Image caption: A glimpse into the general experimental setup utilized. Flasks containing MC-LR, TiO$_2$, and DI water are exposed to UV light while being mixed. Normally, this is covered as to not allow outside light to interfere with the UV treatment. Photo courtesy of Zane Wood.

In Situ Cyanotoxin Mitigation: Net Design to Enhance Photocatalytic Degradation Mechanisms

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Abstract: Harmful algal blooms (HABs) and their associated cyanotoxins cause negative environmental, water quality, and human health impacts, however, no in situ treatment approach currently exists that can treat both HAB cyanobacteria and cyanotoxins. This study investigated the dispersed and immobilized photocatalytic titanium dioxide (TiO$_2$) nanoparticles for the removal of microcystin-LR (MC-LR) cyanotoxin in water. MC-LR adsorption testing on uncoated and 5x coated TiO$_2$ nets resulted in 0% and 13% adsorption, respectively. The 5x coated TiO$_2$ net removed MC-LR by 96%, 64%, and 10% for UV, indirect UV, and no UV exposure over 2 hours. MC-LR removal by 5x coated TiO$_2$ nets fit pseudo first-order kinetics, where the rate constant was k=0.0264 min-1 for 5x coated TiO$_2$ nets. Overall, the study indicated TiO$_2$ photocatalysts coated on nylon supports provide an immobilization approach for in-situ MC-LR removal by TiO$_2$ nanoparticles. Given the prevalence and importance of surface waters in the state of Arkansas for human recreation, environmental health, fresh water supply, and municipal/industrial development, the occurrence of HABs has a direct impact on Arkansas state economic vibrancy and environmental health. This work will benefit environmental quality for the State of Arkansas and the broader Mississippi River Basin by providing a feasible in situ treatment of cyanotoxin caused by HAB.

Key Points:
- A TiO$_2$ spray-coated nylon mesh achieves 96% MC-LR removal in water.
- Pseudo first-order kinetics were established for degrading microcystin-LR with immobilized TiO$_2$ nanoparticles.
- This study demonstrates the feasibility of in situ treatment of cyanotoxins.

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Introduction

Harmful algal blooms (HABs) have been recognized as global phenomenon that can have devastating consequences: they produce cyanotoxins or accumulate biomass that alter the food web dynamics, adversely impacting the health of humans, livestock and wildlife (Anderson et al., 2002). As a result, it is a national interest to prevent, control and mitigate HABs. Currently there are no effective treatment methods for *in situ* and simultaneous treatment of cyanobacteria and cyanotoxin. As eutrophication worsens in water bodies due to excessive nutrients discharge from anthropogenic activities, it is imperative to develop an effective *in situ* treatment method that can inactivate cyanobacteria and degrade cyanotoxins simultaneously, without deleterious impacts on the aquatic ecosystem.

In our initial collaborative work together, we have demonstrated a novel treatment approach that would allow mitigation of both HABs and their cyanotoxins in the water source; our approach is innovative in that it uses the concept of a reusable and retrievable fishing net to apply a catalytic environment locally to an HAB The local application of a net at the HAB/cyanotoxin source prevents the spread of HAB/cyanotoxin and minimizes unwanted catalytic reactions, resulting in point-source treatment with few negative side effects. Our preliminary results suggest that HAB cyanobacteria can be removed via physical coagulation. As a result, the goal of this research is to design a TiO_2 nanoparticle-net configuration that enables optimized cyanotoxin degradation. The cyanotoxin compound, microcystin-LR, was tested as representative of the ten cyanotoxins on the Fourth Unregulated Contaminant Monitoring Rule established by the Environmental Protection Agency (EPA).

