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## Photocatalytic Nanoparticle Treatment of Cyanotoxins in Harmful Algal Blooms

## **An Undergraduate Honors College Thesis**

in the

**Department of Civil Engineering** 

**College of Engineering** 

**University of Arkansas** 

Fayetteville, AR

By

**Brian Wray Baird** 

#### **Abstract**

Harmful Algal Blooms (HAB) have the ability to cause detriment to water bodies, ecosystems, and human health. Furthermore, current evidence suggests that these events are becoming increasingly common due to factors such as increased pollution and global warming. While there are current methods to treat water that is negatively impacted by the bacteria and toxins associated with HABs, these methods are often costly and inefficient. The purpose of this research is to explore a potential treatment option that uses titanium dioxide photocatalytic nanoparticles attached to a retractable net to treat toxins produced by HABs in situ. Specifically, the toxin of interest for these tests is microcystin-LR (MC-LR). While prior research has provided a promising basis for the proposed treatment in sterile conditions, there has been limited research conducted on how the nanoparticles will react in more realistic settings. The goal of this research is to build on the prior research and test the proposed treatment in real lake water samples. Three experiments were designed to achieve this goal. The goal of the first experiment was to observe the effect the nanoparticles have on MC-LR in real lake water samples. The second experiment was to test the role of suspended particles in lake water in MC-LR degradation. The third experiment was to verify past research and to provide a benchmark for MC-LR degradation comparison. Results showed TiO2 nanoparticles could degrade MC-LR in DI water but was not effective in real lake water. Removal of suspended particles by filtration in lake water could enable the MC-LR degradation by nanoparticles, but at a much slower rate compared to DI water. The results of these tests can then be used to determine what the best course of action is for future research on this proposed treatment.

#### **Background**

One issue facing the field of modern water quality and treatment is the increasing occurrence of Harmful Algal Blooms (HAB). Harmful Algal Blooms occur when large groups of certain cyanobacteria congregate in bodies of water. This can be caused by several things including nutrient pollution, the alteration of food webs due to overfishing, eutrophication due to human intervention, climate change, and many other factors - most of which have been drastically increasing in the past few decades (Heisler, 2008). There are many different types of HABs ranging in size, species, and location, but they all have the potential to have drastic consequences. Not only do the bacteria themselves have damaging effects on the environment such as causing many water quality issues, but many cyanobacteria also produce cyanotoxins (Duan, 2017). These toxins have many negative effects on ecosystems and can cause many adverse health issues in humans such as gastroenteritis, kidney and intestine damage, risks of cancer, and even death (Bláha, 2009). Because HABs have such detrimental effects on both humans and the environment, it is a key goal of scientists and environmental engineers to develop methods to treat them.

Current treatments of HABs include many methods such as filtration, flocculation, coagulation, and sedimentation. These methods, while helpful, often have many negative attributes. Most require the complete removal of the bloom in order to treat the HAB, which is not cost effective and requires a waste disposal plan (Meglič, 2017). There are some *in situ* treatments such as ultrasonication, bacterial agents, or chemical agents, but they often have short-lived results or lead to chemical residual accumulation in the ecosystem (Yang, 2018). Within HABs, cyanotoxin removal has been the most challenging due to cyanotoxin's potent toxicity, low concentration, and high resistance to treatment. One particular cyanotoxin of interest is microcystin-LR (MC-LR). MC-LR is the most toxic microcystin and has been shown

to cause many problems in both humans and the environment when high exposure occurs. It is also one of the toxins most commonly associated with HABs. Because of its strong link to HABs and its toxicity levels, the removal of MC-LR specifically is the focus of a lot of current research.

