Evaluating Treatment Processes for Removing Cyanobacterial Toxins from Drinking Water Supplies

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SUMMARY

Cyanobacterial, or blue-green algae, are common in surface waters used as sources for drinking water in Alberta. These organisms complicate water treatment processes and contribute to taste and odor problems. Several species also release water-soluble toxins known as microcystins that, if ingested, are capable of causing liver damage. Microcystin-LR (MC-LR) in the most common variant of these hepatotoxins. The most desirable way to remove MC-LR from water supplies is by removing cyanobacterial cells with the toxin intact (i.e. cellular MC-LR).

Clarification processes (involving chemical coagulation, flocculation, and settling or flotation), together with filtration, often form the first lines of defence for removing cells and other particles. Utility operators must also make provisions for removing dissolved (extracellular) MC-LR because the toxin can be released from cells naturally as they age or as a result of treatment operations. Removing extracellular MC-LR requires special treatment processes such as granular activated carbon or chemical oxidation.

This laboratory study used prepared suspensions of two species of cyanobacteria that are common in Alberta, *Microcystis aeruginosa* and *Anabaena flos-aquae.* Experiments were conducted to test clarification processes involving settling or flotation for removing cellular MC-LR. Clarification trials were conducted with and without pretreatments consisting of UV irradiation or chemical oxidation by potassium permanganate ($KMnO_4$) or ozone. As well, the pretreatments were applied independently to assess direct removal of extracellular or cellular MC-LR. The experimental design consisted of three major components:

- Assessing effects of cyanobacterial species, growth stage, alum dose, and method of clarification (settling or flotation) on cell removal as measured by turbidity reduction
- Assessing direct effects on intracellular and extracellular MC-LR concentrations after treating cyanobacterial suspensions with potassium permanganate, ozone, or UV irradiation
- Assessing effects of potassium permanganate, ozone, or UV pretreatments applied prior to clarification processes (settling or flotation) on over-all reduction in turbidity and MC-LR concentrations.

The results of the study showed that flotation was more effective than settling for removing cyanobacterial cells as measured by turbidity reduction. The efficiency of cell removal from the prepared suspensions by settling or flotation was species specific and greater for *M. aeruginosa* than *A. flos-aquae*. Cyanobacteria of different growth stages responded differently to experimental pretreatments; for example, cells in the late growth phase were more efficiently removed following pretreatment with $KMnO_4$. Water treatment operators needing to control cyanobacteria might, therefore, consider using a flotation process and developing a monitoring program to assess changes in upstream cyanobacterial species and growth stages.

Optimizing the dose of $KMnO_4$ and ozone as pre-oxidants was important for minimizing residual turbidity after clarification. Both $KMnO_4$ and ozone were found to be capable of oxidizing

extracellular MC-LR, consistent with previous research (Hart *et al.*, 1998). Ozone was more powerful in this regard, allowing shorter treatment times to be effective. By maintaining a residual ozone level of >0.25 mg/L, low concentrations of both cellular and extracellular MC-LR (<10 μg/L) were reduced to below the 0.07 μg/L detection limit.

Although UV irradiation at treatment levels up to 120 mJ/cm^2 failed to produce measurable reductions in MC-LR concentrations during pretreatment trials, one set of trials involving UV treatment followed by clarification showed a significant reduction in the fraction of extracellular MC-LR remaining in treated water. This observation requires confirmation by further research, but, if confirmed, would have major implications by way of allowing simultaneous disinfection and control of MC-LR.

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1.0 INTRODUCTION

Cyanobacteria, or blue-green algae, are ubiquitous in Alberta's fresh water bodies. Under appropriate light, temperature and nutrient conditions, they may proliferate to such an extent that blooms form. If a cyanobacterial bloom occurs in a lake or reservoir used as a drinking water source, the water utility may face several water quality problems that require special attention. Taste and odor compounds (e.g., geosmin or methyl isoborneol) may be produced by many species (Rashash *et al*., 1996). In addition, cyanobacterial may be difficult to remove by settling, leading to increased chemical use and shortened filter runs (Westrick, 2003). Furthermore, any carryover of particulate, measured as turbidity, may compromise disinfection processes. However, the greatest concern in terms of human health is the production of low molecular weight metabolites, often referred to as cyanotoxins.

Cyanotoxins have a variety of toxic properties and are categorized according to their modes of action in mammalian test systems. These categories include neurotoxins (e.g. saxitoxin and anatoxin), hepatotoxins (e.g. microcystins and nodularins), and lipopolysaccharides. Hepatotoxins (liver toxins) have the widest distribution in natural waters; of these liver toxins, microcystins are most commonly encountered and most researched. Microcystins are produced by several cyanobacterial genera including *Mycrocystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Nostoc*, and *Phormidium*. Microcystin-LR (MC-LR) is the most common variant of these cyclic peptide hepatotoxins, although 71 variants have been identified (Metcalf and Codd, 2004). While recognizing that other microcystin variants may be as toxic, Health Canada has established a 1.5 μg/L guideline for MC-LR in drinking water, considering this to be protective of human health against exposure to all microcystins (Health Canada, 2003).

In aquatic environments microcystins are produced within cyanobacterial cells and are found primarily in slow-growing, healthy cells. Until a bloom collapses or algal cells are disturbed by a treatment process, most toxins are retained within the cells. Mole *et al* (1997) showed that cultured *Microcystis aeruginosa* began to release microcystin late in the exponential growth phase and that substantially more was released as the integrity of the cells decreased during the stationary phase. More generally, a semi-empirical mathematical model developed by Belov (1998) for phycotoxin release by cyanobacteria cells predicts increases in water toxicity 15-30 days after cell integrity begins to decrease. Dissolved microcystins are quite persistent in natural waters and may require months or even years to break down under dark conditions (WHO, 1999). Slow photochemical decomposition occurs under bright sunlight with the rate enhanced in the presence of cell pigments or humic substances. Observing natural decomposition of MC-LR in Lake Tuusulanjarvi water, Lahti *et al.* (1997) found that 30 days were required to reduce the dissolved toxin by 90% compared to 15 days when the toxin was present in particulate matter. In addition to breakdown under sunlight, microcystins can undergo natural biological decomposition. Biodegradation of microcystin-LR may occur on the Adda side chain, thereby reducing its toxicity (Cousins *et al*., 1996).

In a previous study of the prevalence of microcystin in raw and treated water supplies in Alberta, Zurawell (2002) reported detectable levels (>0.07 μg/L) in 67% of the raw waters sampled and 10% of the treated waters. Concentrations up to15 μg/L were measured in raw water while

microcystin levels in treated water at the same site were at least 25 times lower. Much higher concentrations would be expected within bloom material on Alberta lakes. Elsewhere, microcystin concentrations as high as 25,000 μg/L have been reported in samples of bacterial scums (WHO, 1999).

A priority during water treatment is to remove cyanobacteria cells as suspended solids with most of the toxin intact, thus avoiding the relative complexity of removing soluble toxins resistant to physicochemical treatments. Typical water treatment facilities remove suspended solids by applying chemical coagulants to destabilize particles, flocculating to promote aggregate growth, separating solids by settling or flotation, and finally filtering. Cyanobacteria are naturally buoyant, which makes their treatment by conventional settling processes more difficult. In spite of this, conventional treatment involving settling and filtration can be effective for removing cells and intracellular toxins (Hart *et al.*, 1998). Clarification by dissolved air flotation is particularly effective in removing suspensions of algae and cyanobacteria.

Processes designed to remove suspended particles sometimes inadvertently produce soluble toxins. Pietsch *et al*., (2001) reported an increase in extracellular toxin following flocculation and filtration processes. Some drinking water plants add chemical oxidants such as potassium permanganate, chlorine, or ozone to raw water to enhance removal of suspended solids and to improve disinfection and taste and odor control. Care must be taken with this approach because chemicals used for preoxidation may cause cell lysis, thereby releasing toxins (Kenefik *et al*., 1993; Lam *et al*., 1995). The decision about whether to use a preoxidation process is further complicated because strong oxidants like ozone and chlorine are also effective for breaking down extracellular microcystin (Westrick, 2003).

The removal of dissolved or extracellular toxins cannot be achieved with conventional treatment involving coagulation and settling (Hart *et al.*, 1998). Extracellular MC-LR can be removed by adsorption onto granular or powdered activated carbon, by biologically activated carbon, or by chemical oxidation using ozone, potassium permanganate, or chlorine (Hart *et al.*, 1998). The effectiveness of chlorine, however, was found to be pH and dose dependent (Nicholson, *et al.*, 1994). During chlorination with pH levels greater than 8 (such as found in many of Alberta's raw water sources) the rate of microcystin removal slows due to the prevalence of hypochlorite ion rather than the more powerful oxidizer, hypochlorous acid. Under these conditions, chlorine doses that achieve a residual of 0.5 mg/L for 30 minutes of reaction time are required. The combined chlorine species commonly used to ensure disinfectant residual (chloramines) have been shown to be ineffective in reducing microcystin concentrations.

While ultraviolet (UV) irradiation as sunlight plays an important role in degrading microcystin in natural waters, its application as an engineered (rapid) process may not be cost-effective. Carlyle (1994) found that removing microcystin-LR required a UV fluence two orders of magnitude higher than that generally used for disinfection. Because of the widespread acceptance of UV treatment for inactivating waterborne protozoa such as *Cryptosporidium*, UV has been included as a pretreatment in the current investigation, but using fluence levels only two or three times higher than would typically be used for disinfection purposes. If UV irradiation could be shown to break down or improve removal of cellular microcystin without disrupting cell membranes, UV might find practical application as a pre-clarification process.

2.0 EXPERIMENTAL DESIGN

The purpose of this study was to:

1) Evaluate the effects of alum dose and clarification technique (settling or flotation) on the removal of cyanobacterial cells.
2) Evaluate the effects of UV irradiation and oxidative pretreatments on the above clarification processes with respect to removing cyanobacterial cells and MC-LR.

Prepared suspensions of two species of cyanobacteria that are common in Alberta, *Microcystis aeruginosa* and *Anabaena flos-aquae,* were used in experiments to evaluate potential effects of cell morphology on toxin removal. The experimental design consisted of three major components:

- Assessing effects of cyanobacterial species, growth stage, alum dose, and method of clarification (settling or flotation) on cell removal as measured by turbidity reduction (clarification trials)
- Assessing direct effects on intracellular and extracellular MC-LR concentrations after treating cyanobacterial suspensions with potassium permanganate, ozone, or UV irradiation (pretreatment trials)
- Assessing effects of potassium permanganate, ozone, or UV pretreatments applied prior to clarification processes (settling or flotation) on over-all reduction in turbidity and MC-LR concentrations (pretreatment followed by clarification).

