Adsorption of Microcystin (MC) LA, LR, and RR to Granular Activated Carbon (GAC)

Undergraduate Research Thesis

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Abstract

Microcystins (MCs) are a class of cyanotoxins produced by cyanobacteria found in harmful algal blooms. MCs are hepatotoxins and possible tumor promoters, so removing them during drinking water treatment is essential. There are many different strains of microcystins, but I chose to look at MCLA, MCLR, and MCRR because they are commonly seen in surface waters across Ohio. Conventional methods have proven to be ineffective in removing these toxins in drinking water treatment, thus requiring the efficacy of alternative methods, such as the use of granular activated carbon (GAC), to be evaluated. Because the properties of different MC strains vary, I hypothesized that their adsorption to activated carbon would differ. Specifically, it was expected that MCRR would adsorb the best since it is neutrally charged; since MCLA and MCLR are both negatively charged, they were expected to have lower adsorption capacities as they would experience more electrostatic repulsion from the negatively-charged GAC. To determine the ability of activated carbon to adsorb MCs, equilibrium batch tests were performed with granular activated carbon and three different strains of microcystin. Both individual and competitive batch tests were done with these toxins in order to observe their behavior on their own as well as in a competitive, more realistic environment. The tests used a "model water" (MW) which imitated the properties of Ohio surface water. The data obtained was then fit to the Langmuir isotherm model.

The results indicated that MCLA adsorbs the best to GAC in independent tests. MCLA had an adsorption capacity around 8.5 micrograms of toxin per milligram of GAC in individual tests, while MCRR had a capacity closer to 4μg/mg. MCLR had the lowest capacity with values slightly less than 4μg/mg. This conflicts with the expected result that MCRR would have the highest adsorption capacity, potentially indicating that adsorption to GAC is more complex than

the charge of the toxin. During competitive tests, MCRR had the highest adsorption capacity, followed by MCLA then MCLR. This followed my original hypothesis, which indicates that charge may play a larger role in GAC adsorption during competitive tests. In conclusion, when removing microcystin during drinking water treatment, it is essential to know what strains of the toxin are present. MCLA will require less GAC to remove enough toxin to comply with EPA standards than MCRR or MCLR when it is not competing for adsorption sites with other MCs.

Acknowledgements

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Chapter 1: Introduction

1.1 Background

Harmful algal blooms (HABs) are large colonies of algae that release toxic products into water systems. These products can have adverse health effects for the organisms that live in that water as well as those that use it as a drinking water source. HABs are formed by an excess of phosphorus and nitrogen in a watershed, which creates nutrient-rich runoff to surface waters that, when combined with high water temperatures, provide conditions for widespread eutrophication (NOAA 2018).

Microcystins are cyclic peptides (see Figure 1) produced in HABs by cyanobacteria genera, such as *Microcystis*, *Anabaena*, and *Planktothrix*, as secondary metabolites (de Maagd et al 1998, USEPA 2018). They are hepatotoxins and possible tumor promoters, and they have been responsible for several health-related incidents across the United States (Butler et al. 2009). Though there currently is not a Maximum Contaminant Level (MCL) for microcystin at the federal level, Ohio EPA has set thresholds of 0.3μg/L for sensitive groups and children under the age of six, and 1.6μg/L for children older than six and adults (Ohio EPA 2017). Microcystins are exceptionally stable in water (Butler et al. 2009), therefore conventional treatment processes are often not effective enough to remove microcystin from a drinking water source to the level required by Ohio EPA (Ohio EPA, 2016). Activated carbon (AC) is often used to amend this problem; it is favored because it is inexpensive and able to be incorporated into water treatment without much additional infrastructure. However, its efficacy at removing these toxins depends largely on the types of microcystins and other natural organic matter (NOM) present in the influent (Ohio EPA, 2016).

