2008 Evaluation of the Effectiveness of DMWW Treatment for Removal of Microcystins

The goal of this project was to assess the presence of microcystins in Des Moines Water Works (DMWW) source water and determine the fate of microcystins in the treatment process at the Fleur and McMullen treatment plants.

The source waters for the DMWW, the Raccoon River (RR) and the Des Moines River (DMR), flow through areas of intense agricultural production where nutrient amendments are extensively applied. The Infiltration Gallery (IG), a shallow ground water source for the Fleur Treatment Plant, parallels the RR allowing for naturally filtered RR water to be available as required. Radial collector wells under the influence of the RR provide water to the McMullen Treatment Plant. Crystal Lake, an alternative water source for the McMullen Treatment Plant, is an abandoned gravel pit which is under the influence of the RR. Maffitt Reservoir is also an additional source water option for the McMullen Treatment Plant. Neither Crystal Lake nor Maffitt Reservoir were utilized as source waters at the time of this study, but microcystin data was gathered from these sources.

Cyanobacteria, also known as blue green algae, are a group of oxygenic photosynthetic bacteria that contain chlorophyll a and phycobilins. Cyanobacteria are found primarily in surface waters. Nutrient rich surface waters, receiving nutrients from agricultural runoff, sewage, or storm water, are at the highest risk for cyanobacterial blooms. Cyanobacteria often grow in colonies large enough to see. Toxins synthesized by cyanobacteria may be released when the cells have been compromised (cells die and break open releasing potential toxins). There are three major groups of toxins released from compromised cyanobacteria cells: hepatoxins (toxins targeting the liver), neurotoxins (toxins targeting nerves), and dermatotoxins (toxins targeting skin). Hepatoxins are the most prevalent of all the toxins.

Microcystins and Nodularins are cyclic toxin peptides. Microcystins are fresh water hepatoxins produced by a number of cyancobacterial genera. *Microcystis aeruginosa* (Figure 1) is the primary producer of this dominant class of toxins. Microcystins are composed of seven amino acids (two protein amino acids and five non-protein amino acids). The two protein amino acids distinguish microcystin types from each other. The most prevalent microcystins are: microcystin LR, RR, LA and YR. Nodularins are produced by the genus *Nodularia* and are found in brackish water. To date, approximately 75 different types of microcystins have been discovered. Microcystin-LR is considered the most common toxin found in water supplies around the world. Microcystin LR is comprised of the amino acids Leucine (L) and Arginine (R).

Figure 1. Microcystis aeruginosa

Microcystins have been linked to acute poisoning of humans and animals throughout the world. In Brazil, 55 patients died and another 114 became severely ill from microcystin tainted hemodialysis water. This microcystin tainted water caused liver hemorrhage and liver failure in these patients (Backer 2002). There is supporting evidence that the high incidence of liver cancer in rural regions of China may be caused by drinking water obtained from ditches and ponds with large cyanobacteria loads. Studies on rodents have

shown that microcystins promote liver toxicity, tumors, and birth defects (Falconer et al.1988). It is also speculated that low-level exposure to microcystins may promote the development of cancer and other chronic gastrointestinal disorders. In response to documented health risks and to protect consumers from adverse effects from exposure to microcystins, the World Health Organization (WHO) issued a provisional concentration guideline of 1 microgram per liter microcystin for drinking water. The United States Environmental Protection Agency placed cyanobacteria on its Candidate Contaminant List in 2005.

Nitrogen, phosphorous, carbon, sunlight, warm temperatures and stagnant water are some of the conditions which promote the production of cyanobacteria that produce microcystins.