Factorial experiments were used for all the experiments involving clarification and/or pretreatments. The direct clarification trials (Fig. 1) were conducted with three factors: cyanobacterial species, alum dose, and clarification process (settling vs. flotation). The response parameter was turbidity or percent reduction in turbidity. Individual three-level experiments were used to evaluate direct effects of pretreatments processes (Fig. 2) on intracellular and extracellular MC-LR concentrations. These trials were conducted on both cyanobacterial species, but were not replicated. They were intended to identify any obvious effects and to assist in establishing the two levels of oxidative and UV pretreatments to be used in the subsequent four-factor experiments.

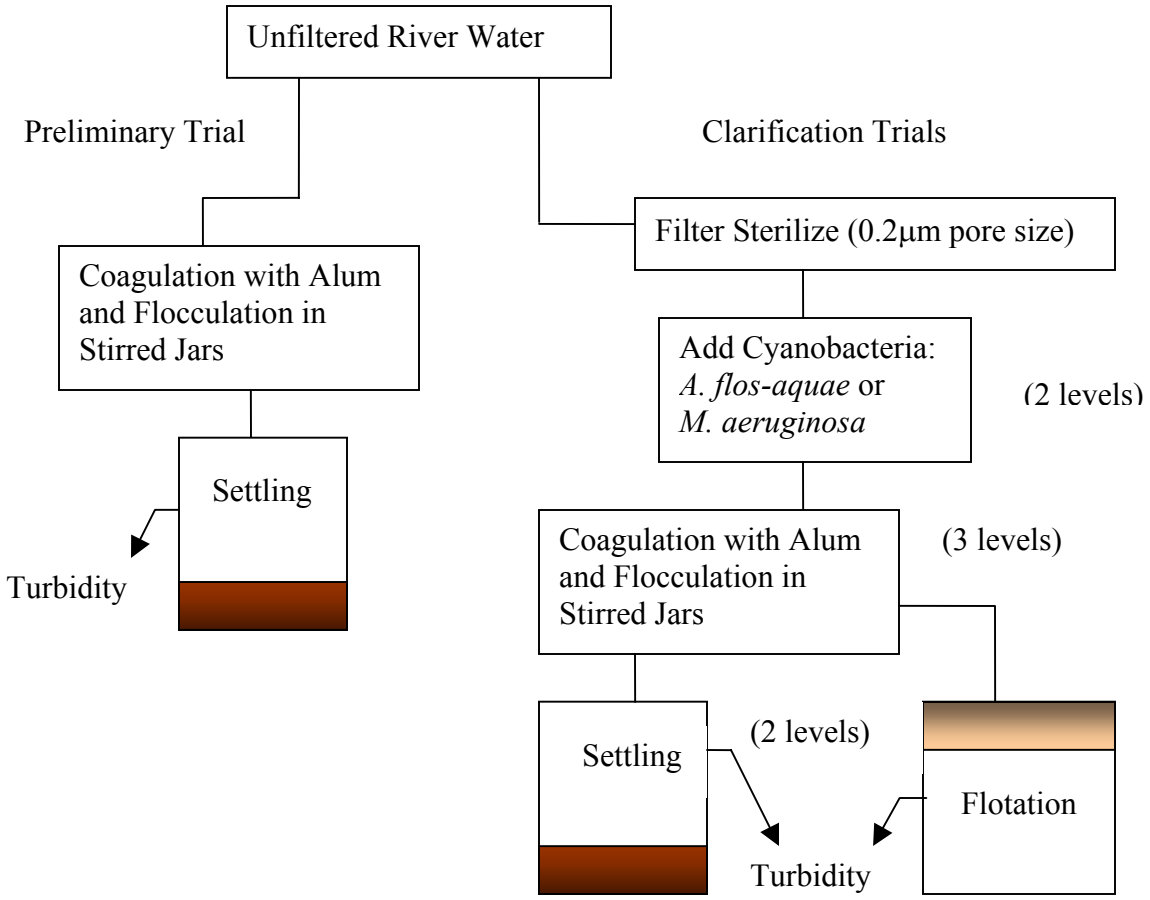

Figure 1 Schematic representation of the clarification trials

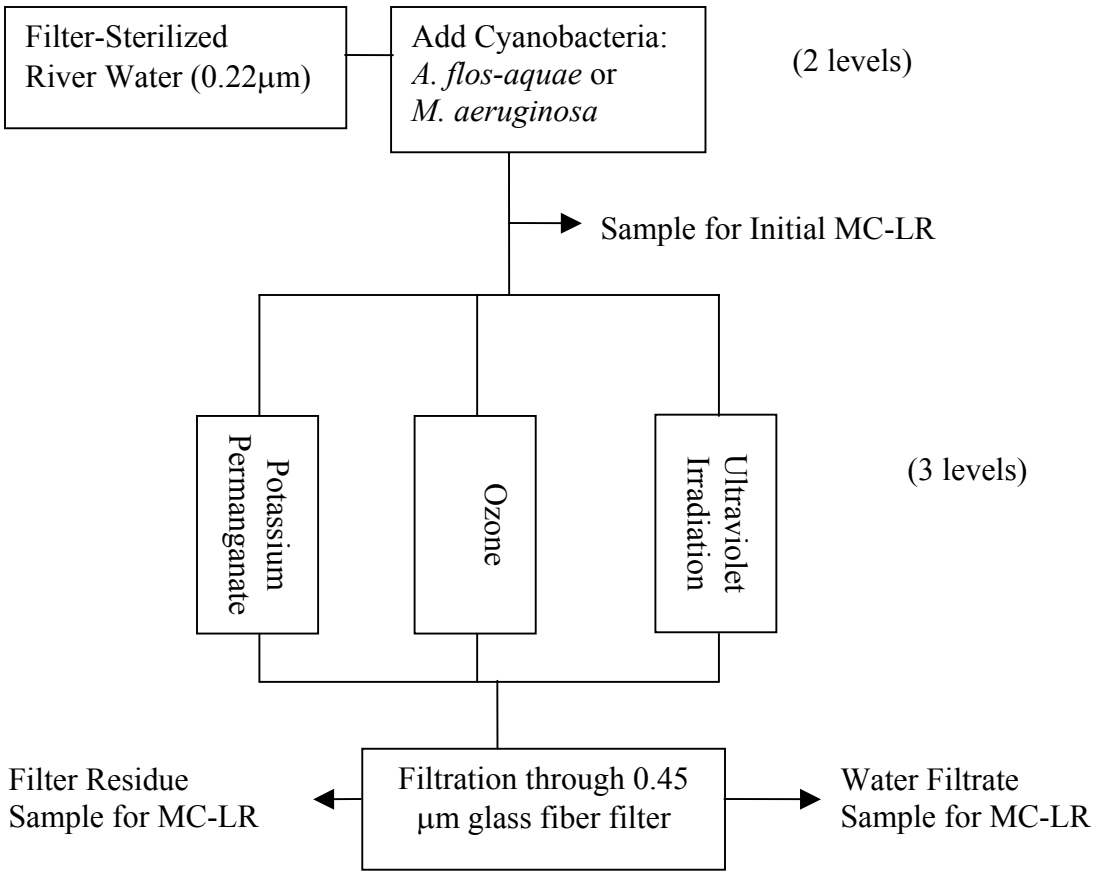

Figure 2 Schematic representation of the pretreatment trials

The combined pretreatment/clarification trials are illustrated in Figure 3. All four factors (species, growth stage, pretreatment dose, and clarification technique) were conducted at two levels.

A separate set of four-factor trials was conducted for each of the three pretreatment techniques. Response parameters included turbidity and microcystin-LR concentration in both filtrate and residue samples.

Two-level designs are considered efficient because they can explore a wide range for each factor, indicate major trends, and determine promising directions for further research (Box *et al.*, 1978). The algorithms used for calculating main effects and interactions (NCSS, 1996) are equivalent to least squares multiple regression analyses. For each regression analysis the null hypothesis was that factors were insignificant. The regression outputs provided linear coefficient estimates (magnitude and direction) and showed whether each factor and interaction was significant (non-zero coefficient) at the specified level of significance (typically α=0.05).