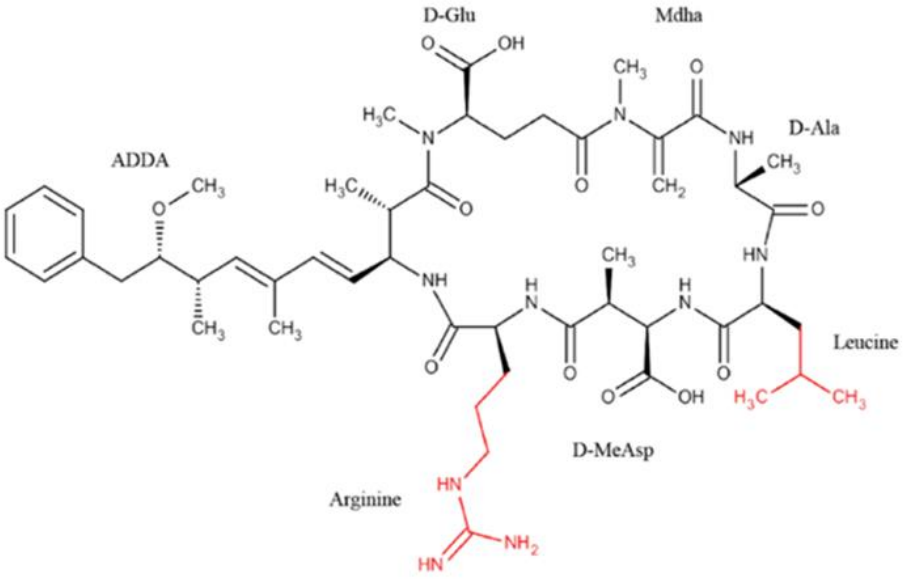

Figure 1: Structure of MCLR molecule. Groups in red denote those that can vary for different MC variants. From Miao et al. (2010) and modified by Liu (2018)

MCLR is the most common strain of microcystin seen in HABs (de Maagd et al, 1998), but there are many other variants of microcystin that are being observed with increasing frequency in surface waters in the U.S. Over 80 different types of microcystin have been recognized in natural algal blooms across the globe, and they are becoming more common in Ohio (Spoof et al, 2009). These different strains vary based on the presence of different variable amino acid groups (see Figure 1). It has been found that AC commonly removes over 80% of MCLR from raw water when combined with conventional water treatment (Lambert et al, 1996), but little research has been done on how well it removes other strains of microcystin. Of the few that have been done with more than one variant, most have been in competitive conditions and found that MCRR has the highest adsorption capacity, followed by MCLR then MCLA (Ho et al 2011, Liu 2018, Newcombe 2003). Of studies that mimicked independent conditions, it was determined that MCRR adsorbed better to AC than MCLR (Zhu et al 2016, Liu 2018). When

MCLA was included in the study, it was found to adsorb the best of the three toxins (Liu 2018). All in all, very few studies have been done on variants other than MCLR, especially with GAC. Therefore, as other variants are becoming a larger problem, further research on them is warranted.

1.2 Objectives

Granular activated carbon (GAC) has been proven effective in removing microcystin in continuous column testing (Liu, 2018). However, equilibrium batch experiments on different toxins have not been run. These tests allow one to see the equilibrium sorption of microcystin to activated carbon, as well as give information on which one is most effectively removed by it. That information can then be used to evaluate activated carbon columns as a treatment method to remove different variations of microcystin.

This research centered on equilibrium batch testing of MCLR, MCLA, and MCRR in both independent and competitive environments (see Table 1). All three of these toxins are frequently detected in surface waters in Ohio and therefore pose a threat to Ohio drinking water safety (Foss and Aubel 2015). Their different properties stem from their variable amino acid sidechains. These sidechains dictate properties such as charge, polarity, and hydrophobic tendencies (Newcombe et al. 2003). They cause MCRR to be neutrally charged and both MCLA and MCLR to carry negative charges. MCLA's variable amino acids of leucine and alanine make it both the smallest and most hydrophilic, while MCLR and MCRR are both larger in size as well as hydrophobic tendencies (Newcombe et al. 2003).