The overall goal of this research is to develop a treatment method for cyanotoxins produced in HABs by using photocatalytic nanoparticles. The proposed treatment is built on the idea of using photocatalytic titanium dioxide nanoparticles to degrade MC-LR *in situ*. The degradation mainly occurs by breaking down the Adda amino acid and ring structure of MC-LR through hydroxyl radical oxidation. Generally, there are six proposed pathways by which this degradation occurs (Ivandic, 2022). Because suspended nanoparticles can have damaging effects such as creating harmful residuals in the water bodies they are in, the nanoparticles are also attached to a retractable net. There has already been significant experimentation done to prove the concept of this method (Ivandic, 2022). As shown in the article from the Journal of Environmental Chemical Engineering, the TiO<sub>2</sub> nanoparticle treatment has proven to be effective at treating MC-LR in deionized water either in suspended or attached forms. The level of UV exposure also greatly affected the removal of toxins in these controlled experiments. Preliminary results, however, showed that constituents in real lake water could negatively impact the efficiency of TiO<sub>2</sub> treatment of MC-LR (unpublished data).

As a result, the objective of this study is to determine the effectiveness of the TiO<sub>2</sub> treatment in MC-LR removal from lake water. Lake water contains various constituents including natural organic matter, nutrients (nitrogen and phosphorus), and microorganisms. It is important to identify key components found in lake water that may potentially affect the treatment and to conduct further research to elucidate these interactions. One constituent that might interfere with the nanoparticle treatment is the presence of suspended solids. Suspended

solids is a term that encompasses a large group of constituents within water such as soil, biological solids, or decaying organic matter. These compounds can originate from runoff, biological growths, algae, pollution, or many other sources. Suspended solids sources can occur naturally in the aquatic system, or they can be transported into the body of water from runoff. An elevated level of suspended solids in water has the potential to greatly decrease the effectiveness of treatment and disinfection (Narkis, 1995). Suspended solids are of special concern for the HAB treatment because the algae causing the production of the toxin itself is classified as a suspended solid. Furthermore, the bodies of water in which HAB typically occur such as lakes are susceptible to high levels of suspended solids. Suspended solids also have the potential to block light from reaching the photocatalytic nanoparticles and thus reduce the effectiveness of the nanoparticles. It is hypothesized that the suspended solids will interfere with the reaction between TiO<sub>2</sub> nanoparticles and the toxin thus reducing the efficiency of MC-LR degradation.

#### **Significance of Research**

The development of an effective *in situ* treatment method for HABs that handles both the toxins and bacteria produced in these events is significant for several reasons. Firstly, as mentioned above, HABs have adverse effects on aquatic systems, the environment, and humans. Furthermore, these events are slowly increasing in frequency due to factors such as climate change (O'Neil, 2012). Current treatment methods exist, but they are often costly and either have negative impacts on other organisms in the water system or require complete removal of the affected water, treatment, and then reentry into the system. The proposed photocatalytic nanoparticle treatment, however, would be a feasible solution to address cyanotoxin issues at the source. The objectives and experiments listed below are significant building blocks in the larger plan to reach the goal to develop this treatment.

#### **Objectives**

The main objectives of this research are to:

- 1. Assess the effectiveness of TiO<sub>2</sub> nanoparticle treatment of MC-LR in real lake water samples. This is significant because it provides a foundation for determining the real-world application of the proposed treatment.
- 2. Observe what effect the filtration of lake water has on the TiO<sub>2</sub> treatment of MC-LR. This is significant because it begins the process of determining what, if any, components in real samples will hinder the application of the treatment.
- 3. Determine the effectiveness of the TiO<sub>2</sub> treatment of MC-LR in deionized water. This is significant because it verifies past research and provides a benchmark for other tests.

#### **Materials and Methods**

Through collaboration with the Arkansas Water Resources Center, lake water was collected from Lake Fayetteville in Fayetteville, Arkansas. 400 milliliters of lake water were obtained using an alpha water sampler submerged three inches below the water surface and stored in 200 milliliter HDPE bottles on ice. Upon arrival at the laboratory, the samples were refrigerated at 4°C.

Solid film of MC-LR was purchased from Enzo Life Sciences (Farmingdale, NY) and dissolved in DI water to make stock solutions at 10 milligrams per liter. The MC-LR was further diluted in the lake water experiment from the stock solution. Aeroxide titanium dioxide P90 nanoparticles were obtained from Evonik Corporation (Piscataway, NJ) and suspended in DI water to achieve a concentration at 1 gram per liter.

