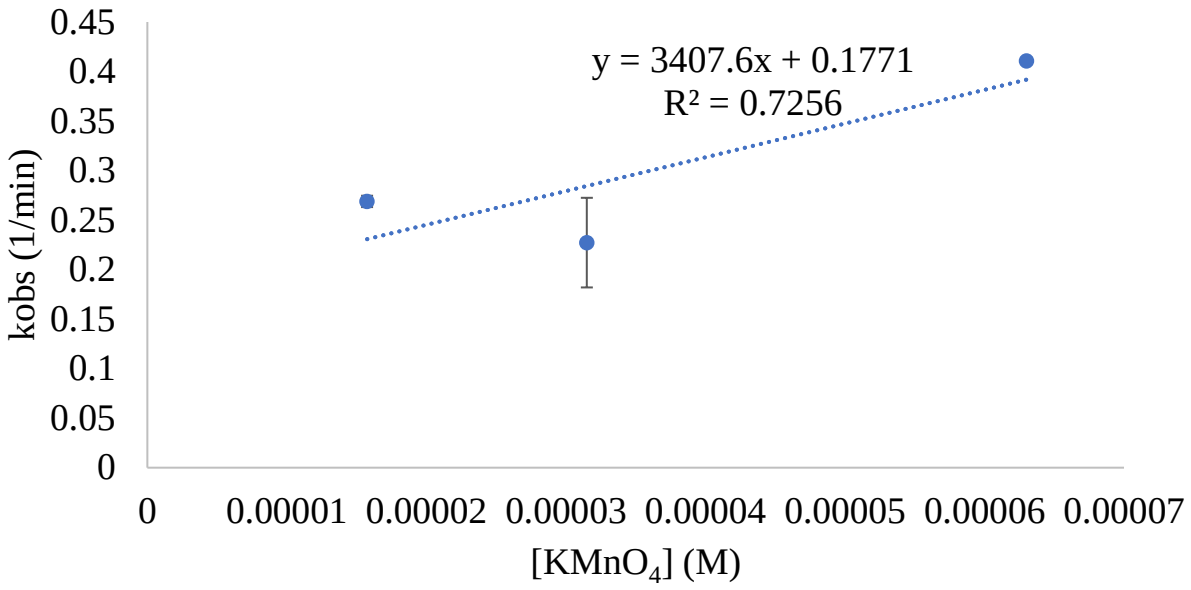

Figure 13: Rate constant between 12 µM and 6 µM of cells estimated using observed relationship between permanganate dose and observed rate constant

Our simulation predicts that, in the presence of DOM only, one dose of 12 µM of permanganate will remove approximately 92% of the total MCLR and two doses of 6 µM will remove approximately 99% of the total MCLR. These predictions are similar to our previous experimental results (Figure 4). In the presence of both DOM and cells however, we predicted that one dose of 12 µM of permanganate will remove 50% of the total MCLR and two doses of 6 µM will remove 58% of the total MCLR.

The decrease in MCLR removal for both a single dose and sequential doses shows that the competition for permanganate reactions caused by cells has the potential to severely impact treatment performance. With the addition of cells into the system, we predict MCLR removal to decrease by approximately 40% of the total MCLR. This dramatic decrease in performance may be due to the difference in average competition rates for both DOM and cells. For all three doses of permanganate,

the k_{DOM} was less than 0.10 1/min and the k_{cells} was at least 0.20 1/min or greater. So, competition caused by the cells was at least twice as great as the competition caused by the cells. In the case of the second dose of 6 µM of permanganate, k_{DOM} was less than one tenth that of k_{cells}.

The difference between MCLR removal simulated with and without consideration for cells may be attributed to the relatively high cell density. With an optical density of *V. fischeri* of 0.85, the sample is cloudy with cells, so this may be unrealistically high for even an intense bloom. Additionally, the cells used in this simulation were not cyanobacteria, which may behave differently in the presence of permanganate. Based on our data, it is unclear how quickly permanganate was consumed by MA cells. There is a need to more closely monitor the consumption of permanganate by MA cells to determine its competition rate. Based on the mathematical model that runs the simulation, whatever the k_{cells} value may be, MCLR removal will be less than in the presence of only DOM as long as k_{cells} is nonzero. This is due to the inclusion of the reaction rate between cells and permanganate in the model.

Conclusions

- Sequential dosing is an alternative dosing technique for permanganate pre-oxidation where the same amount of permanganate is used, but fractional portions of the total permanganate are added over time as opposed to all at once.
- Competition by DOM decreases with each dose of permanganate when using sequential dosing.
- Sequential dosing may increase MCLR removal in microbial-like DOM but may decrease MCLR removal in terrestrial DOM when compared to removal using a single dose of permanganate.
- Sequential dosing predicted to improve MCLR removal in the presence of *V. fischeri* and microbial-like DOM.

Future Work

- Experimental data should be collected to examine how MCLR is removed in the presence of environmentally relevant concentrations of both DOM and cyanobacterial cells
- More work should be done using the propidium Iodide assay with MA to determine the extent to which cells lyse during pre-oxidation

Acknowledgements

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References

Abbas, M. A.-u.-H. (2018). Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review. *Science of the Total Environment, 626*, 1295-1309.

Campos, A. a. (2010). Molecular Mechanisms of Microcystin Toxicity in Animal Cells. *International Journal of Molecular Sciences, 11*, 268-287.

Ding, J. S. (2010). Release and Removal of Microcystins from Microcystis during Oxidative-, Physical-, and UV-Based Disinfection. *Journal of Environmental Engineering, 136*(1), 2-11.

Fan, J. ,. (2013). Evaluating the effectiveness of copper sulphate, chlorine, potassium permanganate, hydrogen peroxide and ozone on cyanobacterial cell integrity. *Water, 47*(14), 5153-5164.

Fan, J. R. (2016). Impact of chlorine on the cell integrity and toxin release and degradation of colonial Microcystis. *Water Research, 102*, 394-404.