In Ohio surface waters, MCLR and MCRR are commonly found in high concentrations in the same surface water sample, but in Ohio, MCLA is usually only found in high concentrations when there are few other MC variants present (H. Raymond, personal

communication, December 11th, 2018). However, this may not be the case everywhere, thus determining MCLA, MCLR and MCRR adsorption capacity in both independent and competitive environments is essential to evaluate GAC's efficacy in MC removal.

Table 1: Microcystin variant descriptions

Toxin	MCRR	MCLR	MCLA
Variable Amino Acid 1	Arginine	Leucine	Leucine
Variable Amino Acid 2	Arginine	Arginine	Alanine
Molecular weight (g/mol)	1038	994	910
Charge	0	-1	-2

At a pH above 6.7, F300 GAC has a slightly negative charge (Villars 2018). Therefore, it was hypothesized that MCRR would adsorb better to GAC than MCLR and MCLA because it theoretically would experience less electrostatic repulsion since it has a neutral charge (as opposed to MCLR and MCLA that both have negative charges). This trend was expected to hold for both independent and competitive tests, though adsorption capacities for all toxins were expected to decrease during competitive tests due to lack of abundant adsorption sites.

Chapter 2: Methodology

2.1 Overview

Two kinds of adsorption experiments were performed with MCs and GAC. One was an individual equilibrium batch test where model water (MW) was spiked with one of the three microcystins. Nine sample jars were then prepared with the same MW dose and varying amounts of GAC. The initial toxin concentration for each of these tests was 100µg/L. For the competitive tests, the MW was spiked with all three toxins. Nine jars were again prepared with the same spiked MW dose and varying GAC doses. Initial concentration was 50µg/L of each toxin in competitive tests. Both tests ran at room temperature for three days at 150rpm on a shaker table.

Samples were then taken from each jar, filtered to remove GAC, and analyzed using Ultra Performance Liquid Chromatography (UPLC).

2.2 GAC Characteristics and Preparation

Filtrasorb-300 (F-300) is a granular activated carbon made from bituminous coal. It was chosen because it is inexpensive and already commonly used at water treatment plants across Ohio. It has a point of zero charge pH (pH_{pzc}) of 6.7, meaning it has a slightly negative charge above a pH of 6.7 (Villars 2018). See Table 2 for more F-300 properties.

Table 2: F-300 GAC properties (Villars 2018)

Property	Source	BET Surface Area	Macropore Volume	Mesopore Volume	pH_{pzc}
Value/Description	Bituminous Coal	$950\frac{m^2}{g}$	$0.40\frac{cm^3}{g}$	$0.06\frac{cm^3}{g}$	6.7

Approximately 5 grams of F-300 was ground in an electric coffee grinder and dry sieved through a #200 sieve (74μm pores) and captured on a #400 sieve (37μm pores). The carbon was then wet sieved through the same size sieves until the water running over the carbon had the same conductivity reading as deionized (DI) water. The #400 sieve that contained the captured carbon was then put in an oven at 70°C for three hours, after which the carbon was transferred to an aluminum weighing dish to dry for a minimum of one day. Finally, once completely dry, the GAC was transferred to a desiccator for long-term storage.

2.3 Model Water Composition

The model water used in these experiments was comprised of DI water and various inorganic salts (see Table 3). It was made to emulate the composition of Ohio surface waters, excluding any natural organic matter (NOM). For the last four MW components, a five-times

concentrated solution was made in order to avoid any error that may arise from weighing out exceedingly small masses of salts.