Methods

Spray Coated TiO₂ Net Preparation

A 1:6 mass ratio Nafion to TiO_2 methanol solution was made by diluting and mixing concentrated TiO_2 nanoparticles and concentrated Nafion ionomer with methanol. Woven, 100 μm hole nylon mesh was cut into 4 cm by 4 cm squares and encased in aluminum foil on all sides but the front. Airbrush tubing was connected to an air supply, which was turned on to a constant and consistent airflow. A 250 μL volume of 1:6 mass ratio Nafion to TiO_2 methanol solution was added to the fluid cup, and a coat of the solution was applied evenly onto the surface of the nylon net. Additional spray coats of solution were applied once a previous coat dried for approximately 2 minutes and was dry to the touch. Nylon nets were spray coated 2, 5, and 8 times with TiO_2 and Nafion solution. Spray coated nets were left overnight in a hood to dry completely before analysis and experiments.

Spray Coated TiO₂ Net Analysis

SEM and EDX were utilized to observe net surfaces and to confirm TiO_2 attachment, and SEM and LA-ICP-MS were used to analyze net stability in a water environment that mimicked MC-LR experimental conditions (e.g., 100 rpm mixing of deionized water on an orbital shaker) for 0, 24, and 72 hours. Prior to SEM and EDX, all nets were sputter coated with gold for 1 minute to ensure charge for imaging and mapping. EDX mapping utilized titanium and carbon elements, which were acquired for 3 minutes. LA-ICP-MS utilized 10-line scans per net to obtain average count rates of Ti47, which was used as the reference material for titanium detection. Since laser ablation is destructive, new net samples were made for all repeats.

Spray Coated TiO₂ Net Experiments

Once thawed overnight, stock MC-LR was mixed then diluted and used for all samples within a batch. Concentrated MC-LR was pipetted into a 100 mL fused quartz Erlenmeyer flask filled with water to reach a 200 ppb starting concentration. The flask was stirred by hand for no more than 20 seconds to ensure complete mixing of MC-LR in water. Once stirred, an initial t=0 min time sample was taken and added to a centrifuge tube. An uncoated or spray coated TiO_2 nanoparticle net was added to the appropriate flask. Once a net was added to the flask, the solution and net were stirred by hand for an additional 20 seconds, and a second t=0 min time sample was taken and added to a centrifuge tube. The flask was capped with a rubber stopper and placed on an orbital shaker rotating at 100 rpm for 60 minutes, and a t=60 min sample was taken to observe possible MC-LR adsorption to net surfaces either free of or spray coated with nanoparticles. Following 60-minute adsorption tests, experimental solutions were exposed to UV light (365 nm wavelength and 230 μW/cm² intensity at 3 inches), indirect UV light (365 nm wavelength and <230 μW/cm² intensity), or no UV light using an angled UV lamp. For experiments using UV light with a spray coated net, samples were taken at 10-minute intervals for 30 minutes followed by 15-minute intervals for 90 minutes and added to centrifuge tubes. For experiments using uncoated nets as well as for experiments without UV light, samples were taken 60 minutes and 120 minutes after the initial 60-minute interval and added to centrifuge tubes. All samples were centrifuged at 7800 rpm for no more than 20 minutes. Sample solutions were transferred from centrifuge tubes to glass autosampler vials and analyzed using LC-MS. MC-LR standards (0, 10, 50, 100, 150, 200 ppb) were used for LC-MS analysis, and the multiple reaction monitoring (MRM) peak values for standards were used to create a standard curve. Sample MRM peak values were compared to the standard curve to obtain a MC-LR concentration profile for each experimental set. All experiments were performed in triplicate.

Results and Discussion

TiO₂ Attachment on Nylon Nets

TiO₂ solution was sprayed onto nylon nets one time (1x), twice (2x), five times (5x), and eight times (8x). With each spray application, the nanoparticles appear agglomerated, where agglomeration is more evident as a function of increased spray coats. To confirm TiO₂ solution attachment to nylon nets, uncoated and TiO2 coated nets were assessed using EDX mapping. In Figure 1, EDX images for a 5x spray coated TiO₂ net are shown at 300 μm, 50 μm, and 10 μm. Significant amounts of agglomerated titanium contrast carbon in the images at all scales, and the net is largely covered in TiO₂. Note that EDX is a qualitative tool, and the goal of EDX mapping images is to indicate relative TiO₂ attachment rather than quantitative results. The EDX for uncoated and 2x spray coated nets demonstrates limited or minimal titanium spectral intensity, which indicates a lack of significant TiO₂ attachment onto the net surface. As a result, 5x spray coated nets were used in the cyanotoxin degradation experiments.