Microcystins have been found to be very resistant to environmental breakdown. They are extremely stable in water because their chemical structure enables them to survive in warm and cold water and endure radical changes in water chemistry such as pH. The amount of daylight required for growth depends upon the species of *Microcystis*. The species *Microcystis aeruginosa* regulates its buoyancy in response to light (Utkilen and Gjolme, 1992). This buoyancy trait allows them to migrate through thermal gradients and use nutrients confined to cooler deeper water. Microcystins have also been shown to raise the pH in waters they inhabit. A study by (Lam et al.1995) demonstrated that microcystin toxin remained in waters even after the cyanobacteria bloom had subsided. In addition to the production of hepatoxins, *Microcystis* may produce geosmin (a taste and odor forming compound).

Drinking water facilities must be vigilant in their detection and removal of microcystin toxins because: (1) greater than 60% of US residents depend upon surface water as their primary source for drinking water (Westick, AWWA, 2002) and (2) microcystins are persistent. The AWWA considers wood based powdered activated carbon the most effective technique for microcystin removal. Coagulation, flocculation, sedimentation and biologically active filters may remove cyanobacteria but may not remove the microcystin toxin. Algicides or pre-oxidants are poor treatment choices for microcystin removal due to their potential to lyse the microcystis cells enabling the release of microcystin toxins. Temporary avoidance of water sources featuring cyanobacteria blooms is considered the treatment strategy of choice (AWWA web broadcast 2008). There is no simple method to distinguish the toxic from the non-toxic cyanobacteria. “The unpredictability of toxin production within any given bloom renders them potentially dangerous and suspect at all times and prevention of cyanobacterial blooms is therefore the key to the control of toxic blooms” (Ressom et al., 1994).

More than 400,000 people rely on Des Moines Water Works for approximately 17 billion gallons of drinking water annually. Therefore, close monitoring of potentially toxic cyanobacteria-tainted surface water is an increasing priority. This project was designed and conducted by the DMWW laboratory. There were two objectives: (1) assess the practicality of instituting a regular microcystin analysis program utilizing an Enzyme-

Linked Immunosorbent Assay (ELISA) method; and (2) evaluate the effectiveness of DMMW treatment for removal of microcystins.

Materials and Methods

Materials.

The ELISA kits used for this study were purchased from Abraxis (Warminster, PA). The assay format was a 96 well microtiter plate based on the recognition of microcystins, nodularins and their congeners by specific antibodies. The Abraxis assay principle is as follows: microcystins, nodularins and their congeners (present in a water sample) compete with microcystin-protein analogues immobilized on the microtiter plate for the binding sites of antibodies in solution. After a washing step and addition of a substrate solution a blue color signal is generated. The intensity of the color is inversely proportional to the concentration of the microcystins present in the sample (Figure2).

Figure 2. Microtiter plate

Figure 3. Plate reader

Additional equipment required for analysis included a microtiter plate reader capable of reading at 450 nm. Dr. Bryan Larsen and Mike Essmann of Des Moines University provided the use of their microtiter plate reader for this project (Figure 3). All samples were analyzed in duplicate. The average analysis time for 41 environmental samples and 7 quality assurance samples (96 total samples) was approximately 3 hours. The cost per kit was $400, or $10.00 per sample. The detection range of the assay was 0.10 – 5 ppb.

Plant assessment.

Both the Fleur and McMullen treatment plants were included in this study. Figure 4 correlates the sample sites at both treatment plants with their abbreviation.

Fleur Treatment Plant

Sample Site	Sample ID
Raccoon River	RR
Des Moines River	DMR
Filter Effluent	FE

McMullen Treatment Plant

Sample Site	Sample ID
McMullen Raw	MR
McMullen Filter Effluent	MFE
Crystal Lake	CL
Maffitt Reservoir	MRES

Figure 4. Sample site identification

Historically, mid- to late-summer and early-fall present favorable environmental conditions for cyanobacteria growth. Therefore, weekly samples were collected July through September, 2008, from both treatment plants for this study.

Remediation Experiments.
Three remediation experiments were conducted to assess microcystin removal during the treatment process. The first assessment evaluated the Fleur plant's chemical dosages at the time of this study. The second assessment evaluated the optimum carbon dosage for microcystin removal. The third assessment evaluated the optimum pH for destruction of microcystin using liquid chlorine.