Table 3: Model water composition

Model Water Component	Model Water Concentration (mg/L)
Sodium Bicarbonate ($NaHCO_3$)	240.5
Calcium Chloride: $CaCl_2.2H_2O$	20
Calcium Sulfate: $CaSO_3.7H_2O$	240
Magnesium Sulfate: $MgSO_4.7H_2O$	300
Potassium Chloride: KCl	5
Ammonium Nitrate: NH_4NO_3	.26
Iron (III) Chloride: $FeCl_3.6H_2O$	2
Disodium Phosphate: Na_2HPO_4	.7

2.4 Calibration Curve Preparation

Liquid analytical standards of each toxin dissolved in methanol were ordered for MCRR, MCLR, and MCLA. Both MCRR and MCLR standards were purchased from Sigma-Aldrich, and MCLA was purchased from Abraxis. These standards were then used to make a standard solution that contained all three toxins. This was done by calculating how much of each liquid toxin and DI water would be required to create a solution that contained 100ppb of each toxin, taking into account the differing densities between water and methanol. A vial was weighed and tarred prior to any additions, and mass was recorded after each addition of toxin and DI. In this way, an exact concentration was calculated for a combined standard solution. From there, varying amounts of standard solution and DI were added to 2mL vials in order to obtain a set of 1mL analytical standards with concentrations that varied from 0-100μg/L. Masses were again recorded after each addition of standard and DI in order to keep track of the exact concentration

in each vial. The standards were then analyzed with UPLC; using the area counts obtained, known concentrations of vials, and standard deviations of area counts, an equation was determined for each toxin that related area count to concentration. Limits of detection and quantitation for UPLC were also determined during this process (see Appendix B, Table 1). All vials were stored at -20°C and run each month to account for any instrumental drift over the course of running the experiments. See Appendix, Figure 4 for a sample calibration curve.

2.5 Toxin Preparation

Toxin was received in dry form in vials containing either 100µg or 500µg of toxin; all vials were purchased from Cayman Chemical Company. In both cases, 1mL of methanol was added to the vial to dissolve the toxin. Next, using a glass syringe, the toxin and methanol were removed from the vial and added to a volumetric flask. 1mL of DI was added to the vial, removed using the glass syringe, and emptied into the same volumetric flask. That procedure was repeated three times. The volumetric flask was then filled with DI in order to make a 2000µg/L stock of toxin. Toxins stocks were stored at 4°C for no longer than a six month period.

2.6 Individual Equilibrium Batch Tests

100mg of GAC was weighed out and added to a small beaker. 50mL of DI water was also added to create a 2g/L GAC slurry. A stir bar was placed in the beaker, and the beaker was set on an electric stirring motor to keep the GAC concentration as consistent as possible throughout the beaker. Next, 1L of model water was spiked with 50mL of 2000µg/L toxin stock to make a solution with 100µg/L of microcystin. 50mL of model water was removed from the volumetric flask prior to this addition to keep volume consistent.

100mL of spiked MW was then added to nine autoclaved amber sample jars. Two jars were set aside without GAC additions to serve as controls for the experiment. Then, a GAC dose

that ranged from 1-100ppm (aliquots of 50-5000µL from 2g/L GAC slurry) was added to each jar. The tops were sealed onto each jar, and the jars were placed on the shaker table for 3 days at 150rpm. After 72 hours were up, 1mL samples were pulled from each jar and filtered through .45µm PVDF syringe filters into 2mL UPLC vials. The nine samples as well as two DI samples were then analyzed using UPLC.

2.7 Competitive Equilibrium Batch Tests

The same procedure followed in section 2.6 was used to make a 2g/L slurry of GAC. For competitive tests, 25mL of each 2000µg/L toxin stocks were added to MW after 75mL had been removed from the volumetric flask. This ensured an initial concentration of 50µg/L for each toxin. 100mL of spiked model water was then added to each amber glass sample jar, as well as varying doses of GAC over a range of 0-100ppm. The procedure from section 2.6 was then followed for the duration of the experiment.

2.8 Ultra Performance Liquid Chromatography

UPLC is a type of chromatography that separates compounds in solution by passing them through a column. The column utilizes different solvents to separate compounds by their chemical and/or physical properties so their concentrations can be analyzed. A method was developed for the analysis of microcystins using UPLC based off of work done by Spoof et al (2009). This method utilized a C18 column (Waters, Acquity UPLC BEH 130Å, 1.7µm, 2.1mm x 50mm) and a 0.45mL/min flowrate. The mobile phase components were DI water and acetonitrile; both were spiked with 0.1% formic acid. The ratio of DI to acetonitrile shifted from 68:32 to 20:80 over the 5.5 minute sample run time, and sample injection volume was 10µL.