MC-LR Adsorption onto Spray Coated TiO₂ Nets

The theoretical TiO₂ particle mass loadings were 2.5 mg, 6.25 mg, and 10 mg, respectively, for the 2x, 5x, and 8x spray nets, and the theoretical mass loading for 0.25 g/L dispersed TiO₂ was 6.25 mg. Therefore, the amount of TiO₂ present on 5 spray net surfaces was comparable to the amount of TiO₂ present for dispersed 0.25 g/L TiO₂ experiments when volume is taken into account. Experimental batches that utilized uncoated and 5x coated TiO₂ nets underwent a 1-hour adsorption test prior to incorporating UV exposure. For uncoated nets and 5x coated TiO₂ nets, the average adsorption percentages were -3% and 13%, respective-

ly. The -3% adsorption value for the uncoated net implies an average 0% adsorption, which is expected since no TiO₂ nanoparticles are present on the nets for adsorption of MC-LR onto TiO₂ to occur. The 13% adsorption percentage for TiO₂ falls within the average calculated standard deviations and is most likely due to experimental losses and fluctuations with MC-LR, such as attachment onto experimental containers or detection using LC-MS instrumentation. The 13% value is not significant enough to signify that adsorption is predominant.

LC-MS data for MC-LR degradation using 5x spray coated and uncoated nets over 2 hours are shown in Figure 2, where 5x coated TiO₂ net and uncoated net experiments are represented in Figure 2a and Figure 2b, respectively. In Figure 2a, the decrease in MC-LR concentration for no UV, indirect UV, and UV exposure using 5x coated TiO₂ nets was 10%, 64%, and 96%, respectively, from 0 minutes to 120 minutes. The indirect UV experiments utilized a less intense light source compared to the UV light experiments, and a MC-LR degradation trend that fell between direct UV and no UV results was observed. For direct UV exposure, results show rapidly decreased MC-LR concentration as a function of time, which indicates significant MC-LR degradation over the 120-minute time period. The average starting concentration was 175 ppb MC-LR, and after 2 hours under direct UV conditions with the 5x spray coated net, the MC-LR concentration decreased to 8 ppb on average. For the 5x spray coated net with UV, error spread decreased as time progressed. Larger spread is observed between 0 minutes and 75 minutes, and minimal error spread occurred for 90, 105, and 120 minutes. Although there is more variability up to 75 minutes, the 5x spray coated net consistently degrades MC-LR below 25 ppb after 75 minutes. Results for uncoated net exposure to no UV, indirect UV, and UV conditions
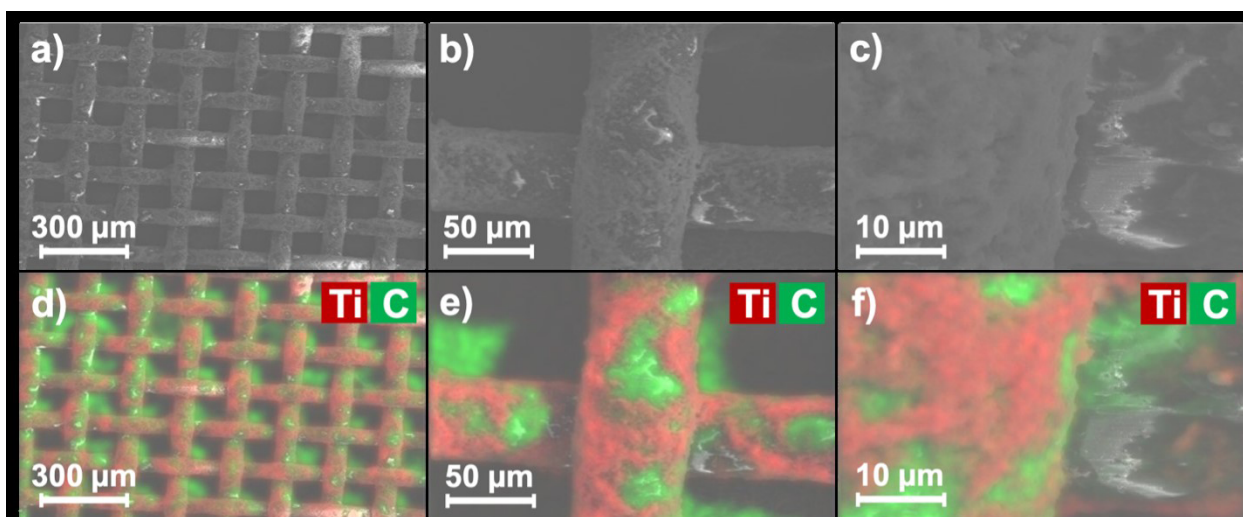