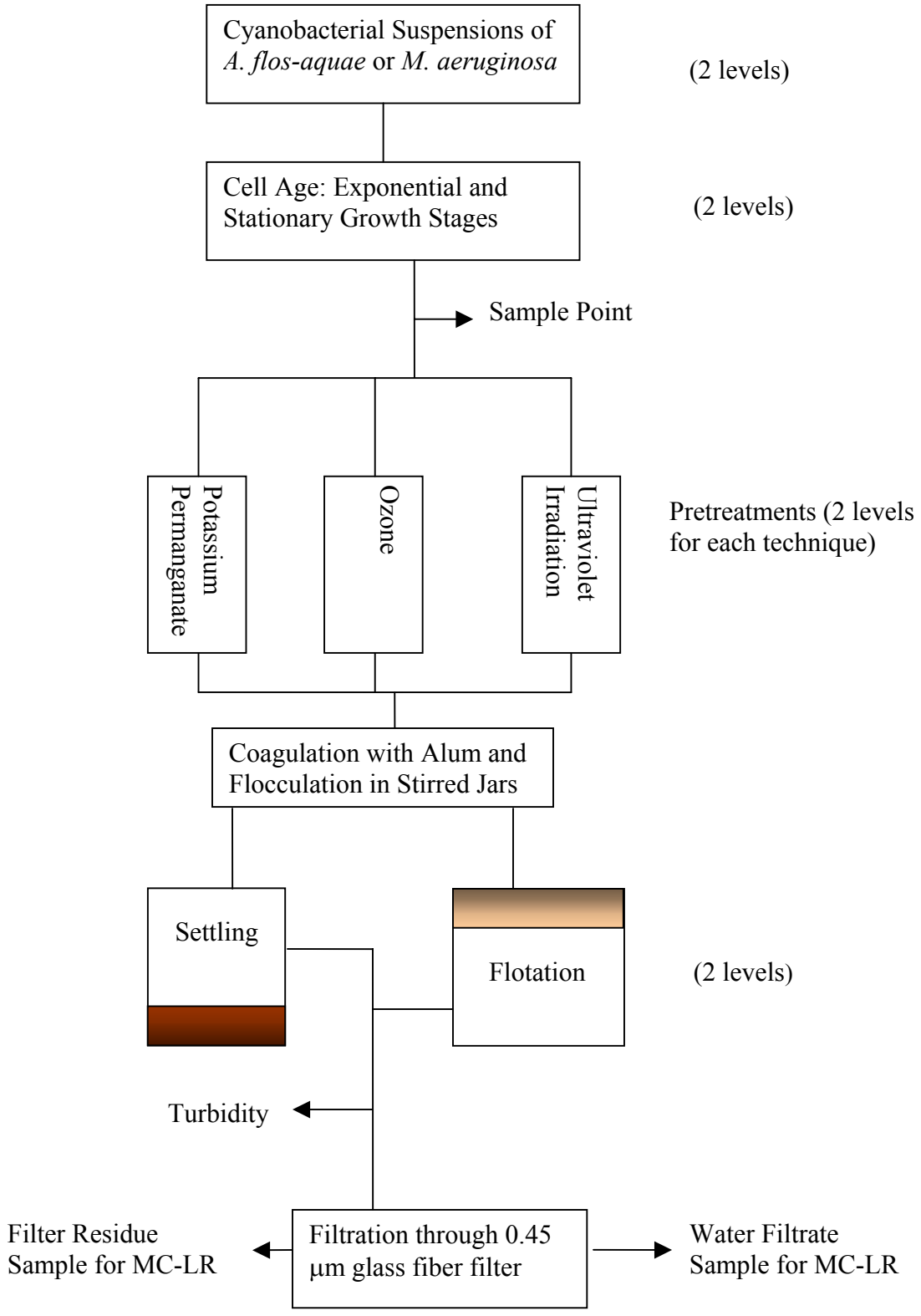

Figure 3 Schematic representation of pretreatments followed by clarification

3.0 MATERIALS AND METHODS

Experiments to examine the effects of pretreatment and clarification processes for removing cyanobacteria and the toxin MC-LR were conducted between June and November 2003 at the Alberta Research Council in Vegreville.

3.1 Preparation of Cyanobacteria Cultures

Pure cultures of *Anabaena flos-aquae* and *Microcystis aeruginosa* were obtained from the University of Toronto Culture Collection (UTCC). The culture strain numbers were UTCC 67 and UTCC 299, respectively. Cultures were grown in BG-11 media in incubators kept between 25° and 28° C, operating with a 16-hour photoperiod (16/8 hour day/night cycle) and a light intensity of 1200 lux. Subsequent cultures were obtained by transferring 10% to 20% of existing cultures to fresh BG-11 media and incubating under the prescribed conditions. Cyanobacteria cell counts in stock solutions were obtained with a haemocytometer using the mean value from 10 squares. Depending on the experimental procedures for each set of trials, stock solutions of up to 6 litres of *M. aeruginosa* and 3 litres of *A. flos-aquae* were required to produce dilutions of approximately 5×10^6 cells/mL. Somewhat lower cell concentrations were used for preliminary experiments, until a relationship with MC-LR production was established. Following dilution and prior to their use in experiments, cell cultures were agitated gently to keep colonies in suspension without causing them to break apart.

Because cell growth phase has been shown to affect microcystin release, two cell growth stages were employed for each species. Cell ages corresponding to late exponential growth and intermediate stationary growth phases were determined for both cultures based on growth curves obtained by plotting cell counts versus time.

3.2 Treatment and Characterization of Dilution Water

Throughout the province cyanobacteria thrive in surface waters with a range of chemical and organic matter compositions. For diluting cyanobacterial suspensions in the current study, it was decided to select a well-characterized potable water source and one that had not been subjected to chemical treatments. Accordingly, North Saskatchewan River (NSR) water was obtained from near the boat launch at Duvernay, Alberta. Raw water was used for preliminary tests to determine a suitable range of alum doses for clarification trials. It was characterized by analyzing for turbidity, alkalinity, pH, dissolved organic carbon (DOC), and UV_{254} absorbance. River water to be used for cyanobacterial dilutions was sterilized by passing it through 0.22 μm filters to ensure that naturally occurring cyanobacteria and other biota would not be present to confound experimental results. Each time water was drawn from the river, a sample of filtered water was analyzed for turbidity, alkalinity, pH, DOC, and UV_{254} absorbance. The water characterizations are summarized in Table 1.

Table 1 Characterization of North Saskatchewan River Water

Sampling Date	Alkalinity (mg/L as $CaCO_3$)	pH	Turbidity (NTU)	UV_{254} Absorbance (cm^{-1})	DOC (mg/L)
August 5th (raw)	123	8.26	21	0.065	3.5
Sept–Oct (0.45 μm-filtered, n = 3)	Not Analyzed	7.8 – 8.5	0.03 – 0.07	0.032 – 0.043	1.5 – 2.0

3.3 Microcystin-LR Assays

MC-LR was differentiated as intracellular or extracellular by filtration, assuming that cells would be retained on 0.45 μm glass fiber filters. Thus any MC-LR detected in filtrate was termed "extracellular" and any detected in filter residue was considered to be cell-bound or "intracellular". Samples to be analyzed for MC-LR were frozen and sent to HydroQual Laboratories where they were analyzed following the protein phosphatase inhibition (PPI) assay (An and Carmichael, 1994).

3.4 Preliminary Clarification Tasks

3.4.1 Clarification of River Water

Prior to conducting settling and flotation trials with suspensions of cyanobacteria, preliminary settling tests were performed using raw NSR water. A sample was subjected to a series of jar tests on June 20th to determine the effects of alum dose on coagulation and settling. The response parameters pH, DOC, UV_{254} absorbance, and turbidity were plotted versus alum dose to determine the level of treatment most likely to be applied in a water treatment facility. Specific UV absorbance (SUVA) calculated by dividing UV absorbance by DOC (100× mg/L-cm) was also plotted because of its possible association with DOC aromaticity and trihalomethane formation potential (Amy *et al*., 2001). Alum concentrations ranged from 0 to 5 mg_{Al}/L. A Phipps and Bird jar test apparatus equipped with 2-L Hudson jars was used for these trials (Hudson and Wagner, 1981). The rapid mix duration was one minute at a rotational speed of 120 rpm, corresponding to a shear rate of approximately 118 s^{-1}. The flocculation time was 20 minutes at a rotational speed of 40 rpm, corresponding to a shear rate of 33 s^{-1}. Results of this preliminary trial were used as the basis for selecting a smaller range of alum doses for subsequent clarification trials that used filtered NSR water for diluting cyanobacteria suspensions.

3.4.2 Clarification of Cyanobacteria Suspensions

Factorial experiments were performed on September 11th and October 9th to assess the effects of alum dose, cyanobacteria species, and clarification technique (settling vs. flotation) on turbidity reduction. Three alum concentrations were used corresponding to 2, 3, and 4 mg_{Al} /L. NSR water filtered through 0.22 μm membrane filters was used to dilute the cyanobacteria

suspensions to approximately 5×10^6 cells/mL. Trials conducted on September 11th made use of cyanobacteria in the late log-growth phase, while trials conducted on October 09th used cyanobacteria in the intermediate stationary growth phase. The different growth phases were expected to exhibit different fractions of intracellular vs. extracellular microcystin-LR. Both sets of trials used turbidity reduction as a response parameter. Samples from the September 11th trials were also analyzed for particle counts (PC) as a measure of cell removal. However, the available PC method only counted particles in the 5 to 100 μm range, which would exclude most individual cells. Therefore this response parameter was not used in the second set of trials.

3.5 Establishing Pretreatment Levels

3.5.1 Determining $KMnO_4$ Demand

Potassium permanganate ($KMnO_4$) was added to stirred beakers of 0.45 μm filtered NSR water to produce doses of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L. After 0, 10, 20 and 30 minutes of contact time the residual concentrations were measured by comparing absorbance readings at 545 nm with a previously determined calibration curve. By plotting residual $KMnO_4$ readings versus time, it was possible to select initial $KMnO_4$ doses to produce known 30-minute residual concentrations. This procedure assumed that the incremental demand created by adding cyanobacteria to NSR water would be small.

During pretreatment trials, $KMnO_4$ was added at initial concentrations up to 1.0 mg/L in filtered NSR water and the residual concentrations were measured after 30 minutes of contact. As shown in Figure 4, a 0.2 mg/L residual was achieved after 15 minutes of contact with an initial $KMnO_4$ dose of 0.4 mg/L. An initial dose of 0.6 mg/L was required to achieve the same residual after 30 minutes of contact time. Based on these results, initial doses were selected at 0.2 and 0.8 mg/L for subsequent trials involving $KMnO_4$ followed by clarification. However, to examine the effects of $KMnO_4$ alone (August 1st trials) on MC-LR removal, a wider range was used with doses of 0, 0.4, 0.8 and 1.2 mg/L.

3.5.2 Determining Ozone Demand

Ozone demand tests were conducted using a semi-continuous apparatus in which ozone was applied to 2.35-L samples in a counter-current, recirculating bubble column. The rate of ozone application and off-gassing were monitored continuously using UV absorbance spectrophotometers calibrated with standard solutions of potassium iodide. Sufficient ozone was applied to produce residual concentrations between 0.2 and 0.4 mg/L. Samples were collected at two-minute intervals and analyzed for residual ozone by the Indigo method (American Public Health Association *et al.*, 1998). Ozone demands were assessed for filtered NSR water and suspensions of *M. aeruginosa* and *A. flos-aquae*.

Experimental observations recorded following the ozonation of 0.45 μm-filtered NSR water are illustrated in Figure 5. The water had very low ozone demand and showed a 0.54 mg/L residual ozone concentration following 5 minutes of ozonation.