This method was used to analyze both individual and competitive samples; it produced chromatograms with peaks that could be integrated to determine the area count of said peak.

These area counts as well as the calibration curve were used to determine the remaining MC concentration in each sample. These concentrations and their accompanying adsorption capacities were then fit to the Langmuir isotherm model.

Chapter 3: Results

3.1 Individual Batch Tests

After collecting area counts for each sample and calculating their respective MC concentrations, the adsorption data was presented in terms of an adsorption isotherm. Though there are many different isotherm models, the Langmuir model was chosen due to its precedence of application to adsorption of MCs to activated carbon (Donati et al. 1994, Pendleton et al. 2001,Marsh and Rodriguez-Reinoso 2006, Campinas and Rosa 2006, Zhu et al. 2016, Villars 2018). The Langmuir model operates on three base assumptions: only monolayer adsorption can occur, the adsorption is localized so there are no adsorbate-adsorbate interactions, and the change in free energy for all sites is equal and independent of surface coverage (Patrick 1995, Crittenden and Montgomery Watson 2012). These assumptions are not explicitly applicable for activated carbons, but the model still provides a good fit to most data sets (Patrick 1995).

When applied to adsorption by activated carbon, the Langmuir model (below) depicts the adsorption of toxin by GAC as a function of MC sample concentration.

$$Q_e = \frac{Q_{max} * K * [C_e]}{1 + K * [C_e]}$$

Where: Q_e=Adsorption (μg/mg)

K=Initial slope (L/mg)

C_e=[MC] (μg/L)

Q_{max}=Maximum adsorption capacity (μg/mg)

The values for Q were calculated by subtracting the sample MC concentration (C_e, µg/L) from the initial MC concentration (µg/L), which was established by the control samples, and dividing by the GAC dose (mg/L) for that jar. These data was then linearized using Excel to calculate the Langmuir constants. Though there are outlying samples in each isotherm, most points follow the trend of the Langmuir isotherm. Additionally, Langmuir fit parameters (Appendix, Table 4) prove that the Langmuir model fits the collected adsorption data well. The lowest R^2 value observed was .67, and the average R^2 value for all tests was .83. Therefore, it can be concluded that the Langmuir adsorption model was a good choice for the data collected over the course of these experiments.

In independent testing, MCLA had the highest adsorption capacity of the three toxins tested. It had a maximum capacity of 8.6µg of toxin per milligram of GAC (Figure 2, a-c). MCRR had the second highest capacity of 4.3µg/mg, and MCLR had the lowest capacity with a value of 3.7µg/mg. However, MCRR had the highest K value in these tests. Since K is the equilibrium constant associated with each reaction, it is an indicator of each toxin's affinity to GAC. Even though MCLA had the highest adsorption capacity, MCRR had the greatest affinity for GAC adsorption. This could possibly be an indicator that MCLA may not adsorb as much to GAC when other toxins or NOM is present.