Figure 1: EDX mapping for 5x spray coated TiO₂ net, consisting of 1:6 mass ratio Nafion ionomer to TiO₂ nanoparticles on nylon netting. Mapping was acquired for roughly 3 min per image. The mapping images show the 5x spray coated TiO₂ net at (a) 300 μm, (b) 50 μm, and (c) 10 μm scales. All samples were sputter coated with gold for roughly 1 min prior to EDX mapping.
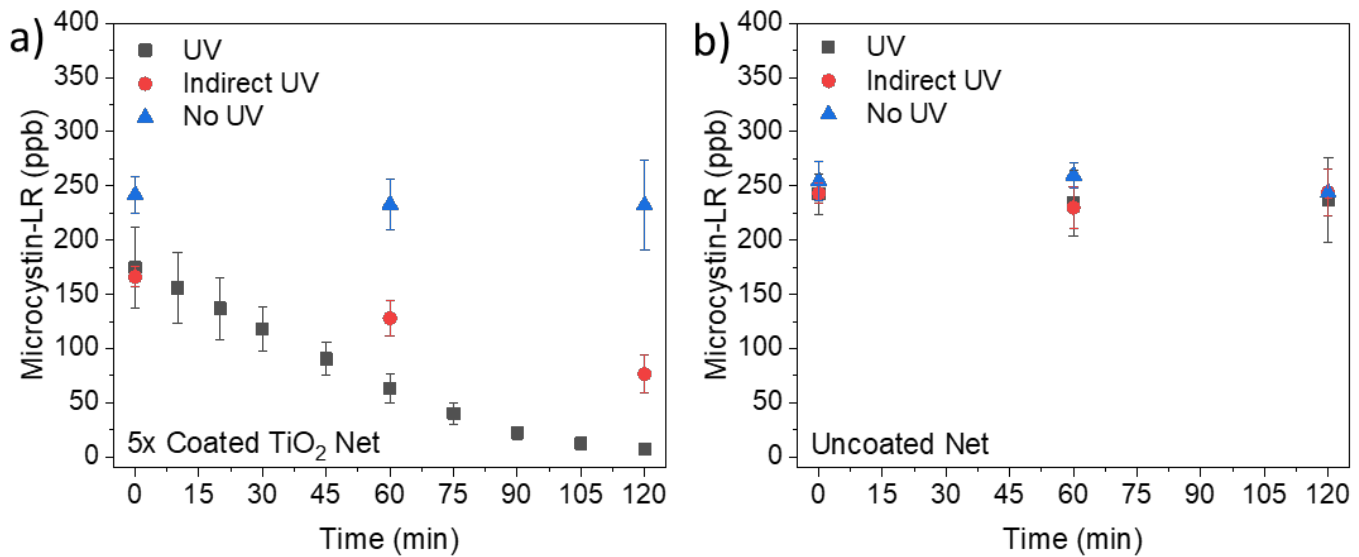
Figure 2: LC-MS data show MC-LR degradation using a MC-LR theoretical starting concentration of 200 ppb and a (a) 5x coated TiO₂ net or (b) uncoated nylon net with UV light (365 nm wavelength with 230 µW/cm² intensity at 3 inches), indirect UV light (365 nm wavelength with <230 µW/cm² intensity), or no UV light exposure. All experiments were done in triplicate.

are shown in Figure 2b. While slight variations in average MC-LR concentrations occur over the 2-hour time period, MC-LR consistently remains at about 250 ppb, where calculated standard deviations for each time point are ≤ ±8%. No photocatalytic nanoparticles were present on the surface of uncoated nets, so no MC-LR degradation was expected.