He, X. L. (2016). Toxic cyanobacteria and drinking water: Impacts, detection, and Treatment. *Harmful Algae, 54*, 174-287.

Hu, L. M. (2010). Oxidation Kinetics of Antibiotics during Water Treatment with Potassium Permanganate. *Environmental Science and Technology, 44*(16), 6416-6422.

Jeong, B. ,. (2017). Elimination of microcystin-LR and residual Mn species using permanganate and powdered activated carbon: Oxidation products and pathways. *Water Research, 114*, 189-199.

Laszakovits, J. R. (2019). Removal of cyanotoxins by potassium permanganate:. *Water Research, 155*, 86-95.

Laszakovits, J. R. (2020). Chemical Alterations of Dissolved Organic Matter by. *Environmental Science and Technology, 54*(6), 3256-3266.

Liu, Y. C. (2020). Impacts of antibiotic contaminants on Microcystis aeruginosa during potassium permanganate treatment. *Harmful Algae, 92*, 1-9.

Massey, I. Y. (2020). A Mini Review on Microcystins and Bacterial Degradation. *Toxins, 12*(268), 1-21.

Moradinejad, S. G. (2019). Using Advanced Spectroscopy and Organic Matter Characterization to Evaluate the Impact of Oxidation on Cyanobacteria. *Toxins , 11*.

OEPA. (2020, Feburary 14). *Ohio Environmental Protection Agency*. Retrieved from Harmful Algal Blooms: Public Water Systems and Other Ohio EPA Surface Water Monitoring: http://wwwapp.epa.ohio.gov/gis/mapportal/HAB_Monitoring.html

Ou, H. G. (2012). Immediate and long-term impacts of potassium permanganate on photosynthetic activity, survival and microcystin-LR release risk of Microcystis aeruginosa. *Journal of Hazardous Material, 219*(15), 267-275.

Qu, F. D. (2015). Control of ultrafiltration membrane fouling caused by Microcystis cells with permanganate pre-oxidation: Significance of in situ formed manganese dioxide. *Chemical Engineering Journal, 279*, 56-65.

Ramseier, M. G. (2011). Kinetics of membrane damage to high (HNA) and low (LNA) nucleic acid bacterial clusters in drinking water by ozone, chlorine, chlorine dioxide, monochloramine, ferrate(VI), and permanganate. *Water Research, 45*, 1490-1500.

Steffan, M. B. (2014). Status, causes, and controls of cyanobacterial blooms in Lake Erie. *Journal of Great Lake Research, 40*, 215-225.

Steffan, M. Z. (2014). Taxonomic assessment of a toxic cyanobacteria shift in hypereutrophic Grand Lake St. Marys (Ohio, USA). *Harmful Algae, 33*, 12-18.

Stiefel, P. ,. (2015). Critical aspects of using bacterial cell viability assays with the fluorophores SYTO9 and propidium iodide. *BMC Microbiology, 15*(36), 1-9.

Szlag, D. S. (2019). Permanganate Oxidation of Microcystin-LA: Kinetics, Quantification, and Implications for Effective Drinking Water Treatment. *Journal of Toxicology*, 1-13.

USEPA. (2021). . Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay.

Wert, E. C. (2013). Using digital flow cytometry to assess the degradation of three cyanobacteria species after oxidation processes. *Water Research, 37*, 3752-3761.

Wert, E. C. (2014). Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species. *Water Research, 52*, 251-259.

Zhang, Z. Z. (2011). Effects of microcystin-LR exposure on matrix metalloproteinase-2/-9 expression and cancer cell migration. *Ecotoxicology and Environmental Saftey, 77*, 88-93.

Supplementary Information

Materials List

Chemicals

Potassium Permanganate – Arcos Organics Potassium Permanganate CAS 7722-64-7

MCLR – Enzo Life Sciences Microcystin-LR ALX-350-012-C100 suspended in 1 mL in methanol

Grand Lake St. Mary's DOM isolate – Isolated by Carissa Hipsher using solid phase extraction

Suwannee River Fulvic Acid DOM isolate – purchased from International Humic Substances Society

Solid Mono Sodium phosphate – Fisher Chemical Sodium Phosphate Monobasic Monohydrate CAS 10049-21-5

Solid Sodium Thiosulfate – Fisher Chemical Sodium Thiosulfate Pentahydrate CAS 10102-17-7

1M HCl

1 M NaOH

1XPBS – Phosphate buffered Saline powder pH 7.4 (Pcode: 1003127976)

Photobroth – 38719 Sigma-Aldrich Photobacterium broth, pH 7.0 +/-0.2 (Pcode:

Propidium Iodide – Biotium Propidium Iodide 1mg/mL solution in water

Solid Copper Sulfate – Fisher Chemicals

Water – Mili-Q

Instruments

UPLC - Waters Acquity Class H UPLC

Plate reader – BioTek Synergy HTX multi-mode reader

Excitation Filter 485/20 - Biotek

Emission Filter 645/40 - Biotek

UV-Vis – Agilent Cary Series UV-Vis Spectrophotometer

TOC – Shimadzu TOC-V CSN Total Organic Carbon Analyzer

Fluorometer – Cary Eclipse Fluorescence Spectrometer

Organisms

Vibrio fischeri pure culture – Natalie Hull, Ohio State University

Microcystis aeruginosa pure culture – Nicholas Dugan, US Environmental Protection Agency, Water Infrastructure Division, Cincinnati OH