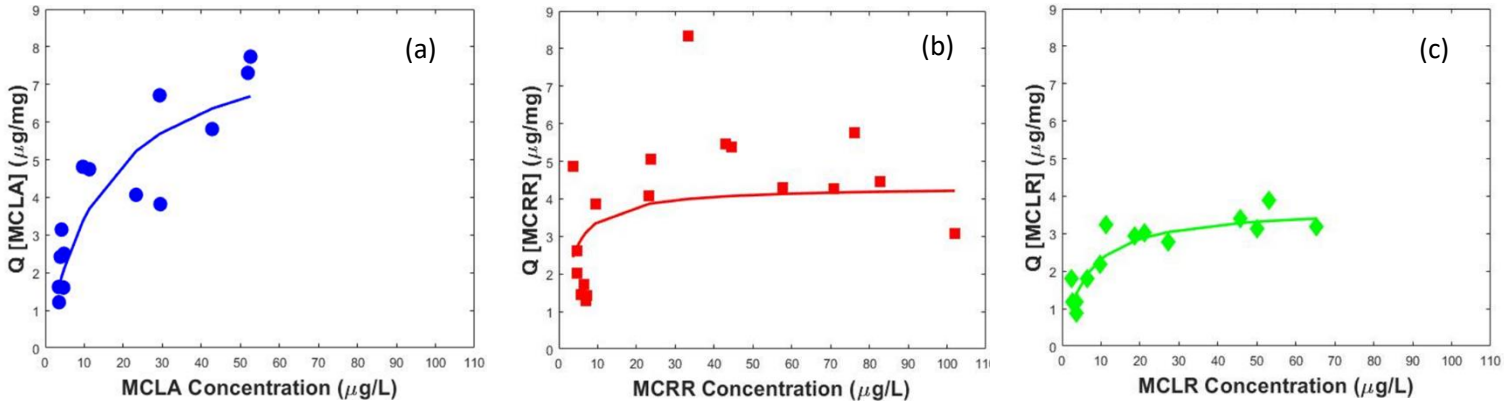

Figure 2: Equilibrium adsorption of MCs to F-300 GAC in model water in independent tests: (a) MCLA adsorption, (b) MCRR adsorption, and (c) MCLR adsorption. Solid symbols represent the experimental data, and the line represents that toxin's fit to the Langmuir isotherm model (other parameter values can be found in the Appendix, Table 4)

Results obtained for MCLR and MCRR are similar to previous work done by Zhu et al. (2016) with coal, shell, and wood-based PAC. Zhu et al. (2016) used a lab-made water that did not contain any NOM, therefore the results, except for the difference in form of carbon, should be comparable. The adsorption capacity of the coal-based PAC was the lowest of the three that they studied; they obtained Q_{max} values of 3.19mg/g and 3.27 mg/g for MCLR and MCRR (respectively) with coal-based carbon. Those values are slightly lower than the ones obtained in this study. This could be due to differences in the type of coal the carbon was sourced from as well as the fact that they utilized PAC instead of GAC. Though they only tested these two MC variants, their result that MCRR had a higher adsorption capacity than MCLR is consistent.

The overall result that MCLA adsorbs the best out of the three toxins tested is consistent with what Liu (2018) saw during competitive column tests using F300 GAC. When GAC was fresh and there was a surplus of it (i.e. at the beginning of a test), MCLA had the highest extent of removal GAC (Liu 2018). Though this was a competitive test, it mimics independent tests in that there was enough GAC available that toxins didn't have to compete with one another for adsorption sites. However, these results do not align with the hypothesis that charge would dictate how each toxin adsorbed to GAC since MCLA has a negative charge yet still had a higher adsorption capacity than the neutrally-charge MCRR.

There is a variety of reasons that MCLA could have had the highest adsorption capacity despite its negative charge. With a molecular weight of 910g/mol, it is significantly smaller than the other two toxins studied, which could allow it to access certain pores in GAC that larger molecules cannot. This may have contributed to its superior adsorption and adherence to GAC. Additionally, each variant of MC has different polarities and hydrophobic tendencies. Newcombe et al. (2003) estimated these properties by assessing each MCs variable amino acids. Alanine is considered nonpolar and hydrophobic, and is only found in MCLA. Both arginine and leucine are considered polar and therefore hydrophilic. For this reason, Newcombe et al. (2003) inferenced that MCLA would be more hydrophobic than MCLR and MCRR and therefore would adsorb the best to AC. This may be the reason that MCLA had a higher adsorption capacity than MCRR and MCLR during independent testing. Though further study is required on the specific MCs themselves instead of exclusively their variable side chains to illuminate exactly how their polarities and hydrophobic tendencies influence adsorption, this insight may play a role in the results seen during independent testing of MCs.