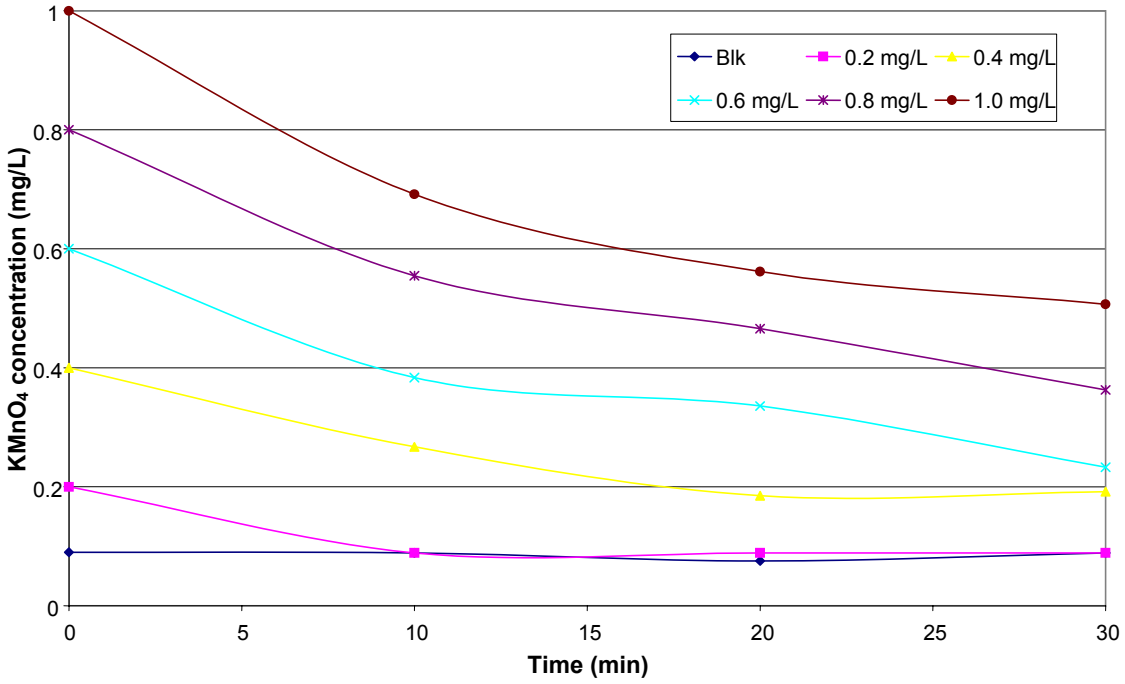

Figure 4 **Potassium permanganate decay curves**

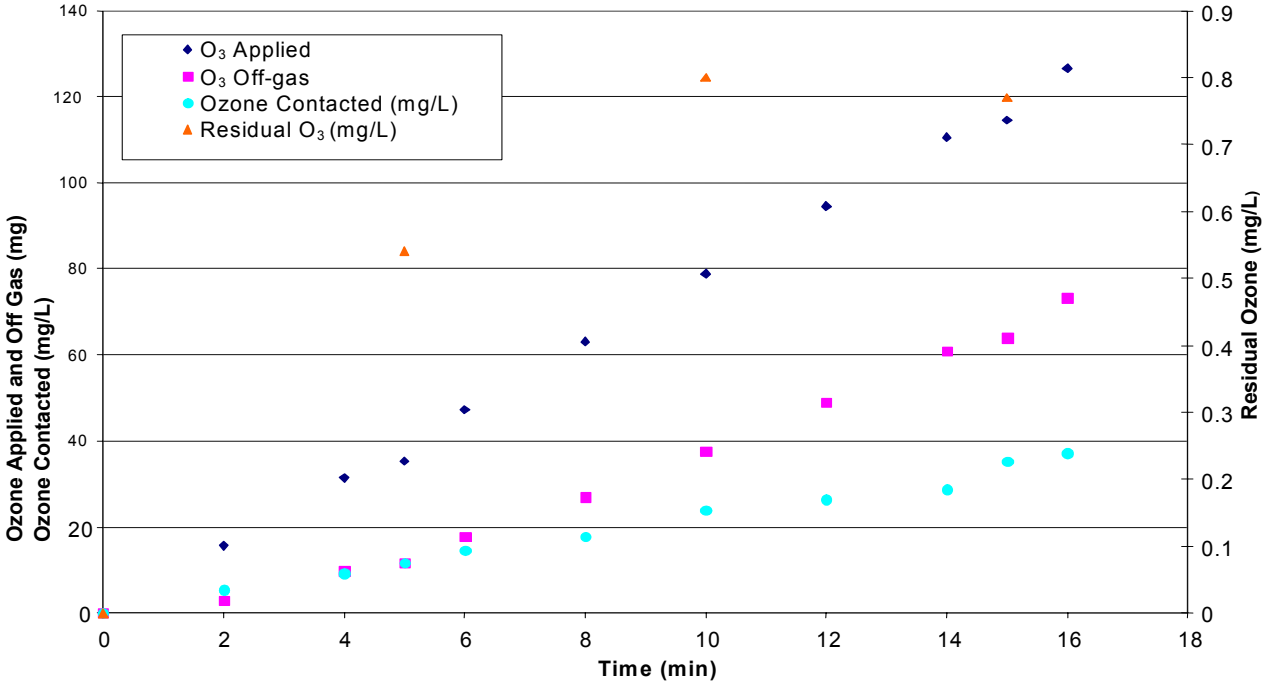

Figure 5 **Ozonation of 0.45 *u*m-filtered North Saskatchewan River water (August 01, 2003)**

Figures 6 and 7 show how the applied ozone was accounted for during trials with suspensions of *A. flos-aquae* and *M. aeruginosa*, respectively. Samples to be analyzed for residual ozone and MC-LR were drawn following 5, 10, and 15 minutes of ozonation, corresponding to residual concentrations of 0.24 to 0.25, 0.37 to 0.38, and 0.39 to 0.40 mg/L, respectively, in each suspension. Notice that more ozone was contacted to achieve these residual concentrations in the *A. flos-aquae* suspension.

3.5.3 Determining UV Fluence

UV irradiation took place using a Katadyn Systems 1-XS reactor with a low pressure, mercury lamp and an effective volume of 1.5 L. The solutions containing cyanobacteria were passed through the UV chamber at rates corresponding to UV fluences between 40 and 120 mJ/cm^2. Previous investigations (James and Skinner, 1994) had defined the UV power intensity in relation to lamp temperature. For example, a lamp temperature of 50° C was typical when water at room temperature was passed through the unit and the corresponding output was 4.8×10^{18} quanta/s. The lamp intensity based on a 0.0274 m^2 quartz sleeve contact area was 13.7 mJ/s·cm^2. With the 1.5 L UV chamber volume, the specified UV fluences ranging from 40 to 120 mJ/cm^2 were achieved using water flows ranging from 30.8 L/min to 10.3 L/min. Sufficient volumes of cyanobacteria solutions were prepared to ensure that at least three volumes of the test solution were flushed through the chamber prior to drawing samples. Flow rates were set using a pre-calibrated progressive cavity pump with an adjustable DC motor controller.

3.6 Assessing Effects of Pretreatments on MC-LR

On August 1st each of the pretreatments was applied to *M. aeruginosa* and *A. flos-aquae* suspensions diluted to approximately 1.5×10^5 cells/mL in order to determine any direct removal of MC-LR (i.e., without a clarification process). Three levels were applied for each pretreatment as shown in Table 2. The ozone levels represent dissolved residual concentrations measured in water containing the cyanobacteria suspensions after 5, 10, and 15 minutes of ozonation.

Table 2 Levels used to assess the effects of pretreatment processes on microcystin-LR concentrations

Pretreatment Process	Low Level	Intermediate Level	High Level
$KMnO_4$	0.4 mg/L	0.8 mg/L	1.2 mg/L
Ozone	0.24-0.25 mg/L	0.37-0.38 mg/L	0.39-0.40 mg/L
UV_{254}	40 mJ/cm^2	80 mJ/cm^2	120 mJ/cm^2

Samples were frozen and sent to HydroQual Laboratories to be analyzed for microcystin-LR.

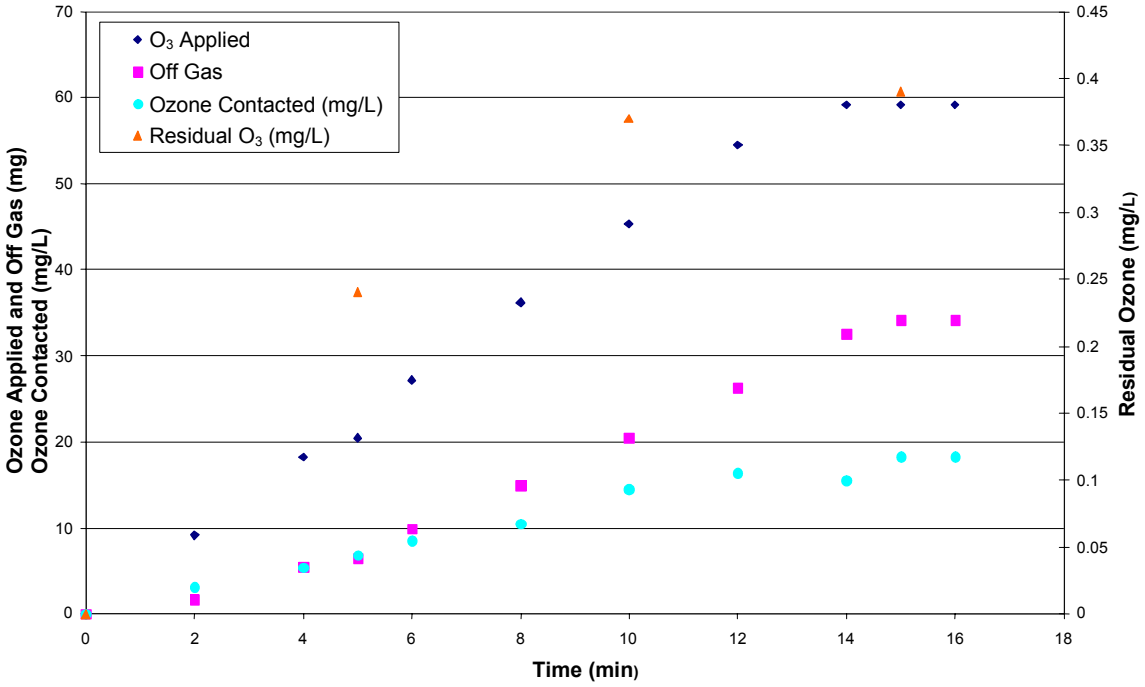

Figure 6 **Ozonation of river water with an *A. flos-aquae* suspension of 1.5x10^5 cells/mL (August 01, 2003)**