Other Materials

MicroSolv nylon 0.2 µM syringe filters

Appendices

Appendix A

Propidium Iodide Controls Read on Fluorometer

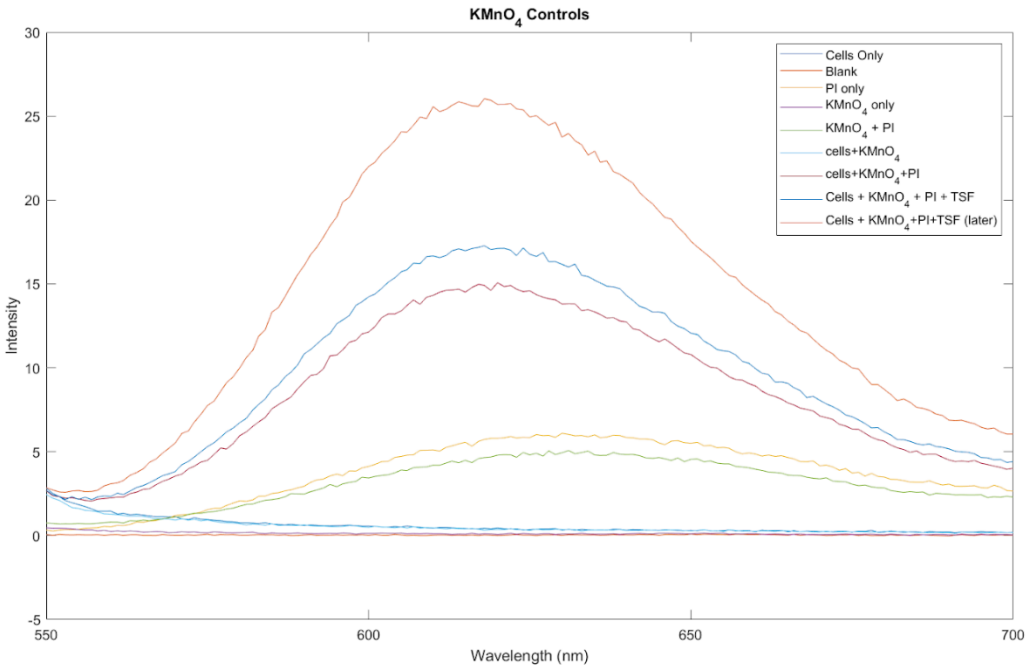

Figure 14: Emission spectra of Propidium Iodide (15 µM) in solutions of V. fischeri cells (OD600 = 0.44, in 1X PBS) after exposure to 10 ppm (63 µM) $KMnO_4$ and 433 µM of thiosulfate. The "later" indicates the sample was read on the fluorometer again after 30 minutes.

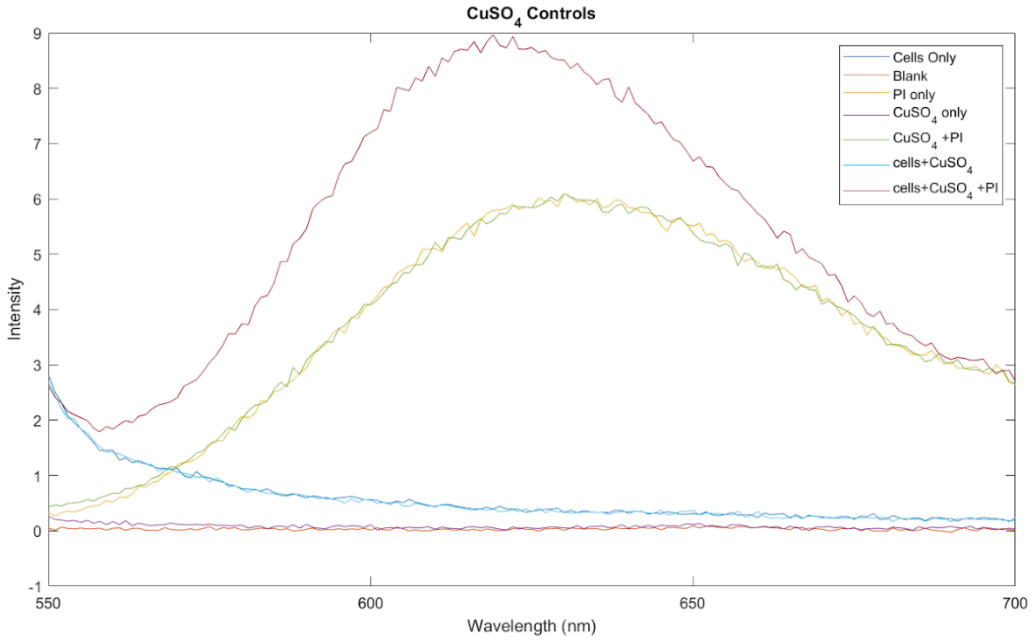

Figure 15: Emission spectra of Propidium Iodide (15 µM) in solutions of V. fischeri cells (OD600 = 0.44, in 1X PBS) after exposure to 10 ppm $CuSO_4$.