Although systems used between authors are variable, studies have repeatedly shown and proven the ability of dispersed TiO₂ nanoparticles to degrade MC-LR under UV conditions. Pinho et al. utilized a pilot scale solar photoreactor to degrade 100 µg/L MC-LR with 200 mg/L TiO₂ in roughly 32 minutes (Pinho et al., 2015). Lawton et al. observed rapidly degraded MC-LR, where 200 µg/mL MC-LR was degraded to nearly zero after 20 minutes of UV light exposure in the presence of TiO₂ catalyst (1% m/v) (Lawton et al., 1999). In addition to further showing the ability of dispersed TiO₂ to degrade MC-LR under light conditions, the work presented in this study solidifies the capability of spray coated nanoparticle nets to perform equivalently to dispersed nanoparticles.

Kinetic Analysis

Data representing MC-LR degradation by 5x coated TiO₂ nets were fitted to pseudo zeroth, first, and second order kinetics. First order kinetics have the correlation coefficient (i.e., R^2 value) closest to 1 (i.e., 0.965) compared to zeroth and second order. Using pseudo first-order kinetics, the rate constant (k) was 0.0264 min⁻¹ for 5x coated nets. Shephard et al. utilized a photoreactor using immobilized TiO₂ on a fiber glass sheet to degrade 55 g/mL MC-LR with TiO₂ ranging from 0.2-1 g/L and fit degradation data to first

order kinetics, where the rate constant was 0.117 min⁻¹ (Shephard et al., 2002). Feng et al. fit 20 µg/L MC-LR degradation data using nano-TiO₂ thin film to pseudo first-order kinetics and obtained a rate constant of 0.0157 min⁻¹ (Feng et al., 2006). This work demonstrates that the rate constant for 5x coated TiO₂ nets is similar to dispersed TiO₂ data in literature studies.

Conclusions

This study investigated the development and performance of immobilized TiO₂ nets utilizing nylon mesh supports for direct microcystin-LR degradation in comparison to dispersed TiO₂. Nets spray coated with a 6.25 mg (5x spray coats) theoretical mass loading were observed to be more homogeneous and evenly coated with TiO₂ solution compared to nets with 2.5 mg (2x spray coats) and 10 mg (8x spray coats) particle loading. 5x spray coated TiO₂ nets were tested under various UV exposure conditions, where 96% and 64% MC-LR removal was observed for 5x coated nets with direct (230 µW/cm²) and indirect (<230 µW/cm²) UV over a 2-hour time period. MC-LR removal was not indicated for uncoated nets under varying UV and 5x coated TiO₂ nets with no UV applied. The final MC-LR concentration was 8 ppb after degradation. The study has shown that the degradation of MC-LR followed pseudo first-order reaction kinetics for 5x coated TiO₂ nets. Overall, 5x coated nets are capable of degrading MC-LR similarly to dispersed TiO₂ nanoparticles under UV conditions, suggesting that immobilized TiO₂ does not hinder degradation ability for MC-LR. In addition, varying UV conditions and UV intensity

play crucial roles in MC-LR degradation, where sufficient UV light intensity and TiO_2 nanoparticle concentrations are necessary to effectively degrade MC-LR over time.

The feasibility of utilizing TiO_2-immobilized nets in a water environment for the treatment of MC-LR cyanotoxins has implications for both natural and engineered water systems. Direct, in-situ MC-LR treatment using retrievable TiO_2 immobilized netting could degrade toxins in natural surface waters (e.g., lakes and ponds). Direct treatment could help alleviate cyanotoxin level spikes in recreational water over blooming periods, making water safer. Additionally, since water treatment facilities struggle with treating cyanotoxin-rich waters, direct treatment method could aid in decreasing microcystin toxins prior to transport and processing at facilities.

Acknowledgements

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