1. Treatment Assessment.
Individual remediation experiments were conducted to assess the microcystin removal capability for each treatment chemical utilized at the Fleur Drive treatment plant at the time of this study. Chemical concentrations used for the study replicated concentrations utilized at the treatment plant (Figure 5). The removal capability of sand filtration and UV were also examined.

Treatment Chemical	**Concentration Used mg/L, unless noted**
Powdered Activated Carbon, PAC	10
Ferric Chloride, $FeCl_3$	5
Alum	15
Liquid chlorine	2.3
Lime (calcium oxide slurry)	To pH 10.8

Figure 5. Treatment chemical concentrations

Two sample sets were designed. Each set was concurrently dosed with treatment chemicals to achieve the concentrations listed in Figure 5. Set one was spiked to a concentration of 2 ppb microcystin. Set two represented the sample controls or background (ruling out interference of the treatment chemicals with the ELISA).

A sample containing 5mL IG water spiked to a concentration of 2ppb microcystin was paired with an unspiked IG water sample and placed under a UV light for 30 minutes (Figure 6).

Figure 6. Sample under

A 250 mL capacity container was layered (bottom to top) with large rocks, small rocks, pebbles, and sand to simulate the rapid sand filters at the Fleur Plant. An aliquot of 250 ml of IG water was passed through this sand filter and captured. Another aliquot of IG water spiked to a concentration of 2ppb microcystin and passed through the same sand filter and captured (Figure 7).

Figure 7. Sand Filtration

A duplicate, unspiked IG control, and a 2ppb microcystin control (in IG water) were included in this assessment. An aliquot of each sample was frozen until a

batch of 40 samples was collected. All samples were analyzed by the ELISA method and microcystin removal was calculated. Figure 8 represents the paired sample sets. The experiment was repeated for confirmation purposes and to evaluate precision.

Set One Treatment Chemicals	**Set Two Treatment Chemicals**
5mL IG + 10mg/L PAC + 2ppb microcystin	5mL IG + 10mg/L PAC
5mL IG + $FeCl_3$+ 2ppb microcystin	5mL IG + $FeCl_3$
5mL IG + 15mg/L Alum + 2ppb microcystin	5mL IG + 15mg/L Alum
5mL IG + 2.3mg/L liquid chlorine + 2ppb microcystin	5mL IG + 2.3mg/L liquid chlorine
50mL IG + lime to pH 10.8 + 2ppb microcystin	50mL IG + lime to pH 10.8
250 mL IG + 2ppb microcystin through sand filter	250mL IG through sand filter
5mL IG + 2ppb microcystin under UV light	5mL IG under UV light
5mL IG + 2ppb microcystin	5mL IG

Figure 8. Paired Sets

2. Optimum Carbon Dosage Assessment for Microcystin Removal.

An assessment of carbon at a variety of dosages was performed to determine the carbon dosage which yields maximum microcystin removal. Two series of 7-100 ml volumetric flasks were filled with IG water. Flasks in each series were respectively dosed with 0, 5mg/L, 10mg/L, 15mg/L, 20mg/L, 25mg/L and 30mg/L PAC. Microcystin was added to the first series to produce a concentration of 2 ppb. Microcystin was not added to second series (control sets). A 2ppb microcystin-spiked IG water control and an unspiked IG water control were included in this study. All samples were filtered through 45µm filters. Filtration was performed to simulate the PAC in the treatment plant after undergoing sedimentation and filtration. An aliquot of each sample was frozen pending ELISA analysis. The experiment was repeated for confirmation purposes and to evaluate precision.