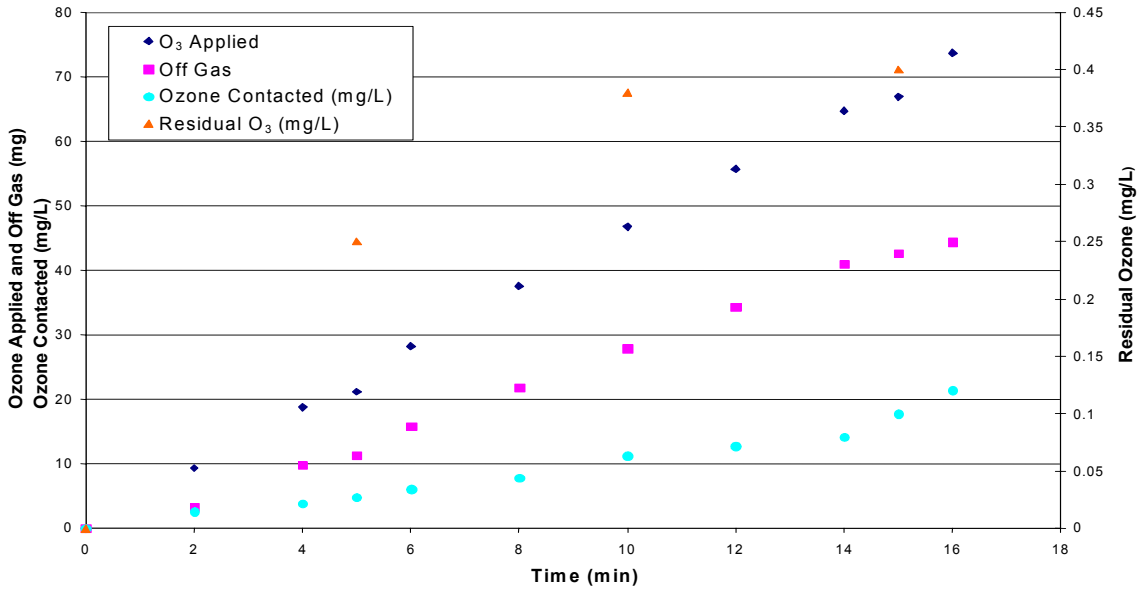

Figure 7 **Ozonation of river water with a *M. aeruginosa* suspension of 1.5×10^5 cells/mL (August 01, 2003)**

3.7 Clarification by Settling and Flotation

Clarification procedures were similar following all of the pretreatments. A common apparatus, the ECE DAF jar tester (EC Engineering, 2003) illustrated in Figure 8, was used for coagulation, flocculation, settling, and flotation processes. This apparatus had six 1-L square section jars, paddle stirrers, and a manifold that allowed pressurized air to be released into jars designated for the flotation process. Alum was added to each jar to provide the equivalent of $3mg_{AL}/L$. One minute of rapid mix at 300 rpm was followed by 20 minutes of flocculation time at 40 rpm. The corresponding shear rates for rapid mix and flocculation were 1000 and 45 s^{-1}, respectively. After 20 minutes of flocculation, the paddles were stopped and a super-saturated charge of water was added to the jars designated for flotation. A 10-minute interval was allowed prior to sampling flotation jars and an additional 10 minutes prior to sampling settling jars.

Figure 8 The EC Engineering dissolved air flotation batch tester

3.8 Clarification Following Potassium Permanganate Pretreatments

3.8.1 August 13th Experiments

Based on low (near detection limit) extracellular MC-LR concentrations measured during the August 1 pretreatment trials, more concentrated suspensions of cyanobacteria were prepared for the August 13th trials. By making best use of the available incubation chambers, suspensions of 5.7×10^5 cells/mL for *M. aeruginosa* and 2.1×10^6 cells/mL for *A. flos-aquae* were obtained. A two-level design was used to test the effects of four factors: initial $KMnO_4$ dose, cyanobacteria species, cell age, and clarification process. The initial concentrations of $KMnO_4$ added to cyanobacteria solutions were 0.2 and 0.8 mg/L. After adding the appropriate dose of $KMnO_4$ to each 1-L jar, agitation was continued for 30 minutes at 60 rpm prior to adding alum and beginning the clarification process.

Samples of clarified water were analyzed for turbidity, pH, DOC, UV_{254} absorbance and MC-LR. DOC and UV_{254} absorbance samples were included to provide additional means of

evaluating the clarification processes and to determine any effects of pretreatment. Both DOC and UV_{254} absorbance samples were filtered through 0.45 μm filters prior to analysis. Samples for MC-LR were filtered through 0.45 μm glass fiber filters and both the filtrate and filter residue were frozen. Because no MC-LR had been detected in *A. flos-aquae* samples following the August 1st pretreatment trials, only clarified *M. aeruginosa* samples and untreated dilutions of both species were submitted to HydroQual Laboratories to be analyzed for MC-LR.

3.8.2 October 8th Experiments

The procedures followed on October 8th were very similar to those of August 13th, with the exception that cyanobacteria dilutions of 5×10^6 cells/mL were prepared for both species and cell ages.

3.9 Clarification Following Ozone Pretreatments

3.9.1 September 10th Experiments

Cyanobacteria dilutions of 5×10^6 cells/mL were prepared for both species and cell ages. A two-level design was used for the experiments with four factors: ozonation time (dose), cyanobacteria species, cell age, and clarification process. A preliminary ozonation trial using filtered NSR water was performed to provide a baseline for comparing ozone demands in subsequent trials with cyanobacteria suspensions. Ozonation times were set at four and eight minutes. Ozone application and off-gas concentrations were measured using online monitors. Dissolved residual ozone concentrations were measured by the Indigo method.

The coagulation, flocculation, settling and flotation processes were carried out in the same way as described in the preceding section. DOC and pH analyses were performed only on untreated samples and on a selected sample from each culture that had been subjected to 8 minutes of ozonation prior to flotation. All samples were processed for turbidity and UV_{254} analyses. Clarified *M. aeruginosa* samples and untreated dilutions of both species were submitted to HydroQual Laboratories for MC-LR analysis.

3.9.2 October 9th Experiments

Another two-level factorial experiment was conducted using older (13 and 14 day old) dilutions of *M. aeruginosa* and *A. flos-aquae*, respectively. The factors were ozone dose (2 or 4 minutes of ozone application), cyanobacteria species, and clarification method (settling or flotation). The ozonation and clarification procedures were the same as described above. All samples were analyzed for turbidity, UV_{254}, and DOC. Clarified *M. aeruginosa* samples were submitted to HydroQual Laboratories to be analyzed for MC-LR.

3.10 Clarification Following Ultraviolet Pretreatments

Cyanobacteria suspensions of approximately 5×10^6 cells/mL (both species and growth stages) were prepared for September 24th. A two-level, four-factor experiment was conducted that

included pretreatments with UV_{254} at a fluence of either 60 or 120 mJ/cm^2. UV fluence was controlled as described in section 2.3.3. Coagulation, flocculation, settling and flotation procedures were conducted as previously described. Samples were drawn and prepared for turbidity, UV_{254}, and particle count assessments. DOC and pH were measured only in samples representing older cyanobacteria cultures clarified by flotation.

3.11 Statistical Procedures

Two-level factorial designs were used to estimate main effects (magnitude and direction) and their interactions for each response variable (e.g., MC-LR). One useful feature of two-level designs is the ability to evaluate both quantitative and qualitative variables. The investigations evaluated cyanobacterial species and clarification technique (settling or flotation) as qualitative variables. Estimates of variance, and hence the significance of effects, were obtained by assuming higher order interactions to be due to experimental noise (Box *et al.*, 1978). Because no two cultures of cyanobacteria were considered identical, each set of trials was treated as a separate experiment and no attempt was made to combine data from similar experiments conducted on different dates.

4.0 RESULTS

4.1 Cyanobacteria Cultures

Growth curves for *A. flos-aquae* and *M. aeruginosa* are shown in Figures 9 and 10, respectively. Using these curves, the late log-growth stages were determined to be 6 days for *A. flos-aquae* and 5 days for *M. aeruginosa*. The intermediate stationary growth stage was then assumed to be one week later for each species (i.e., 13 and 12 days, respectively).

4.2 Clarification Trials

The results of preliminary jar tests conducted on June 20th using unfiltered NSR water are plotted in Figure 11. Increasing the alum dose resulted in lower pH, UV_{254} absorbance, and SUVA. DOC was also reduced, but the response appeared to diminish for alum doses greater than 3 mg_{AL}/L. The lowest turbidity readings were achieved using alum doses of 2 or 3 mg_{AL}/L. Based on these results, an alum dose of 3 mg_{AL}/L was selected as the intermediate point for subsequent clarification trials involving cyanobacteria suspensions and filtered NSR water.