3.2 Competitive Equilibrium Batch Tests

During competitive testing, results adhered closely to the original hypothesis that charge dictates a toxin's adsorption affinity to GAC. In these tests, MCRR had the highest adsorption capacity with a maximum capacity of 5.98µg/mg (Figure 3). MCLA adsorbed the second most out of the toxins tested with a maximum capacity of 3.62µg/mg. Finally, as in independent testing, MCLR had the lowest adsorption capacity with a maximum value of 3.43µg/mg. During these tests, K values were all very similar for each toxin which means they all demonstrated similar affinities for GAC adsorption in a competitive environment.

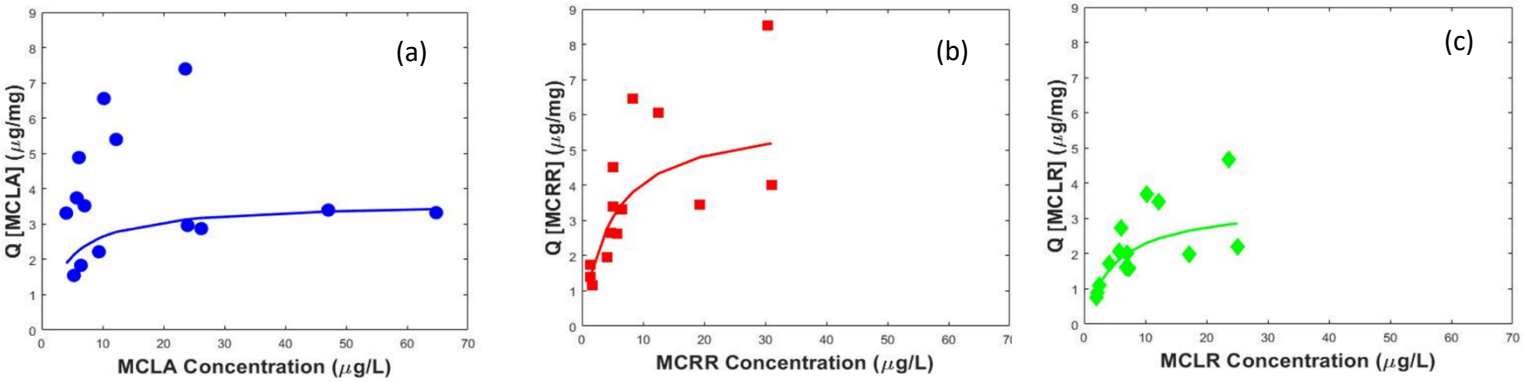

Figure 3: Equilibrium adsorption of MCs to F-300 GAC in model water in competitive tests: (a) MCLA adsorption, (b) MCRR adsorption, and (c) MCLR adsorption. Solid symbols represent the experimental data, and the line represents that toxin's fit to the Langmuir isotherm model (other parameter values can be found in the Appendix, Table 5)

The Q_{max} value obtained for MCRR is more consistent with both the original hypothesis that it would adsorb the best to GAC due to its lack of charge as well as previous work done with MC's and PAC. In experiments done by Ho et al (2011) with coal-based PAC and MCRR, MCLR, and MCLA in natural, unfiltered waters, it was found that MCRR had the highest

adsorption capacity in a competitive environment. However, in the same study, Ho et al (2011) found that MCLA had the lowest capacity, which contradicts these results. This could be due to small sample size; in order to comply with time restraints, only two tests were run on MCs in competitive environments. Additionally, experimental drift could have played a part in this result. The limit of detection and limit of quantitation changed significantly over the course of experimental runs. During the competitive tests, around half of the MC concentrations were near the UPLC detection limit. This may have resulted in inaccurate measurements of smaller MC concentrations, which may have slightly skewed the adsorption results in competitive tests since initial MC concentrations were lower during these trials.