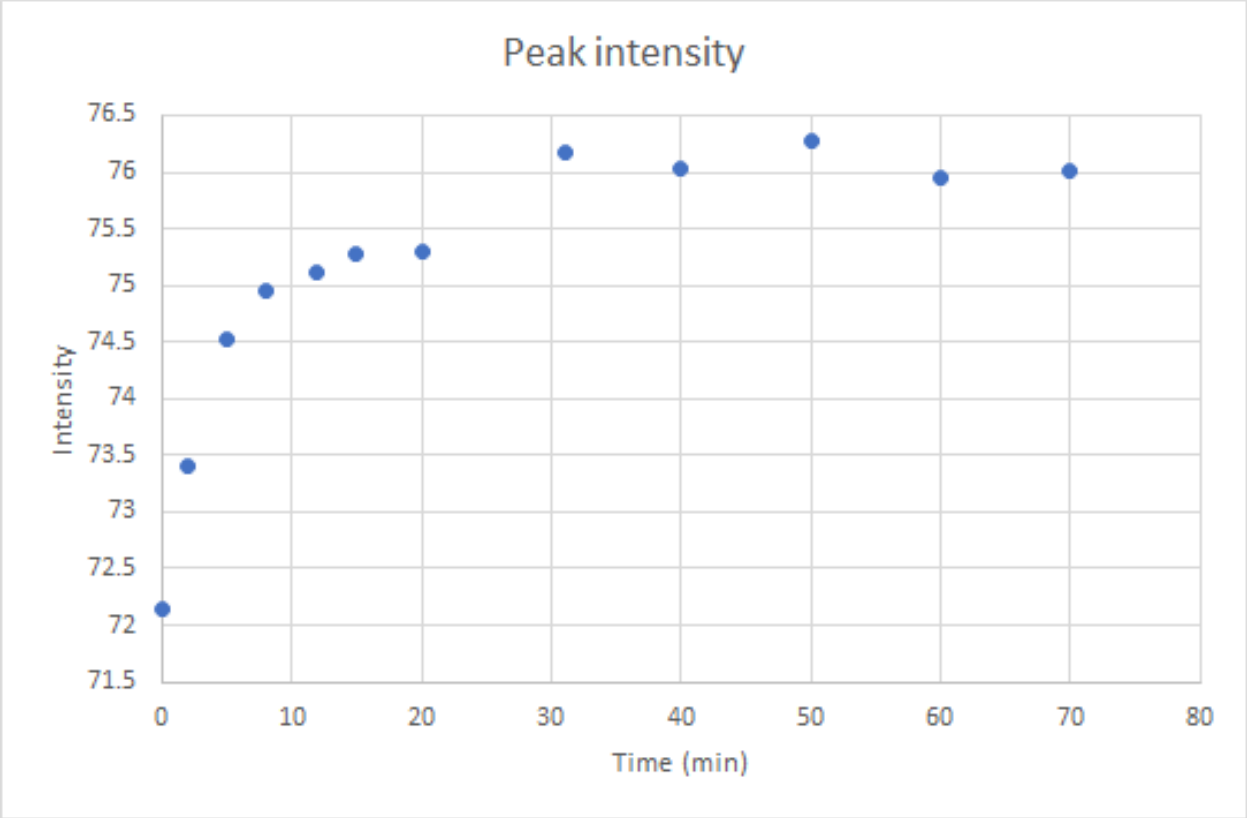

Figure 16: Maximum intensity of Propidium Iodide (15 µM) Signal vs. time after V. fischeri cells (OD600 = 0.44, in 1X PBS) were exposed to 10 ppm (63 µM) $KMnO_4$ for 30 minutes

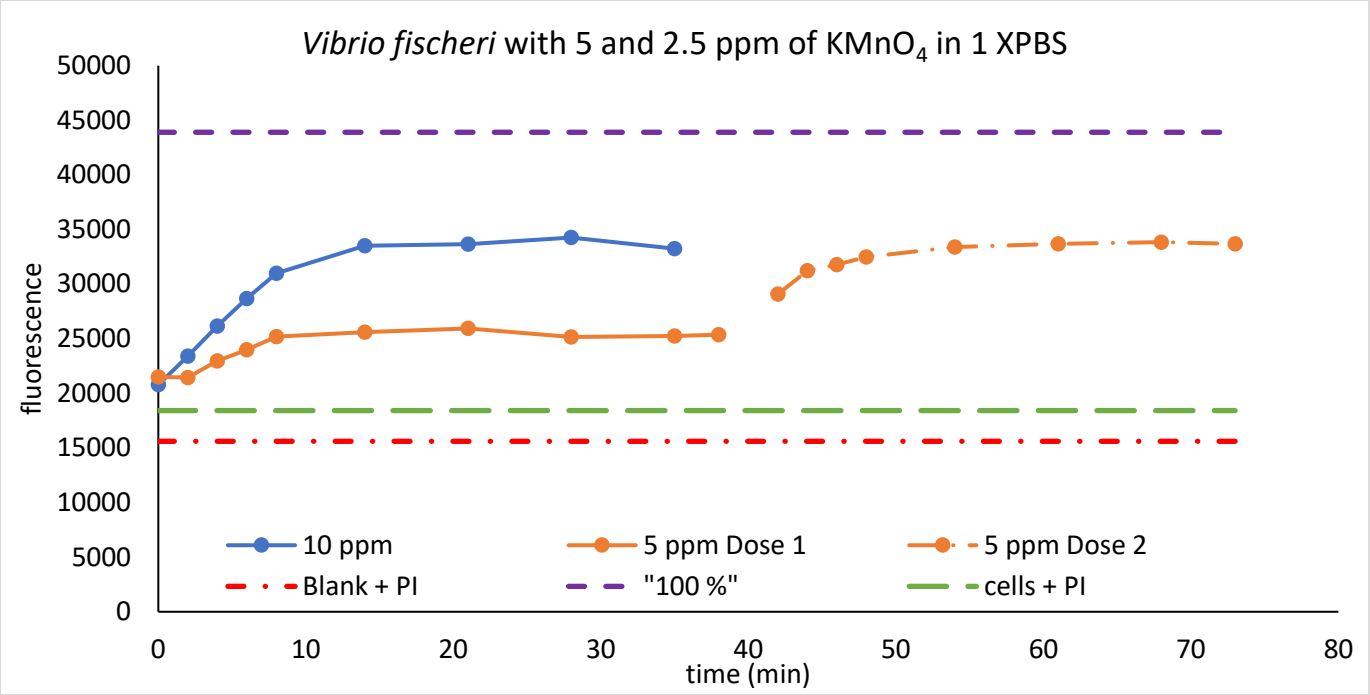

Figure 17: Raw fluorescence data from plate reader. Propidium Iodide (15 uM) signal of V. fischeri cells (OD600 = 0.44, in 1X PBS) after exposure to either 10 ppm (63 µM) or 5 ppm (31.5 µM) of $KMnO_4$. "100%" control represents cells exposed to 83 µM of $KMnO_4$ for 30 mins. "Blank" refers to 1X PBS only.