#### **Experiment One:**

This experiment addresses the objective to assess the effectiveness of the TiO<sub>2</sub> nanoparticle treatment in real lake water. To obtain a test sample that was most representative of the lake, the test sample was taken from a mixture of multiple alpha sampler samples. For this round of testing, four 150 milliliter Erlenmeyer flasks were used – two control flasks and two flasks with the TiO<sub>2</sub> nanoparticles. In order to simulate an increased level of cyanotoxin in the sample, MC-LR stock solution was used to spike the lake water until the desired concentration of 200 parts per billion was met for the experiment in each flask. It was also determined in prior experimentation that the optimal concentration of nanoparticles for treatment is 0.25 grams per liter. This condition was met in the two flasks with nanoparticles. Because of this, the following amounts of solution were used in the experiment one:

- Control (Flasks 1 and 2):
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - 49 milliliters of lake water
- Flasks with TiO<sub>2</sub> nanoparticles (Flasks 3 and 4):
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - o 12.5 milliliters of 1 gram per liter TiO<sub>2</sub> nanoparticle solution
  - o 36.5 milliliters of lake water

After the addition of the nanoparticles, all flasks were stirred by hand for twenty seconds, and initial 1.5 milliliter samples were taken. The flasks were then placed on a low-speed orbital shaker. Due to the fact that prior experimentation had proven the necessity of UV light for the photocatalytic nanoparticles to be effective, two UV lights were then placed on either side of the

flasks for the duration of the experiment. Once the flasks were exposed to UV light, a timer was started to track samples. Samples were taken from all four flasks at times of 20, 40, 60, 90, and 120 minutes. Once two hours had passed, all twenty-four samples were centrifuged at a speed of 7,800 rotations per minute for twenty minutes. The liquid portion of the samples were then transferred to separate vials and delivered to University of Arkansas mass spectrometry lab for MC-LR analysis.

#### **Experiment Two:**

The purpose of this experiment is to begin the process of identifying what constituents in real lake water samples interfere with the reactions of MC-LR and the titanium dioxide nanoparticles. Specifically, this experiment focused on the effect the removal of suspended solids from the lake water samples had on the treatment. The experimental setup was similar to experiment one. To begin, mixed water samples from the same sampling trip were filtered using 25-millimeter PES membrane filters with a pore size of 0.2 microns attached to a syringe. This step removed suspended particles with sizes larger than 0.2 microns such as sediment, particles, and microorganisms from the raw lake water sample. After filtration, four 150 milliliter Erlenmeyer flasks were prepared for testing - two for control tests containing just MC-LR and filtered lake water, and two containing MC-LR, filtered lake water, and titanium dioxide nanoparticles. Similarly to experiment one, the samples were spiked with toxin to reach 200 parts per billion to represent elevated levels of MC-LR associated with algal blooms. Furthermore, the condition of 0.25 grams per liter of TiO<sub>2</sub> nanoparticles was met in the relative test flasks as well to optimize treatment similarly to experiment one. To meet these conditions, the following amounts of each solution were added to the flasks for experiment two:

- Control (Flasks 1 and 2)
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - o 49 milliliters of filtered lake water
- Flasks with TiO<sub>2</sub> nanoparticles (Flasks 3 and 4):
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - 12.5 milliliters of 1 gram per liter TiO<sub>2</sub> nanoparticle solution
  - o 36.5 milliliters of filtered lake water

After the test solutions were formed, all four flasks were mixed for twenty seconds by hand. A 1.5 milliliter sample was then taken from each flask to indicate an initial MC-LR concentration for each flask. The solutions were then placed on a low-speed orbital shaker and exposed to direct UV light from two directions. A timer was started, and additional samples were taken from the four flasks at times of 20, 40, 60, 90, and 120 minutes. After the 120-minute mark, all twenty-four 1.5 milliliter samples were centrifuged at a speed of 7,800 rotations per minute for twenty minutes to separate the nanoparticles from the liquid portion of the samples. The liquid portion of the samples were then transferred to separate vials and delivered to University of Arkansas mass spectrometry lab for MC-LR analysis.