The results of this research provides information on the maximum adsorption capacity one particular GAC has with MCLR, MCLA, and MCRR. However, in order to determine a proper dose of GAC for each toxin, the adsorption of each toxin to GAC over time in both independent and competitive conditions must be analyzed. These kinetic tests would simulate more realistic water treatment conditions and would allow one to see exactly how much carbon must be in contact for a certain amount of time to achieve desired removal. This is outside the scope of this research, however it is essential in determining how much GAC must be used to effectively remove MCs to achieve the 1ppb required by Ohio EPA's regulation.

Chapter 4: Conclusion

In this study, it was found that MCLA has the highest adsorption capacity, MCRR has the second highest, and MCLR has the lowest capacity when they are the only toxins in solution. However, MCRR had the highest affinity for GAC adsorption during these tests. During competitive tests, MCRR had the highest adsorption capacity, MCLA had the second highest, and MCLR had the lowest capacity of the three toxins tested, and all three toxins demonstrated

similar affinities for GAC adsorption. The results for the individual tests contradicted the hypothesis that charge would dictate adsorption capacity, indicating that another property (possibly size, polarity, etc.) dictates adsorption. However, adsorption during competitive tests did seem to be more heavily influenced by charge. Based on these results, MCLA will require less GAC to remove it from a water system than MCLR or MCRR when found without the presence of other toxins. However, it was found in competitive tests that toxins have different adsorption capacities when they must compete with other toxins for adsorption sites.

These results align with some previous studies, yet they contradict others. Overall, more research must be done both in independent and competitive environments to determine the exact adsorption trends of these toxins with GAC. Through further testing of these conditions, batch tests with NOM, and kinetic sorption tests, a proper dose of GAC can be determined that would remove enough MC to be compliant with Ohio EPA. This would help establish whether or not GAC is an effective and efficient treatment technology for microcystins going forward.

Appendix: Supplemental Information

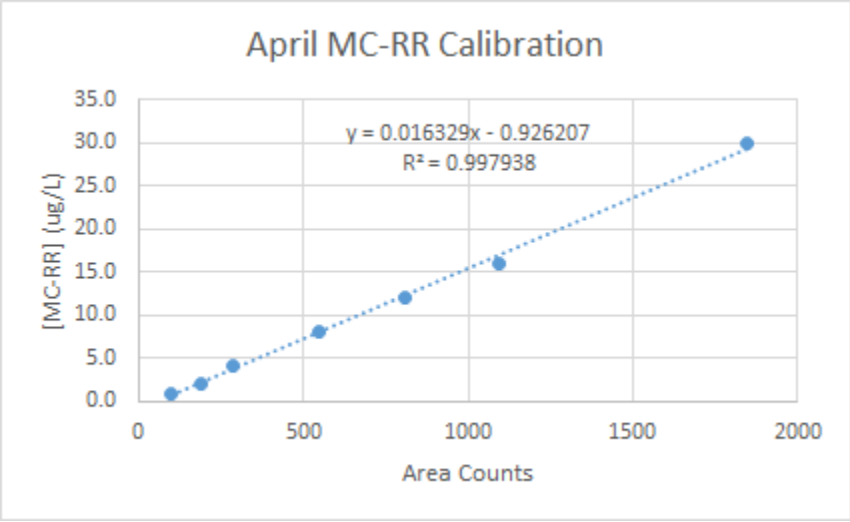

Figure 4: Sample Calibration Curve for MCRR

Table 4: Summary of adsorption fit to Langmuir isotherm model for individual batch tests

Toxin	Q_{max} (µg/mg)	K (L/mg)	Coefficient of Determination (R^2)
MCRR	4.33	0.36	0.85
MCLR	3.72	0.17	0.97
MCLA	8.58	0.07	0.81

Table 5: Summary of adsorption fit to Langmuir isotherm model for competitive batch tests

Toxin	Q_{max} (µg/mg)	K (L/mg)	Coefficient of Determination (R^2)
MCRR	5.98	0.21	0.73
MCLR	3.43	0.20	0.67
MCLA	3.62	0.27	0.96

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