Appendix B – MATLAB Code

Main Code – MCdsing_new.m

```
clear all
clc

%MC DOSING CODE NEW
%this program will be used to analyze the data using cell structures


%convert excel sheets to cell array using MCxl2cell function
MCD = MCxl2cell('MCdosingSumConc.xlsx', 'MCdosingERRORS.xlsx');


%              exp1          exp2          exp3        ...
%  dose 1    [6x8 array]   [6x8 array]   [6x8 array]
%  dose 2    [6x8 array]   [6x8 array]   [6x8 array]
%  dose 3    [6x8 array]   [6x8 array]   [6x8 array]

%within each 6x8 array
%Col 1= dose #
```

```
%Col 2= time (min)
%Col 3= KMnO4 dose (mM)
%Col 4= [MC-LR] in nM (ppb)
%Col 5= Standard Deviations (errors) in ppb
%Col 6= Dose added time (min)
%(these two rows will be added later in code)
%Col 7= kobs (1/min) (to be added)
%Col 8= "theoretical" [MC-LR] based on kobs (ppb) (to be filled in)

%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% k observed values %%%%%%%%%%%%%%%%

%create placeholder vector matrix for kobs values
[rows, cols] = size(MCD);

kobs_mat = zeros(3, cols);

NumExp = cols ;%number of experiments based on how many columns in the
cell matrix


%% Find k observed of each dose

for d = 1:1:3
    for e = 1:1: NumExp

        t = (MCD{d,e}(:,2))-(MCD{d,e}(1,6)); %col 6 of each cell is
DOSE TIME vector
        %must subtract the
        %so that t_1 = 0
        conc = MCD{d,e}(:,4); %col 4 of each cell is [MC] vector
        MnO4 = MCD{d,e}(1,3); %col 3 of each cell is [MnO4] vector
        %only need the first value of the vector though

        if d ==1
            %if it is the 1st dose, the initial value will be the 1st
[MC]
            %measurement
            MC_int = MCD{d,e}(1,4); %Initial [MC] is the first element
in the [MC] vector
        else
            %Initial [MC] value will be the last measured [MC] value
of the
            %previous dose

            %in some doses, the last [MC] value of the previous dose
is
            %NaN... if thats the case, we need to use NEXT to last
value
            %from previous dose as MC0

            if isnan(MCD{d-1,e}(end,4))
```

```
                MC_int = MCD{d-1,e}(end-1,4)
            else
            MC_int = MCD{d-1,e}(end,4);
            end
        end

        [fitresult,gof] = kobscurvecode(t, conc, MnO4, MC_int);
        kobs_mat(d,e) = coeffvalues(fitresult);
    end %end e loop
end %end d loop
close all


%% assign kobs values
%assign first value in the last column of each cell as the k observed
for
%that dose
for e = 1:1:NumExp
    for d = 1:1:3
        MCD{d,e}(1,7) = kobs_mat(d,e);
    end %end d loop
end %end f loop


%% Calculate "Theoretical Values" of [MC] based off of kobs



for e = 1:1:NumExp
    for d = 1:1:3
        %calc a value at every timepoint
        for k = 1:1:length(MCD{d,e}(:,1))
            %define variables
```

```
            time = (MCD{d,e}(k,2))- (MCD{d,e}(1,6)) ; %col 2 is time
vector
            %must subtract time of dose (from column 6)
            MnO4_0 = MCD{d,e}(1,3); %col 3 is MnO4 dose
            kobs = kobs_mat(d,e); %kobs will be the same for every
timepoint in one dose

                if d ==1
                     %if it is the 1st dose, the initial value will be
the 1st [MC]
                     %measurement
                     MC_0 = MCD{d,e}(1,4); %Initial [MC] is the first
element in the [MC] vector
                else
                    %Initial [MC] value will be the last measured [MC]
value of the
                    %previous dose

                    MC_0 = MCD{d-1,e}(end,4);

                end

             %plug in variables to get theoretical concentration
             MCD{d,e}(k, end) =  TheoMCconc(time, MnO4_0,kobs, MC_0);

        end %end k loop

    end %end d loop

end %end e loop

%% PLOTTING %%%%%%


%%  12 v 6 v 4 uM in SRFA and GLSM
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%12 uM in SRFA
subplot(2,3,1);
AltDosePlot(MCD, 18)
hold on
title('12 uM x 1 Dose in SRFA')

hold off

%6 uM in SRFA
subplot(2,3,2);
AltDosePlot(MCD, 14)
hold on
title('6 uM x 2 Doses in SRFA')
hold off
```

```
%4 uM in SRFA
subplot(2,3,3);
AltDosePlot(MCD, 17)
hold on
title('4 uM x 3 Doses in SRFA')
hold off

%12 uM in GLSM
subplot(2,3,4);
AltDosePlot(MCD, 27)
hold on
title('12 uM x 1 Dose in GLSM')
hold off

%6 uM in GLSM
subplot(2,3,5);
AltDosePlot(MCD, 25)
hold on
title('6 uM x 2 Doses in GLSM')
hold off

%4 uM in GLSM
subplot(2,3,6);
AltDosePlot(MCD, 30)
hold on
title('4 uM x 3 Dose in GLSM')
hold off

%%  6 v 3 v 2 uM in SRFA and GLSM
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%6 uM in SRFA (mannualy plot)

subplot(2,3,1);
t1 = MCD{1,21}(:,2);
MC1 =  MCD{1,21}(:,4);
Th1 =  MCD{1,21}(:,8);
scatter(t1, MC1, 20, 'ko', 'filled');
hold on
%Plot Theoretical Values of dose 1
plot (t1, Th1, 'r');
%errors
errorbar(t1, MC1, MCD{1,21}(:,5) ,'LineStyle', 'none');
legend ('Dose 1')

title('6 uM x 1 Dose in SRFA')

xlabel('Time (min)');