#### **Experiment Three**

The goal of experiment three is to verify the effectiveness of the titanium dioxide nanoparticle treatment in DI water samples. The process for this experiment is closely related to experiments one and two. Four 150 milliliter Erlenmeyer flasks were prepared for testing - two control flasks, and two flasks that would include TiO<sub>2</sub> nanoparticles. Deionized water was added to each flask, and then nanoparticles were added to the test flasks. The flasks were then spiked

with MC-LR to reach an elevated concentration of the toxin. The final concentrations of the flasks were 0.25 grams per liter of titanium dioxide in the test flasks and 200 parts per billion MC-LR in every flask. To achieve these values, the following volumes of solutions were used:

- Control (Flasks 1 and 2)
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - o 49 milliliters of deionized water
- Flasks with TiO<sub>2</sub> nanoparticles (Flasks 3 and 4):
  - o 1 milliliter of 10 milligram per liter MC-LR solution
  - o 12.5 milliliters of 1 gram per liter TiO<sub>2</sub> nanoparticle solution
  - o 36.5 milliliters of deionized water

After the MC-LR was added to all flasks, the flasks were mixed by hand for twenty seconds and an initial 1.5 milliliter sample was taken to determine the initial conditions. After sampling, all flasks were placed on a low-speed orbital shaker and exposed to direct UV light. A timer was started, and further sampling occurred at 20, 40, 60, 90, and 120 minutes. All twenty-four samples were then centrifuged at a speed of 7800 rotations per minute for 20 minutes to remove the nanoparticles. The liquid portion of the samples were then transferred to separate vials and delivered to University of Arkansas mass spectrometry lab for MC-LR analysis.

#### **Data Analysis**

Once LC/MS/MS data was returned from the University of Arkansas mass spectrometry lab, thorough analysis of the data was conducted. This included a t-test for every experiment conducted using Excel. Because duplicate flasks were used for each type of sample in these

experiments, the inputs for the t-test were the average of the duplicate flasks. The tests were conducted using ten degrees of freedom and a significance level of five percent to determine whether a significant difference in degradation of MC-LR occurred under the experiment's conditions or not.

#### **Results and Discussion:**

#### **Experiment One**

The results from the LC/MS/MS analysis for experiment one can be found in Figure 1 and Figure 2 below. Figure 1 shows the graphical trends of the data, and Figure 2 depicts the percent change for the concentration of MC-LR for each test flask. Table 1 also displays the results of the t-test for experiment one.