3. Optimum pH Assessment for Destruction of Microcystin by Chlorine.

An assessment of microcystin destruction by chlorine at a variety of pH levels was performed to determine the optimum. Two sets of 7-100ml volumetric flasks were filled with IG water. Each flask in set 1 was spiked to a concentration of 2 ppb microcystin. Set two, the control set, was not spiked with microcystin. Both sets were dosed with 2.3 mg/L chlorine (13% sodium hyporchlorite) and the pH was adjusted in each pair to pH 7, 8, 9, 10, 11, and 12 with sodium hydroxide and/or HCl. One pair (spiked and unspiked) remained unadjusted for pH (about 7.5) and acted as a control. An aliquot of each sample was frozen pending ELISA analysis. The experiment was repeated for confirmation purposes and to evaluate precision.

Results

Treatment Assessment.

The average results from two separate studies (using current treatment doses in the Fleur Drive Plant) for the removal or destruction of microcystin are presented in figure 9. Sand filtration did not exhibit any microcystin removal. Lime, carbon, and ferric chloride exhibited the greatest microcystin removal (greater than 50%) with lime demonstrating greater than 60% removal potential. Chlorine dosed at 2.3 mg/L mg/L resulted in reductions of microcystin less than 30%. Interferences with ELISA were not produced by any of the treatment chemicals. The recovery of the 2ppb microcystin-spiked IG water was 102%.

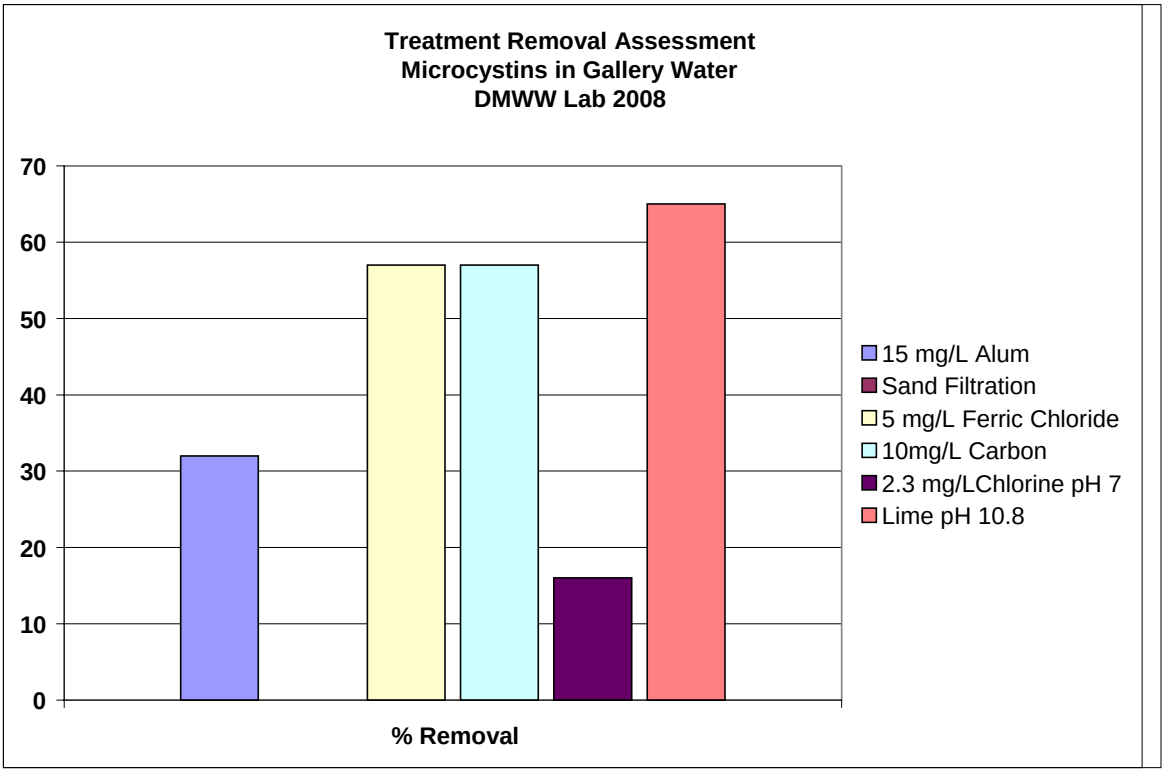

Figure 9. Microcystin Coagulant Removal Assessment

Optimum Carbon Dosage Assessment for Microcystin Removal.