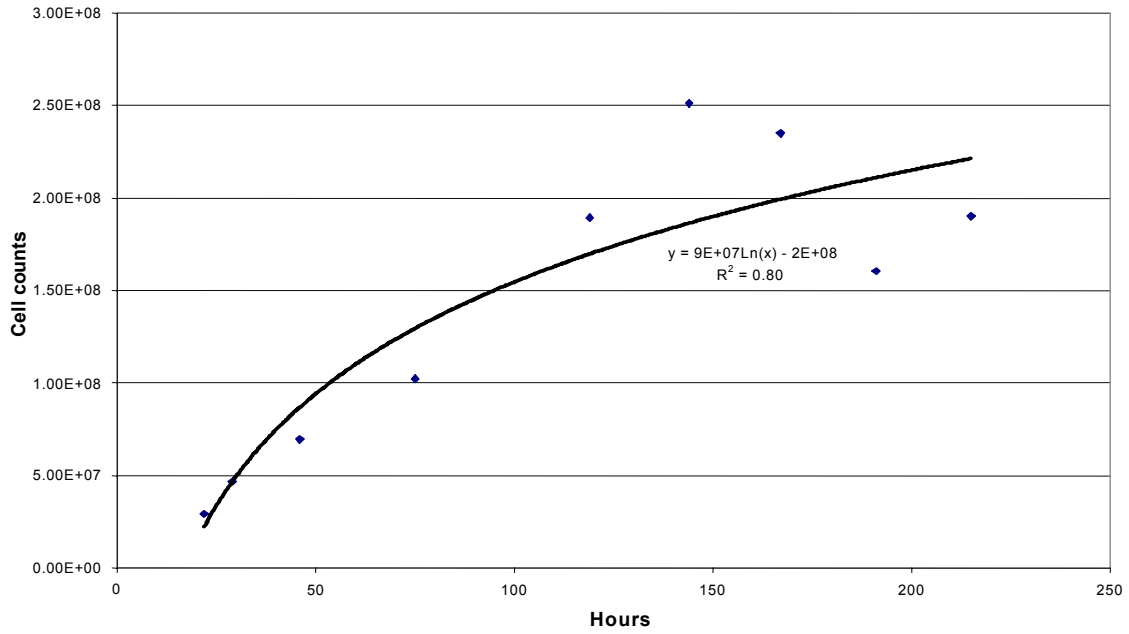

Figure 9 ***Anabaena flos-aquae*** **growth curve**

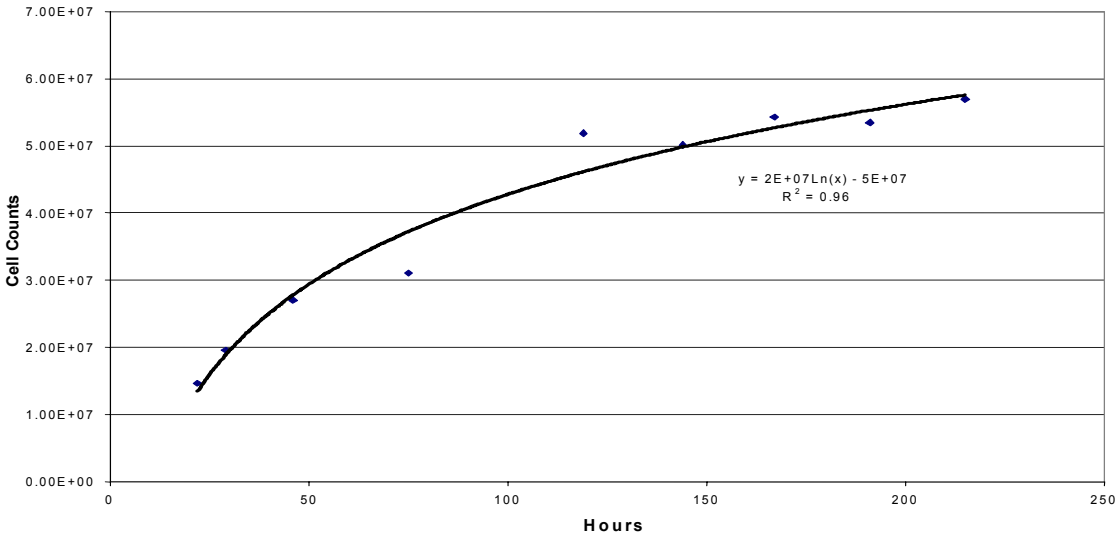

Figure 10 ***Microcystis aeruginosa*** **growth curve**

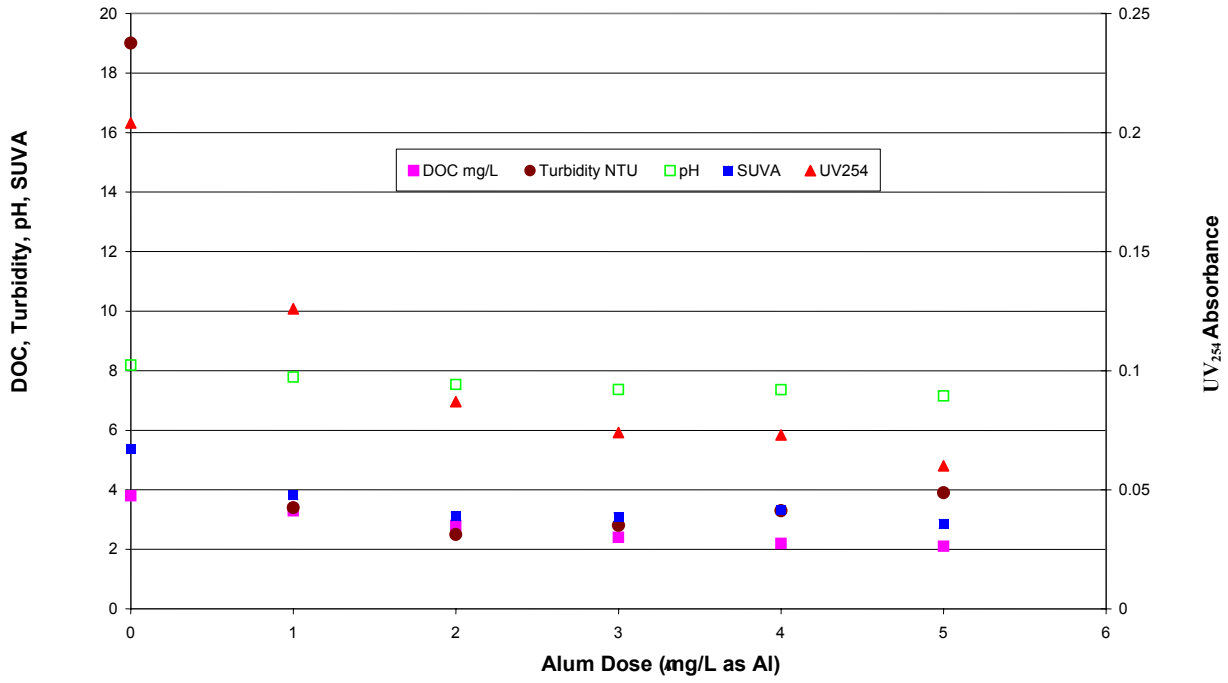

Figure 11 **Response of selected variables to alum coagulation in preliminary jar tests**

Regression analyses showed significant treatment effects for turbidity reduction for each set of trials (Table 3). Because of the way the design matrix was set up, a positive coefficient for the qualitative factor "Species" implies a greater response (i.e., turbidity reduction) for *A. flos-aquae* than *M. aeruginosa*. Similarly, a positive coefficient for "Clarification" implies a greater response for flotation versus settling.

On both trial dates, flotation proved more effective than settling in removing turbidity. Cyanobacteria species was significant on both dates, but the results appeared to depend on cell age: younger *M. aeruginosa* was more readily removed in September and older *A. flos-aquae* was more readily removed in October. The effect of alum dose alone (evaluated by setting all other factors equal to their average value) was significant only during the October trials. Significant interactions were noted between species and alum dose on both dates, and hence for both cell ages. The interaction between species and clarification method was significant only for younger cultures. These interactions are illustrated by comparing Figures 12 and 13.

Figure 12 combines data from both trial dates by showing percent turbidity removal versus alum dose for suspensions of *M. aeruginosa*. Measured increases in turbidity are plotted here as zero-removal. Generally, turbidity removal was greater for increasing alum dose, flotation versus settling, and younger cells.

Figure 13 shows percent turbidity removal versus alum dose for suspensions of *A. flos-aquae*. Again, increases in turbidity are plotted as zero-removal. Increasing alum doses did not generally result in greater turbidity reduction. Flotation produced greater percent turbidity removal than settling. Turbidity associated with older cells was better removed than that associated with younger cells for both clarification processes.

Particle counts in the 5 to 100 μm size range were assessed in clarified samples following the September trials. Linear regressions using either particle count data or percent removal of these counts as the response variable showed no significant treatment effects. This may be because the cyanobacteria cells were smaller than the counter was set to detect, so that only particle flocs would have been counted.

4.3 Effects of Pretreatments on MC-LR

Untreated suspensions of *A. flos-aquae* did not produce detectable concentrations of MC-LR in either filtrate or filter residue samples. Therefore, the effects of the pretreatments on this species were not tested. The *M. aeruginosa* control sample had a concentration of 0.22 μg/L in the filtrate and 0.76 μg/L in the filter residue.

Table 3 Factors and interactions significantly (α= 0.05) affecting turbidity removal during clarification trials

Date	Factor or Interaction	Coefficient	Standard Error	Probability	Interpretation
Sep. 11th (Younger cells)	Species	-54.75	9.82	0.005	*M. aeruginosa* more readily removed
	Settling/Flotation	56.58	9.82	0.0045	Flotation more effective than settling
	Species × Alum Dose	-33.63	12.03	0.049	Effects of species and alum dose are interrelated: e.g., *M. aeruginosa* removal improved with higher alum dose
	Species × Settling/Flotation	45.92	9.82	0.0095	Effects of species and clarification technique are interrelated: e.g., *A. flos-aquae* was better removed than *M. aeruginosa* by flotation
Oct. 9th (Older cells)	Species	13.37	2.31	0.0044	*A. flos-aquae* more readily removed
	Settling/Flotation	20.65	2.31	0.0009	Flotation was more effective than settling
	Alum Dose	17.09	2.83	0.0038	Higher alum dose was more effective
	Species × Alum Dose	-18.69	2.83	0.0027	Effects of species and alum dose are interrelated: *M. aeruginosa* removal improved with higher alum dose

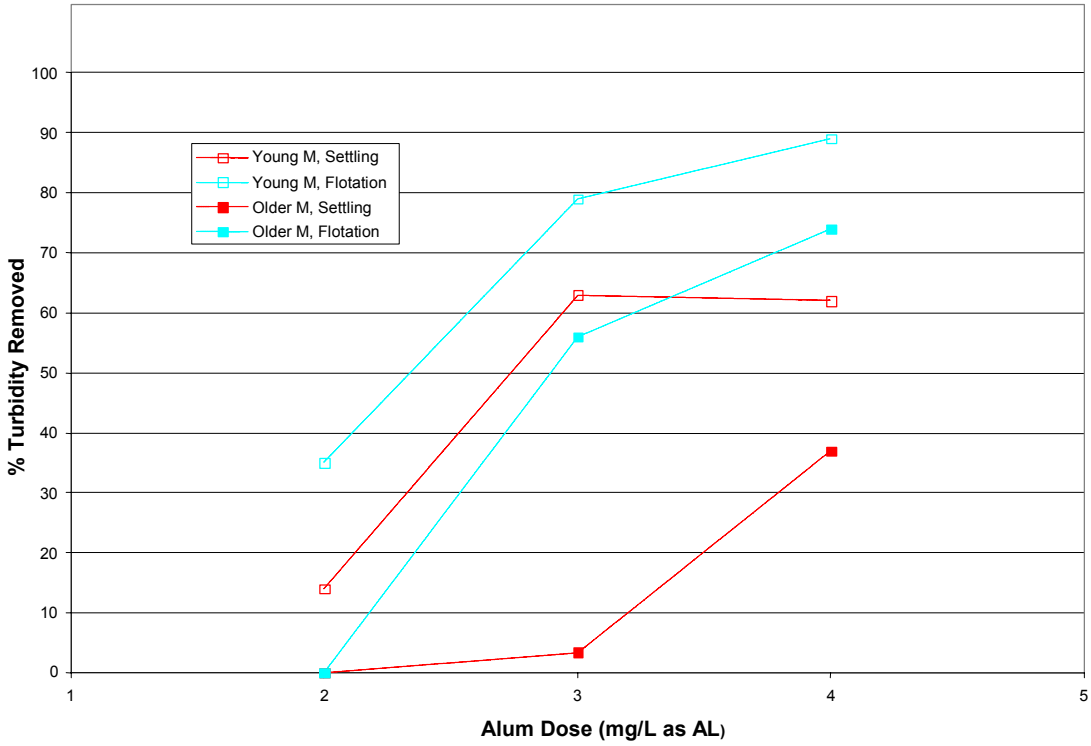

Figure 12 **Turbidity reduction *in M. aeruginosa* suspensions during September and October clarification trials**