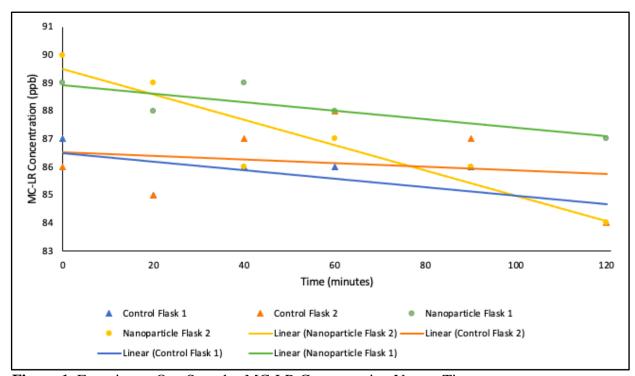


Figure 1. Experiment One Samples MC-LR Concentration Versus Time

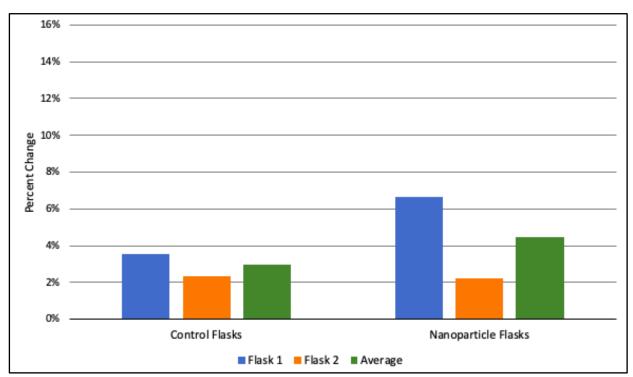


Figure 2. Experiment One Percent Change of MC-LR Over Experiment Duration

**Table 1.** Results of t-Test for Experiment One.

	Control	Nanoparticle
Mean	86.16666667	87.41666667
Variance	2.166666667	2.241666667
Observations	6	6
Pooled Variance	2.204166667	
Hypothesized Mean Difference	0	
df	10	
t Stat	-1.458305203	
P(T<=t) one-tail	0.087718572	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.175437145	
t Critical two-tail	2.228138852	

As seen in Figure 1 and 2, all four test flasks followed the same general trend of having minimal degradation over time. The average percent change in the toxin for the control flasks and flasks with the nanoparticles were roughly 3 percent and 4 percent, respectively. While there is a slight increase in degradation of MC-LR with the addition of TiO<sub>2</sub> nanoparticles, this

increase is not significant. This is supported by the p-value in Figure 1 being well above the value of 0.05. This falls in line with the expected results. It was hypothesized that while the TiO<sub>2</sub> nanoparticles were preliminarily shown to reduce MC-LR in deionized water samples, there are many constituents in lake water that would block this process by reacting with the nanoparticles before the toxin could. The results of experiment one seem to support this hypothesis because there is no significant difference between the trendlines for the control flasks and the flasks with the presence of the nanoparticles.

The MC-LR concentrations at time zero also necessitate further analysis. As mentioned in the materials and methods section, the target starting concentration was 200 parts per billion. The maximum starting concentration was observed to be only 90 parts per billion, though.

Because all of the starting values are roughly equivalent, it is likely that there was a systematic error that occurred during experiment one. There are two probable causes that may have had this impact. Firstly, it is possible that the original stock solution and standard of MC-LR had degraded below the assumed value of 10 milligrams per liter. If this is true, then the amount of toxin in the flasks would have been significantly lower from the start of the experiment, leading to the results that were obtained. It is also possible that during the centrifugation process a certain volume of the toxin could have been adsorbed to the surface of the centrifuge tubes. This adsorption of MC-LR would yield the lower concentrations in the final samples that are seen. Experiment three will explore this further.

Altogether, the results of experiment one support the hypothesis that there are constituents in lake water that hinder the use of TiO<sub>2</sub> nanoparticles for treatment of harmful algal blooms. The next step in this research is to conduct experiments to identify specific components that affect the treatment and then to explain why they have the observed effect. This is precisely what was done in experiment two.

## **Experiment Two:**

The results from the LC/MS/MS analysis for experiment two can be found in Figure 3 and Figure 4 below. Figure 3 shows the graphical trends of the data, and Figure 4 depicts the percent change for the concentration of MC-LR for each test flask. Table 2 displays the results of the t-test for the second experiment.