ylabel('[MC-LR] in nM');
xlim([0 120]);
ylim([0 110]);
```

```
kobs1 = strcat('kobs Dose 1 =  ', num2str(MCD{1, 21}(1,7), '%.2f'),
'(1/min)');

 perc_rem = MC1(1)-MC1(end);
    percent = strcat('Percent Removal =  ', num2str(perc_rem, '%.0f'),
'%');
    txt = {kobs1, percent};
    text(50,50, txt)
hold off

%3 uM in SRFA
subplot(2,3,2);
AltDosePlot(MCD, 24)
hold on
title('3 uM x 2 Doses in SRFA')
hold off

%2 uM in SRFA
subplot(2,3,3);
AltDosePlot(MCD, 20)
hold on
title('2 uM x 3 Doses in SRFA')

legend({'Dose 1', 'Dose 2', 'Dose 3'}, 'Location', 'west')
hold off




%6 uM in GLSM (mannualy plot)


subplot(2,3,4);
t1 = MCD{1,25}(:,2);
MC1 =  MCD{1,25}(:,4);
Th1 =  MCD{1,25}(:,8);
scatter(t1, MC1, 20, 'ko', 'filled');
hold on
%Plot Theoretical Values of dose 1
plot (t1, Th1, 'r');
%errors
errorbar(t1, MC1, MCD{1,25}(:,5) ,'LineStyle', 'none');
legend ('Dose 1')

title('6 uM x 1 Dose in GLSM')

xlabel('Time (min)');
ylabel('[MC-LR] in nM');
xlim([0 120]);
ylim([0 110]);
```

```
kobs1 = strcat('kobs Dose 1 =  ', num2str(MCD{1, 25}(1,7), '%.2f'),
'(1/min)');

 perc_rem = MC1(1)-MC1(end);
    percent = strcat('Percent Removal =  ', num2str(perc_rem, '%.0f'),
'%');
    txt = {kobs1, percent};
    text(50,50, txt)
hold off



%3 uM in GLSM
subplot(2,3,5);
AltDosePlot(MCD, 29)
hold on
title('3 uM x 2 Doses in GLSM')
hold off

%2 uM in GLSM
subplot(2,3,6);
AltDosePlot(MCD, 28)
hold on
title('2 uM x 3 Dose in GLSM')
hold off

%%

%12 uM in GLSM
subplot(1,3,1);
AltDosePlot(MCD, 27)
hold on
title('12 uM x 1 Dose in GLSM')
hold off

%6 uM in GLSM
subplot(1,3,2);
AltDosePlot(MCD, 25)
hold on
title('6 uM x 2 Doses in GLSM')
hold off

%4 uM in GLSM
subplot(1,3,3);
AltDosePlot(MCD, 30)
hold on
title('4 uM x 3 Dose in GLSM')
hold off


%% Tables of info %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
%Find average kobs values

%5 mg/L of SRFA %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%initialize parameters

sums = zeros(5,3);
counts = zeros(1,5);

%count the number of each types of doses

for i = [ (14:1:24),36,38];

    if MCD{1,i}(1,3) == 12

         counts(1) = counts(1) +1;

    elseif MCD{1,i}(1,3) == 6

        counts(2) = counts(2) +1;

    elseif MCD{1,i}(1,3) == 4

        counts(3) = counts(3) + 1;

    elseif MCD{1,i}(1,3) ==3

        counts(4) = counts(4) +1;

    else MCD{1,i}(1,3) == 2

        counts(5) = counts(5) +1;

    end
end


%% sum up kobs values of each dose type


for i = [ (14:1:24),36,38];

    for k = [1 2 3]

         if MCD{k,i}(1,3) == 12;

             sums(1,k) = sums(1,k) + MCD{k,i}(1,7);

         elseif MCD{k,i}(1,3) == 6;
```

```
            sums(2,k) = sums(2,k)+ MCD{k,i}(1,7);

        elseif MCD{k,i}(1,3) == 4;

            sums(3,k) = sums(3,k) +MCD{k,i}(1,7);

        elseif MCD{k,i}(1,3) == 3;

            sums(4,k) = sums(4,k) +MCD{k,i}(1,7);

        else MCD{k,i}(1,3) == 2;

            sums(5,k) = sums(5,k) + MCD{k,i}(1,7);

        end
    end
end

%% percent removals

percremov = zeros(1, length([14:1:24,36,38]))
percremovavg = zeros(1,5);

for i = [ (14:1:24),36,38];

    if MCD{1,i}(1,3) == 12

         percremov(i) = MCD{1,i}(1,4)- MCD{1,i}(end,4);
         percremovavg(1) = percremovavg(1) + percremov(i);

    elseif MCD{1,i}(1,3) == 6

        percremov(i) = MCD{1,i}(1,4)- MCD{2,i}(end,4);
        percremovavg(2) = percremovavg(2) + percremov(i);

    elseif MCD{1,i}(1,3) == 4

        percremov(i) = MCD{1,i}(1,4)- MCD{3,i}(end,4);
        percremovavg(3) = percremovavg(3) + percremov(i);

    elseif MCD{1,i}(1,3) ==3

        percremov(i) = MCD{1,i}(1,4)- MCD{2,i}(end,4);
        percremovavg(3) = percremovavg(3) + percremov(i);

    else MCD{1,i}(1,3) == 2

        percremov(i) = MCD{1,i}(1,4)- MCD{3,i}(end,4);
        percremovavg(2) = percremovavg(2) + percremov(i);

    end
end
```

```
%% avg values


kobsavgdose1 = sums(:,1)'./counts;

kobsavgdose2 = sums(:,2)'./counts;

kobsavgdose3 = sums(:,3)'./counts;


SRFAavgKobs = [kobsavgdose1; kobsavgdose2; kobsavgdose1];
percremovavgSRFA = percremovavg./counts;



%% perc remove in GLSM

percremovg = zeros(1, length([25:1:35,37]))
percremovavg = zeros(1,5);

for i = [25:1:35,37];

    if MCD{1,i}(1,3) == 12

         percremovg(i) = MCD{1,i}(1,4)- MCD{1,i}(end,4);
         percremovavg(1) = percremovavg(1) + percremovg(i);

    elseif MCD{1,i}(1,3) == 6

        percremovg(i) = MCD{1,i}(1,4)- MCD{2,i}(end,4);
        percremovavg(2) = percremovavg(2) + percremovg(i);

    elseif MCD{1,i}(1,3) == 4

        percremovg(i) = MCD{1,i}(1,4)- MCD{3,i}(end,4);
        percremovavg(3) = percremovavg(3) + percremovg(i);

    elseif MCD{1,i}(1,3) ==3

        percremovg(i) = MCD{1,i}(1,4)- MCD{2,i}(end,4);
        percremovavg(3) = percremovavg(3) + percremovg(i);

    else MCD{1,i}(1,3) == 2

        percremovg(i) = MCD{1,i}(1,4)- MCD{3,i}(end,4);
        percremovavg(2) = percremovavg(2) + percremovg(i);

    end
end
```