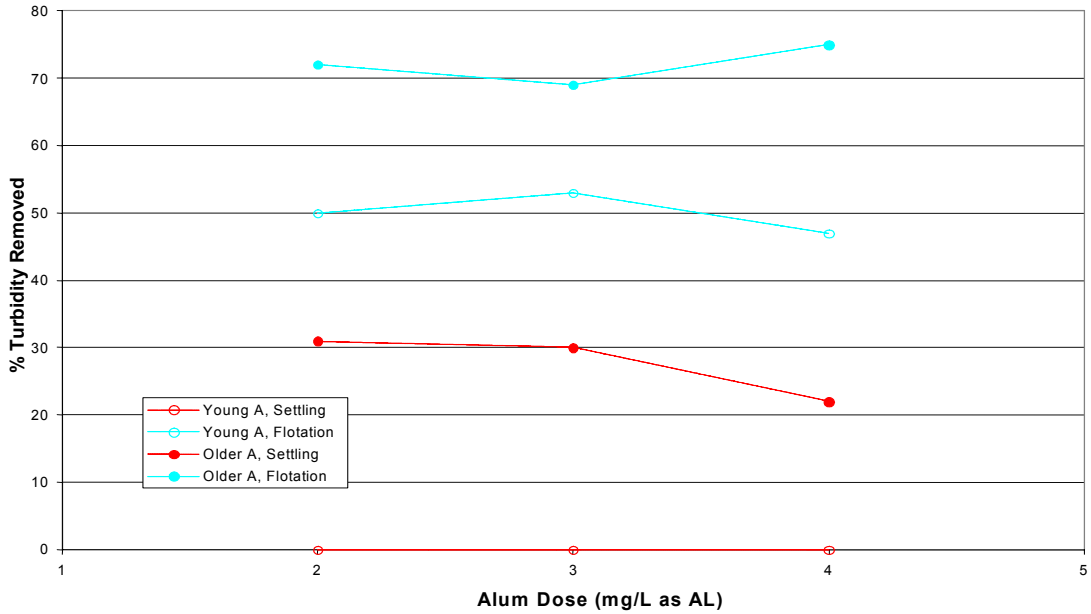

Figure 13 **Turbidity reduction *in A.Flos-aquae* suspensions during September and October clarification trials**

4.3.1 Potassium Permanganate Pretreatments

The effects of $KMnO_4$ pretreatments on MC-LR in *M. aeruginosa* suspensions are illustrated in Figure 14. Initial doses of 0.8 mg/L or greater resulted in MC-LR concentrations less than the detection limit in filtrate samples (assumed to represent extracellular MC-LR). Based on earlier demand trials, these doses were associated with >0.4 mg/L $KMnO_4$ residual after 30 minutes of contact time. Thus, doses of $KMnO_4$ that produced a sustained residual greater than 0.4 mg/L removed extracellular MC-LR effectively. The highest $KMnO_4$ doses also appeared to produce a downward trend in MC-LR in filter residue samples (assumed to represent cellular MC-LR), but the large variance renders these results insignificant. Thus, it may only be concluded that the $KMnO_4$ treatments were significant for removing extracellular MCLR. These results were used to bracket the range of $KMnO_4$ doses to be used in subsequent experiments.

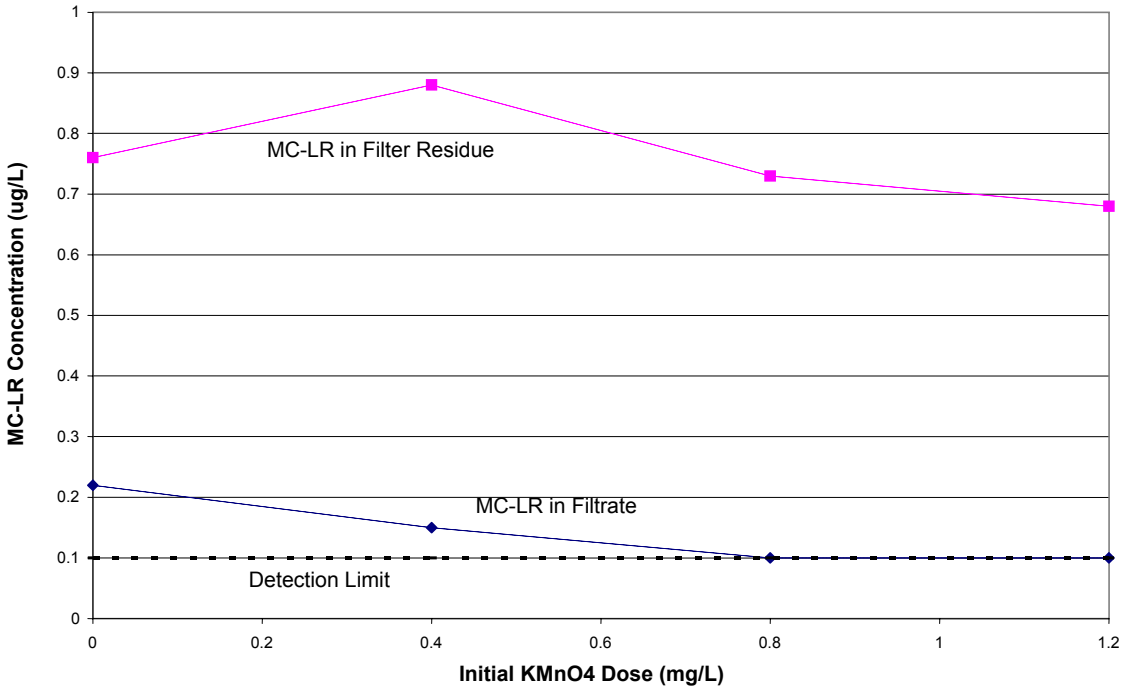

Figure 14 Microcystin-LR concentrations detected in *M. aeruginosa* samples following $KMnO_4$ treatments

4.3.2 Ozone Pretreatments

Ozonation proved very effective in reducing MC-LR concentrations in suspensions of *M. aeruginosa.* Detectable MC-LR (0.13 µg/L) was reported for only one sample of filtrate corresponding to the lowest ozone dose. No MC-LR was detected in samples of filter residue. Pretreatments that produced ozone residual concentrations greater than 0.25 mg/L reduced MC-LR concentrations to less than the detection limit (<0.10 µg/L) in both filtrate and filter residue samples, implying that both extracellular and cellular MC-LR were effectively oxidized.

4.3.3 Ultra-Violet Light Pretreatments

The concentrations of MC-LR in filtrate and filter residue samples of raw and treated *M. aeruginosa* suspensions exposed to UV pretreatments are shown in Figure 15. These results show higher concentrations of MC-LR associated with the filter residue (cellular) than with the filtrate (extracellular). UV irradiation at the levels tested here (up to 120 mJ/cm^2) had no significant effect on concentrations of either cellular or extracellular MC-LR.

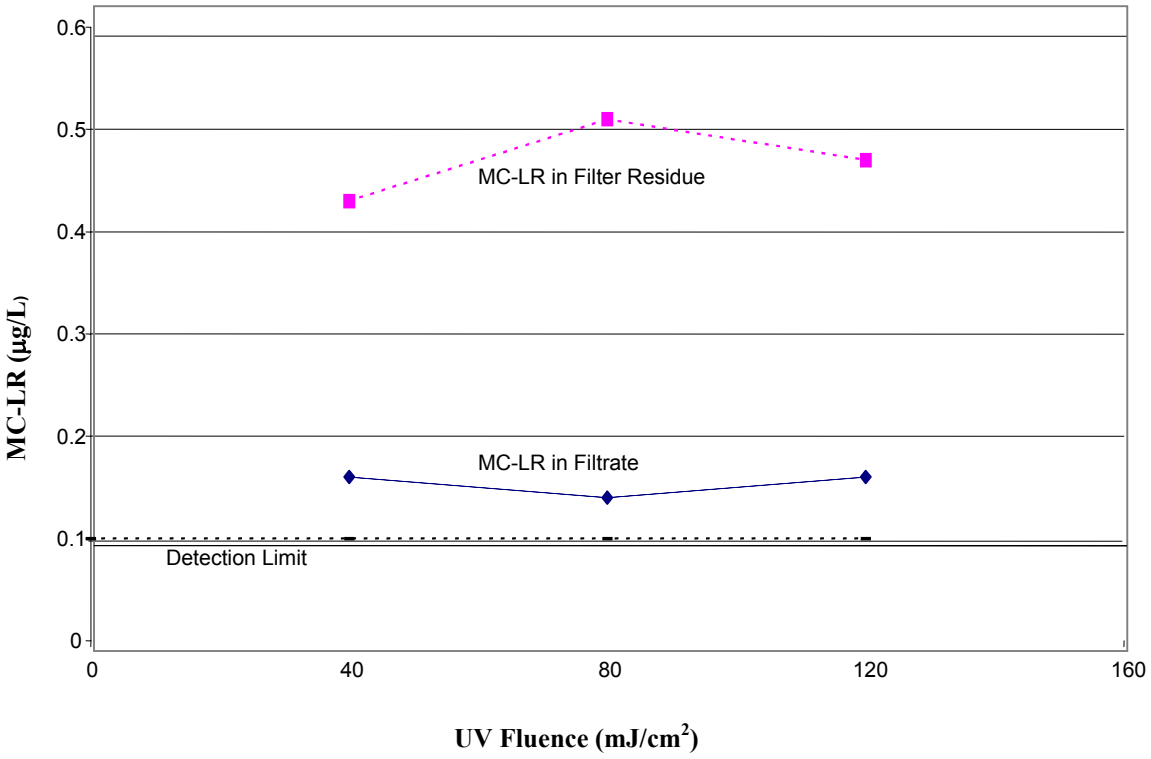

Figure 15 Microcystin-LR detected in UV-pretreated suspensions of *M. aeruginosa*

4.4 Clarification Following $KMnO_4$ Pretreatments

During $KMnO_4$/clarification trials conducted on August 13th and October 8th, MC-LR was not consistently detected in raw samples of *A. flos-aquae* suspensions and, therefore, treated samples of this species were not analyzed for MC-LR. MC-LR was detected in post-treatment *M. aeruginosa* filtrate samples except for the four subjected to higher levels of pretreatment on August 13th (Table 4). Where values were reported as < 0.07, the analytical detection limit, a value of 0.07 was used in the regression analysis. This is a conservative statistical approach in dealing with non-detectable results. The removal of extracellular MC-LR (i.e., in the filtrate) was significantly improved by higher $KMnO_4$ dose and younger cell age during both sets of trials. MC-LR was detected in too few filter residue samples on August 13th to allow the partitioning between extracellular and cellular toxin to be regressed. On October 8th, both lower $KMnO_4$ dose and older cells were associated with higher fractions of extracellular MC-LR.