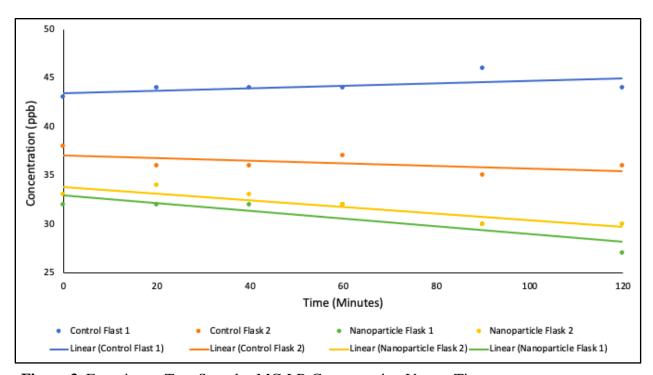


Figure 3. Experiment Two Samples MC-LR Concentration Versus Time

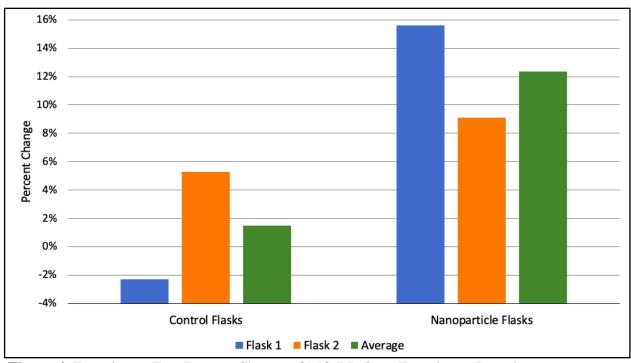


Figure 4. Experiment Two Percent Change of MC-LR Over Experiment Duration

**Table 2.** Results of t-Test for Experiment Two

	Control	Nanoparticle
Mean	40.25	31.41666667
Variance	0.075	3.141666667
Observations	6	6
Pooled Variance	1.608333333	
Hypothesized Mean Difference	0	
df	10	
t Stat	12.06416352	
P(T<=t) one-tail	1.38923E-07	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	2.77847E-07	
t Critical two-tail	2.228138852	

There are many observations that can be made from Figure 3 and 4. As seen in Figure 3, there appears to be a significant difference in the degradation of MC-LR between the control flasks and nanoparticle flasks when the experiment is conducted with filtered lake water. The control flasks experience minimal degradation of the toxin (similar to control flasks in experiment 1), while the samples with the TiO<sub>2</sub> treatment show larger amounts of toxin removed.

This is made more evident in Figure 4. The control flasks experienced an average percent change of MC-LR of roughly 1 percent, while the nanoparticle flasks had an average change of roughly 12 percent. This is a significant difference that supports the idea that there are constituents in natural lake water that hinder the reaction between the TiO<sub>2</sub> nanoparticles and MC-LR. The p-value shown in Table 2 supports the claim that there is a significant difference between the two types of flasks as well. The data also indicates that whatever constituents are interfering with the treatment are at least partially filtered out by the 0.2-micron syringe filter. Potential causes could thus be any of the constituents listed above such as DOM or microorganisms, but further tests would need to be conducted to specify constituents confidently.

Once again, it is important to note that the starting concentrations of MC-LR are well below the targeted value of 200 parts per billion. The hypothesized potential causes are the same as in experiment one. Experiment three will help indicate what potentially is causing this large undershoot in initial toxin concentrations.

#### **Experiment Three:**

The results from the LC/MS/MS analysis for experiment three can be found in Figure 5 and Figure 6 below. Figure 5 shows the graphical trends of the data, and Figure 6 depicts the percent change for the concentration of MC-LR for each test flask. Table 3 displays the results of the t-test for experiment three.