The average results from two filtered carbon assessments for the removal of microcystins are presented in figure 10. The removal potential of the microcystin toxin didn't significantly increase with carbon dose until 25 mg/L PAC. The author speculates the ELISA kit utilized for this assessment contributed to the variance in the results (ELISA kit incorporates a margin of error of : +/- 15%) Filtration did not introduce any error. A dosage of 30mg/L carbon exhibited the greatest microcystin removal: 57%. The recovery of the 2ppb microcystin spiked Gallery water was 110%.

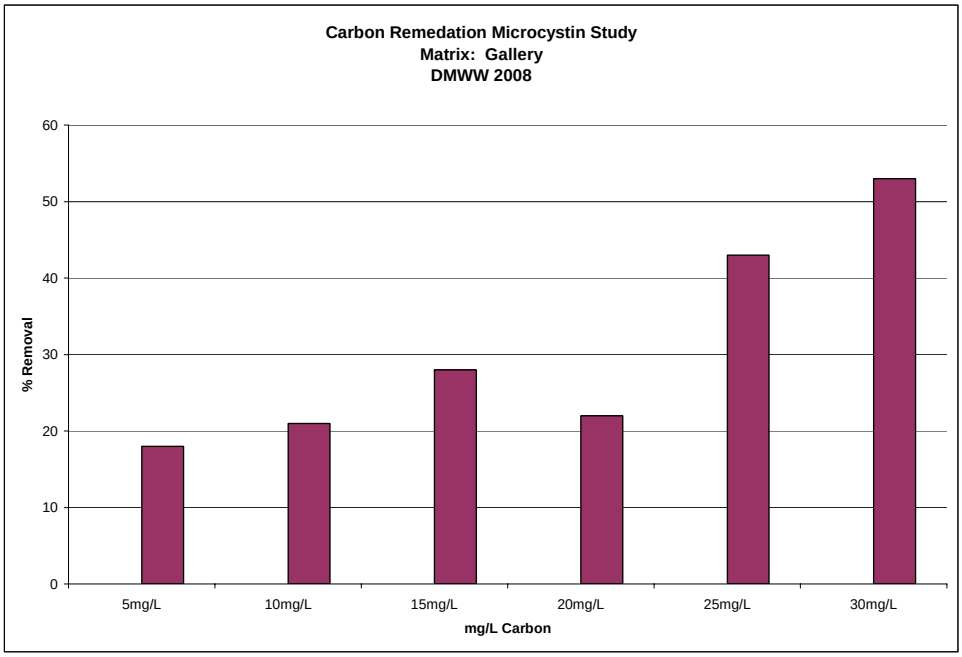

Figure 10. Microcystin Remediation – Carbon

Assessment of Optimum pH for Destruction of Microcystin by 2.3 mg/L chlorine.
The average of 2 microcystin removal assessments at various pH values indicated that in order for a chlorine concentration of 2.3mg/L to be considered an effective remediation tool, the pH of the sample should less than 9 and ideally be at a pH 8 or less (figure 11). These findings concurred with a presentation given by Judy Westrick PhD of Lake Superior State University, Michigan (Westrick, Judy. 2002. ***Everything a Manager Should Know About Algal Toxins but was Afraid to Ask.*** AWWA Annual Conference Proceedings. New Orleans, LA.) Westrick proposed chlorination as an effective treatment tool for destroying microcystins. The presentation also suggested that the inactivation of microcystins by chlorination takes place at pH 9 or less and ideally at pH 8.

Chlorine interference was not detected with the ELISA screen. The recovery of the 2ppb microcystin spiked Gallery water was 102%.