Determining DOM competition rate constant – kobscurvecode.m

```
function [fitresult, gof] = createFit(time, MC_conc, MnO4_0, MC_int)
%CREATEFIT(TIME,MC_CONC)
%  Create a fit.
%
%  Data for 'kobz' fit:
%      X Input : time
%      Y Output: MC_conc
%  Output:
%      fitresult : a fit object representing the fit.
%      gof : structure with goodness-of fit info.
%
%  See also FIT, CFIT, SFIT.

%  Auto-generated by MATLAB on 04-Mar-2020 12:11:56


%% Fit: 'kobz'.
[xData, yData] = prepareCurveData( time, MC_conc);



%convert from number to string

MC_int = num2str(MC_int);

%Convert MnO4 dose to string

MnO4_0 = num2str(MnO4_0);

%Concatinate input variables into fitted equation

% Set up fittype and options.
ft = fittype( strcat(MC_int,'*exp(-272*60*1e-6*',MnO4_0,'*((exp(-
kobs*x)/-kobs)+(1/kobs))')','independent', 'x', 'dependent', 'y' );
opts = fitoptions( 'Method', 'NonlinearLeastSquares' );
opts.Algorithm = 'Levenberg-Marquardt';
opts.Display = 'Off';
opts.StartPoint = 1e-5;

% Fit model to data.
[fitresult, gof] = fit( xData, yData, ft, opts );
```

```
% Plot fit with data.
figure( 'Name', 'kobz' );
h = plot( fitresult, xData, yData );
legend( h, 'MC_conc vs. time', 'kobz', 'Location', 'NorthEast' );
% Label axes
xlabel time
ylabel MC_conc
grid on
```

Theoretical MCLR concentration Calculations – TheoMCconc.m

```
function [theo_val] = TheoMCconc(time, MnO4_0,kobs, MC_0)

%function that finds the theoretical MC_t in the competition model
based on
%time (min), MnO4- conc (M), the K_obs(1/min), and the initial MC conc
for
%that dose (M)

theo_val = MC_0*exp(-272*60*MnO4_0*1e-6*((exp(-kobs*time)/-
kobs)+(1/kobs)));
```

Load in Data from excel – MCx12cell.m

```
function [NewCell] = MCxl2cell(MCdatasheet, MCerrsheet)

% This function will combine the two excel sheets used in MCdosing.m
into a
% cell array similar to the one used in AltDosingMC.m
%enter the names of the excel sheets as strings
%MCdatasheet = excel sheet w MC concentration values
%MCerrsheet = excel sheet w stdev data for error bars


%% read sheet and form arrays
%Read main data sheet
MC_d = readtable(MCdatasheet);

%read error data sheet
MC_e = readtable(MCerrsheet);

%convert both tables to arrays
MC_d = table2array(MC_d);
MC_e = table2array(MC_e);


%first column of MAIN data array is time vector, exclude this vector
time = MC_d((2:1:end),1);
```

```
MC_d = MC_d(:, 2:1:end);

%First row of the MAIN data array contains the [pmgnt] vector
%extract the row and then exclude it

pmgnt = MC_d(1, :);

MC_d = MC_d(2:1:end, :);


%% Create cell array

%create a placeholder array of zeros that will later be partitioned
into
%cells

[row, col] = size(MC_d);

%each experiment will contain 8 columns of data
%Col 1= dose #
%Col 2= time (min)
%Col 3= KMnO4 dose (uM)
%Col 4= [MC-LR] in nM (ppb)
%Col 5= Standard Deviations (errors) in ppb
%Col 6= Dose added time (min)
%(these two rows will be added later in code)
%Col 7= kobs (1/min)
%Col 8= "theoretical" [MC-LR] based on kobs (ppb)

NewCell = zeros(row, col*8);

%% Place data in array

%first create dose # vector

one = ones(1,6);
two = 2*one;
three = 3*one;
dosenum = [one, two, three];

%use another index that will multiply by k after every iteration
%initialize h
h = 1;

for k = 1:1:col

    %input dose number
    NewCell(:, h) = dosenum' ;
    %time vector (min)
    NewCell(:, h+1) = time' ;
    %KMnO4 dose (uM)
```

```
    NewCell(:, h+2) = pmgnt(k)*ones(1,row)'; %pmgnt will be the same
in each element of the vector
    %MC-LR data in nM
    NewCell(:,h+3) = MC_d(:, k); %k is the "exp number"
    %stdev data
    NewCell(:, h+4) = MC_e(:, k);
    %dose added time
    NewCell(:,h+5) = [zeros(1,6), 40*ones(1,6), 80*ones(1,6)]'; %for
these experiments, all of the doses will have been
    %added at the same times

    %seventh and eight rows in this iteration are intentionally left
blank

    %add 8 to h to go to next set of rows

    h = h+8;

end



%% partition into cells

%separate vertically into 3 groups of 6 rows each
%separate horizontally into "col" groups of 8 columns each
NewCell = mat2cell(NewCell, [6 6 6], [(8*ones(1,col))]);


%% Convert non-existant doses to zero vectors
%12 uM exps only have 1 dose
%6 and 3 uM exps only have 2 doses
%4 and 2 uM exps have all three doses
%converting to zero vectors is makes using plotting functions easier

[row, col] = size(NewCell);

for i = 1:1: row
    for j = 1:1: col

        if NewCell{i,j}(1,4) == 0

            NewCell{i,j} = zeros(1,8);

        end
    end
end
```

```
end
```