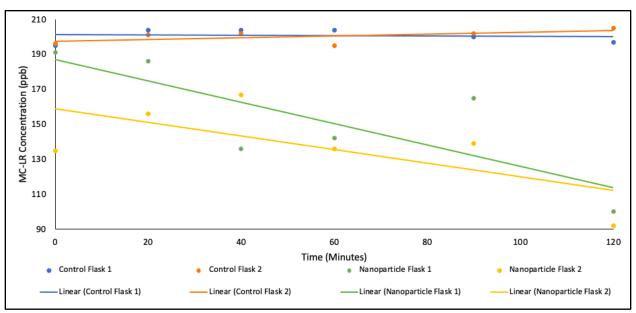


Figure 5. Experiment Three Samples MC-LR Concentration Versus Time

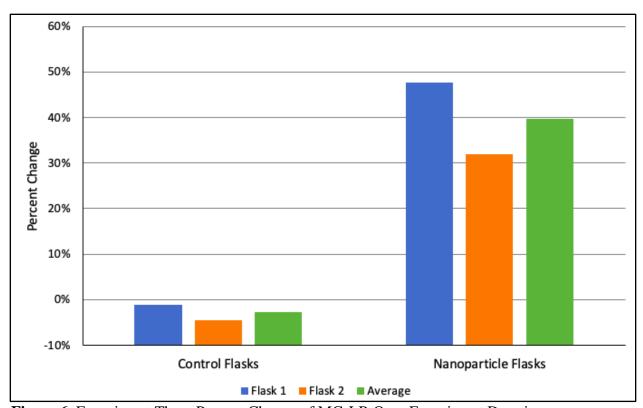


Figure 6. Experiment Three Percent Change of MC-LR Over Experiment Duration

**Table 3.** Results of t-Test for Experiment Three

	Control	Nanoparticle
Mean	200.4166667	145.4166667
Variance	7.341666667	705.4416667
Observations	6	6
Pooled Variance	356.3916667	
Hypothesized Mean Difference	0	
df	10	
t Stat	5.046142918	
P(T<=t) one-tail	0.00025104	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.00050208	
t Critical two-tail	2.228138852	

The results of experiment three yield very helpful conclusions. Firstly, as shown in Figure 5 and Figure 6, there is a much larger trend of MC-LR degradation in the flasks including nanoparticles compared to the flasks with just the toxin and DI water. The average percent change of MC-LR in the control flasks is calculated to be about negative three percent. This is clearly impossible, but it leads to the conclusion that no degradation occurred. The flasks with nanoparticles, however, had an average percent change of forty percent. This is by far the largest amount of degradation seen amongst all three of the experiments conducted. This difference is confirmed as significant by the p-values shown in Table 3. These results confirm the results of prior testing and support the claim that TiO<sub>2</sub> nanoparticles effectively break down MC-LR in DI water samples. Furthermore, because the amount of degradation in experiment three is so much greater than the amount of degradation seen in experiment two with filtered lake water, it is reasonable to conclude that there are some constituents in filtered lake water that still interfere with the treatment. These could potentially be dissolved organic matter (DOM), chemical compounds, or several other constituents. Further tests should be conducted to determine what specific components of filtered lake water may be responsible for this observed difference.

The initial MC-LR concentrations are much closer to the target value of 200 parts per billion for experiment three. The centrifugation process was identical to the first two experiments, so it is unlikely that the observed undershoot of concentration in prior experiments was due to adsorption to the centrifuge tubes. One important difference in experiment three was a new MC-LR standard was used to obtain LC/MS/MS data. Because this value yielded results closer to the expected value, it is likely that there was an issue with the first standard. This is most likely due to degradation of MC-LR in the first standard.

#### **Future Research**

The results of these three experiments are a small part of a much larger project. Because of the results of experiments one and two specifically, it is clear that more experiments need to be done to determine what suspended solids are specifically hindering the nanoparticle treatment. This can be done in a similar method to these three experiments but with DI water spiked with specific suspended solids such as soil particles or algae. Another step in this research process is to observe the effect the nanoparticles will have on MC-LR degradation in lake water when attached to portions of the retractable net. Due to the fact that the MC-LR standard for experiments one and two was more than likely faulty, rerunning experiments one and two with a new standard would be more accurate. The expected trends would be the same, though. Lastly, it is likely that there were still components in the filtered lake water that interfere with the treatment. Because of this, another good next step would be to conduct experiments focusing on DOM or other chemical compounds found in lake water.

#### **Conclusion**

There are many conclusions based on the results of the three experiments that were conducted. Firstly, experiment one showed no statistical difference in the breakdown of MC-LR in unfiltered lake water between the samples that were exposed to TiO<sub>2</sub> and those that were not. This means that there are components of lake water that significantly hinder the effectiveness of the photocatalytic titanium dioxide nanoparticle treatment. Experiment two demonstrated that the filtration of lake water leads to a statistically significant difference in degradation of MC-LR, although the rate at which this occurs is less than in DI water as shown in experiment three. This means that there are likely both constituents in lake water that are filtered out (such as suspended solids) and constituents that pass-through filtration (such as DOM) that hinder the treatment. In order for the retractable nanoparticle net method of treating HAB to be feasible, it is important to determine what specific compounds are causing this effect. The next tests conducted in this research based on these experiments should focus on identifying the specific components of lake water that hinder the breakdown of MC-LR.

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