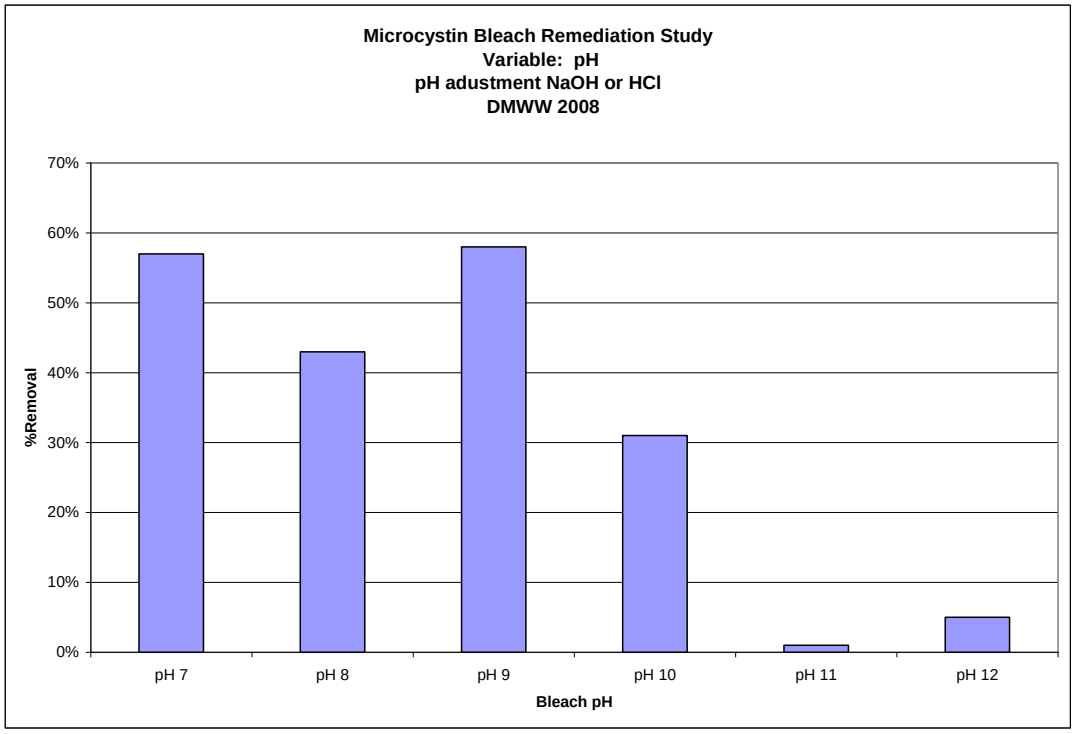

Figure 11. Microcystin Remediation – Bleach

Discussion

All chemicals utilized in the DMWW treatment plants at the time of this study contributed to the removal or destruction of microcystins. Powdered activated carbon (dose of 30mg/L), lime (pH 10.8), and ferric chloride (dose of 5mg/L) all exhibited microcystin removals greater than 50%. The combination of the chemicals in the treatment process was not studied in this assessment. The author can only assume that complete microcystin removal was achieved since microcystins were not detected in the filtered effluent water at either treatment plant.

The 2008 microcystin assessments demonstrated that ELISA kits are useful as a microcystin screen for environmental water samples. ELISA strengths are: reliability, specificity to microcystins, low cost, high sensitivity, and ease of analysis (analysis without sample cell lysing and pre-concentration). However, reverse phase HPLC or HPLC/MS analysis should be employed for the quantification and classification of individual microcystin congeners. Although selective and highly sensitive, both reverse phase HPLC and HPLC/MS analysis are expensive to perform, demand extraction, and require concentration of the extract.

Source water management, including monitoring of nitrogen and phosphorus fertilizer usage, sewage, wastewater, storm water, and flow rate, should remain essential operating practices of DMWW. Weekly monitoring of source water by ELISA, during the summer months following microscopic cyanobacteria detection, would be a proactive and inexpensive approach to detect microcystins. ELISA would also be a useful tool for the evaluation of the treatment process to ensure safe drinking water for the customers of the Des Moines Water Works.