Plot Experiments – AltDosePlot.m

```
function AltDosePlot (Cell_array, ExpN)
%Plotting function to plot a single experiment from the "Alternate
Dose
%Timing" experiments
%The input cell array "Cell_array" will always have 3 rows of cell, 1
for
%each dose
%ExpN = Experiment #, referencing particular column of the cell array

%Each cell within the array will have 7 columns and various row
numbers

%Col 1= dose #
%Col 2= time (min)
%Col 3= KMnO4 dose (mM)
%Col 4= [MC-LR] in nM (ppb)
%Col 5= Standard Deviations (errors) in ppb
%Col 6 = Dose time (min)
%Col 7 = kobs (1/min)
%Col 8 = "theoretical" [MC-LR] based on kobs (ppb)

%% Assign Variables for each dose

%Dose 1

D1 = Cell_array{1,ExpN};

t1= D1(:, 2)'; %time vector
p1 = D1(1,3); %KMnO4 dose
MC1 = D1(:, 4)'; %[MC-LR] vector
Er1 = D1(:, 5)'; %errors vector
Th1 = D1 (:, 8)'; %Theoretical [MC] vector

%Dose 2

D2 = Cell_array{2,ExpN};

t2= D2(:, 2)'; %time vector
p2 = D2(1,3); %KMnO4 dose
MC2 = D2(:, 4)'; %[MC-LR] vector
Er2 = D2(:, 5)'; %errors vector
Th2 = D2 (:, 8)'; %Theoretical [MC] vector

%Dose 3

D3 = Cell_array{3,ExpN};
```

```
t3= D3(:, 2)'; %time vector
p3 = D3(1,3); %KMnO4 dose
MC3 = D3(:, 4)'; %[MC-LR] vector
Er3 = D3(:, 5)'; %errors vector
Th3 = D3 (:, 8)'; %Theoretical [MC] vector

%% what buffer?

if ExpN<= 6
    buffname = ' in SRFA';
else
    buffname = ' in GLSM';

end

%% Compostite vectors based on number of non-zero doses in the exp

if D2(1,1) == 0
    %both doses 2 and 3 dont exist
    time = [t1];
    MC_conc = [MC1];
    Errors = [Er1];

elseif D3 == 0
    %Dose 3 is a zero dose and doesnt exist
    time = [t1 t2];
    MC_conc = [MC1 MC2];
    Errors = [Er1 Er2];

    else
    %There is a third dose
    time = [t1 t2 t3];
    MC_conc = [MC1 MC2 MC3];
    Errors = [Er1 Er2 Er3];

end


%% Plots

%Plot raw data of first dose
scatter(t1, MC1, 20, 'ko', 'filled');
hold on


if D3(1,1) ~= 0

    %Plot raw data of second dose
    scatter(t2, MC2, 20, 'k^', 'filled');

    %Plot raw data of third dose
```

```
    scatter(t3, MC3, 20, 'ks', 'filled');

    %Plot Theoretical Values of dose 1
    plot (t1, Th1, 'r');

    %Plot Theoretical Values dose 2
    plot ([D2(1,6),t2], [TheoMCconc(0,p2,D2(1,7), D1(end, 4)),Th2],
'g', 'LineWidth', 2);


    %Plot Theoretical Values dose 3
    plot ([D3(1,6),t3], [TheoMCconc(0,p2,D3(1,7), D2(end, 4)),Th3],
'c', 'LineWidth', 2);

elseif D2(1,1) ~= 0
    %Plot raw data of second dose
    scatter(t2, MC2, 20, 'k^', 'filled');

    %Plot Theoretical Values of dose 1
    plot (t1, Th1, 'r');

    %Plot Theoretical Values of dose 3
    plot ([D2(1,6),t2], [TheoMCconc(0,p2,D2(1,7), D1(end, 4)),Th2],
'g', 'LineWidth', 2);

else
    %Plot Theoretical Values of dose 1
    plot (t1, Th1, 'r');

end

%Add error bars to each dose
errorbar(time, MC_conc, Errors ,'LineStyle', 'none');




%Determine if 3rd dose plot is needed
if D3(1,1) ~= 0

    legend ('Dose 1', 'Dose 2', 'Dose 3')
    %title
    title (strcat(num2str(D1(1,3)), strcat('uM KMnO4 x 3 doses',
buffname)))
elseif D2(1,1) ~=0
  legend ('Dose 1', 'Dose 2')
  title (strcat(num2str(D1(1,3)), 'uM KMnO4 x 2 doses', buffname))

else
    title (strcat(num2str(D1(1,3)), 'uM KMnO4 x 1 dose', buffname))

end
```

```
%Axes + Axes Labels

xlabel('Time (min)');
ylabel('[MC-LR] in nM');
xlim([0 120]);
ylim([0 110]);

%% additional info

kobs1 = strcat('k_{DOM} Dose 1 =  ', num2str(Cell_array{1, ExpN}(1,7),
'%.2f'), '(1/min)');
kobs2 = strcat('k_{DOM} Dose 2 =  ', num2str(Cell_array{2, ExpN}(1,7),
'%.2f'), '(1/min)');
kobs3 = strcat('k_{DOM} Dose 3 =  ', num2str(Cell_array{3, ExpN}(1,7),
'%.2f'), '(1/min)');

if D3(1,1) ~= 0

    perc_rem = MC1(1)-MC3(end);
    percent = strcat('Percent Removal =  ', num2str(perc_rem, '%.0f'),
'%');
    txt = {kobs1, kobs2, kobs3, percent};

    text(80,65, txt)

elseif D2(1,1) ~= 0

    perc_rem = MC1(1)-MC2(end);
    percent = strcat('Percent Removal =  ', num2str(perc_rem, '%.0f'),
'%');
    txt = {kobs1, kobs2, percent};

    text(60, 50, txt)


else

    perc_rem = MC1(1)-MC1(end);
    percent = strcat('Percent Removal =  ', num2str(perc_rem, '%.0f'),
'%');
    txt = {kobs1, percent};
    text(50,20, txt)


end


hold off
end
```