Table 4 MC-LR measured during August 13th and October 8th $KMnO_4$ and clarification trials with *M. aeruginosa*

			MC-LR		MC-LR	
KMnO4 Dose	**Cell Age**	**Clarification**	**Filtrate (μg/L)**		**Residue (μg/L)**	
			Aug. 13th	**Oct. 8th**	**Aug. 13th**	**Oct. 8th**
0.2	Older	Settling	0.57	3.11	ND	0.87
0.8	Younger	Flotation	< 0.07	0.34	ND	2.13
0.2	Older	Flotation	0.53	2.67	ND	0.42
0.8	Older	Flotation	< 0.07	1.29	ND	0.43
0.8	Older	Settling	< 0.07	1.47	ND	0.92
0.2	Younger	Settling	0.24	1.56	ND	2.31
0.2	Younger	Flotation	0.26	1.12	ND	2.77
0.8	Younger	Settling	< 0.07	0.36	ND	2.24
0	Older	Settling	0.54	3.10	ND	0.77
0	Older	Flotation	0.43	2.95	ND	0.50

Significant effects for reduction of the response parameters turbidity and extracellular MC-LR are shown in Table 5. An additional response parameter, the fraction of extracellular compared to total cellular MC-LR, is also included for the October data.

$KMnO_4$ dose, species, and clarification process were significant factors affecting turbidity removal on both trial dates. Cell age was significant for the October trials. Increased turbidity removal occurred with lower $KMnO_4$ dose, *M. aeruginosa* vs. *A. flos-aquae*, flotation vs.

settling, and older cells. The importance of species to turbidity removal is underscored by its inclusion in each of the significant interaction terms.

The effects of $KMnO_4$ dose, cell age, and the interaction between them were all significant for extracellular MC-LR removal. Higher $KMnO_4$ doses resulted in greater removal of extracellular MC-LR; treatments involving older cells resulted in less removal. During the October trials, the fraction of extracellular MC-LR was significantly affected by $KMnO_4$ dose and cell age. None of the four treatment factors proved significant for reducing DOC or UV_{254} absorbance.

4.5 Clarification Following Ozone Pretreatments

Following ozone/clarification trials conducted on September 10th and October 9th, multiple regression analyses were used to determine the significant effects of ozone pretreatment dose, cyanobacteria species, cell age, and clarification process (settling vs. flotation) on response parameters including percent reduction in extracellular MC-LR, turbidity, and UV_{254} absorbance. As with the $KMnO_4$ pretreatment trials, percent removal of particle counts did not show a significant response to treatments. Significant effects are summarized in Table 6.

Species and clarification process were significant factors affecting turbidity removal during both sets of trials. More turbidity was removed from *M. aeruginosa* suspensions than from *A. flos-aquae* suspensions and from flotation versus settling trials. The interaction of species and clarification was also significant.

All of the treatment factors had significant effects on UV_{254} absorbance removal. Better removal was attributable to higher ozone dose, *A. flos-aquae* vs. *M. aeruginosa*, older cell age, and settling vs. flotation. The only significant interaction was species × cell age during the September trials. SUVA removal was similarly affected by ozone dose, species, and clarification process.

Species, clarification technique, and the interaction between these two variables significantly affected DOC removal during the October trials. More DOC removal was evident with *A. flos-aquae* vs. *M. aeruginosa* and with flotation vs. settling. The enhanced removal of DOC by flotation vs. settling may be an analytical anomaly since a clarification process should not affect dissolved substances. However, the possibility of dissolved substances being removed in association with particulate matter (e.g., by adsorption) cannot be discounted.

During regression analysis performed using extracellular MC-LR as a response parameter, values reported as <0.07 μg/L, were entered as 0.07 μg/L. Ozonation time (or dose) was the only significant factor affecting the concentration of extracellular MC-LR during both sets of trials. For the September trials cellular MC-LR was only detected in unozonated samples, but with the reduced ozone doses applied during the October trials, cellular MC-LR was detectable. However, cellular MC-LR concentrations were less than 5% of the extracellular concentrations in ozonated samples.

Table 5 Significant effects (α= 0.05) in clarified, $KMnO_4$ pretreated samples (August 13th and October 8th results)

Response	Factor or Interaction	Coefficient	Standard Error	Probability	Interpretation
% Turbidity Removed	$KMnO_4$ dose	-26.63, -11.68	2.09, 0.55	0.006, 0.030	Greater removal with lower $KMnO_4$ doses
	Species	-35.70, -14.98	2.09, 0.55	0.003, 0.023	*M. aeruginosa* cells more readily removed
	Cell Age	4.78, 14.55	2.09, 0.55	Not significant, 0.024	Older cells more readily removed
	Settling/Flotation	28.38, 28.15	2.09, 0.55	0.005, 0.012	Greater removal by flotation
	$KMnO_4$ dose × Species	-6.30, -11.45	2.09, 0.55	Not significant, 0.031	Effects of $KMnO_4$ dose and species may be interrelated
	Species × Settling/Flotation	9.75, 17.98	2.09, 0.55	0.043, 0.019	Effects of species and clarification technique are interrelated: E.g. greater removal of *A. flos-aquae* by flotation
% Extracellular* MC-LR Removed	$KMnO_4$ dose	68.27, 3.13	0.93, 0.02	0.009, 0.005	Greater removal with higher $KMnO_4$ doses
	Cell Age	-38.77, -8.67	0.93, 0.02	0.015, 0.002	Less removal from suspensions of older cells
	$KMnO_4$ dose × Cell Age	35.68, -0.77	0.93, 0.02	0.017, 0.021	Effects of $KMnO_4$ dose and cell age are interrelated
	$KMnO_4$ dose × Settling/Flotation	2.88, -0.42	0.93, 0.02	Not significant, 0.037	Effects of $KMnO_4$ dose and clarification technique may be interrelated
	Cell Age × Settling/Flotation	0.92, -1.63	0.93, 0.02	Not significant, 0.010	Effects of cell age and clarification technique may be interrelated
Fraction Extra-cellular: Total MC-LR**	$KMnO_4$ dose	-0.17	0.02	0.056	Lower fraction of extracellular MC-LR with higher $KMnO_4$ doses
	Cell Age	0.51	0.02	0.019	Higher fraction of extracellular MC-LR with older cells

* Based on *M. aeruginosa* suspensions, only

** October results, *M. aeruginosa* suspensions, only

Table 6 Significant effects (α= 0.05) in clarified, ozone pretreated samples (September 10th and October 9th results)

Response	Factor or Interaction	Coefficient	Standard Error	Probability	Interpretation
% Turbidity Removed	Species	-39.56, -36.45	3.04, 0.55	0.006, 0.010	Greater turbidity removal with *M. aeruginosa*
	Settling/Flotation	25.26, 24.95	3.04, 0.55	0.014, 0.014	Greater turbidity removal by flotation
	Species × Settling/Flotation	21.86, 27.45	3.04, 0.55	0.019, 0.013	Effects of species and clarification technique are interrelated. E.g. *A. flos-aquae* better removed by flotation
% UV_{254} Removed	Ozone dose	4.64, 3.33	0.24, 0.41	0.033, 0.015	Greater UV absorbance removal with higher ozone dose
	Species	19.16, 6.38	0.24, 0.41	0.008, 0.004	Greater UV absorbance removal with *A. flos-aquae*
	Settling/Flotation	-3.31, -2.22	0.24, 0.41	0.046, 0.032	Greater UV absorbance removal by settling
	Cell Age (Sept.)	3.71	0.24	0.041	Greater UV absorbance removal with older cell suspensions
	Species × Cell Age (Sept.)	-7.66	0.24	0.020	Effects of species and cell age are interrelated. E.g. Greater removal from older *M. aeruginosa*
% DOC Removed	Species	8.02	1.23	0.023	Greater DOC removal with *A. flos-aquae*
(October)	Settling/Flotation	9.38	1.23	0.017	Greater DOC removal by flotation
	Species × Settling/Flotation	-9.33	1.23	0.017	Effects of species and clarification technique are interrelated. E.g. More DOC removed from *M. aeruginosa* by flotation
% Extracellular* MC-LR Removed	Ozone dose	12.13	0.78	0.041, 0.05	More extracellular MC-LR removed at the higher ozone dose

Based on *M. aeruginosa* suspensions, only

Figure 16 shows MC-LR concentrations in relation to contacted ozone during the September 10th trials with *M. aeruginosa*. Cellular MC-LR (measured in filter residue) was only detected above 1 μg/L in untreated samples and a single unozonated, clarified sample. This suggests that clarification processes removed most cellular MC-LR and the remainder was effectively removed by ozonation. The positive regression coefficient in Table 6 and the trend line of Figure 16 indicate the importance of ozone for removing extracellular MC-LR. The equation predicts contacted ozone doses between 8 and 10 mg/L to reduce extracellular MC-LR to below the method detection limit (0.07 μg/L). Corresponding residual ozone measurements were between 0.24 and 0.33 mg/L. These results compare favourably with those of Shawwa and Smith (2001) who tested MC-LR reduction in water with <0.1 mg/L DOC and found that a residual ozone concentration of 0.25 mg/L after two minutes of ozonation resulted in virtually complete removal of initial MC-LR. Their research also showed MC-LR to be rapidly oxidized by ozone in waters of neutral pH, but that the reaction showed a distinct lag in the presence of natural organic matter.

An interesting characteristic of Figure 16 is that points above the extracellular MC-LR trend line corresponded to older cell suspensions and points below it to younger cell suspensions. However, interactions involving cell age were not found to be significant in the regression analysis.

Observations for the ozone-clarification process indicate that effective clarification would remove most cellular MC-LR. Given that ozonation may lyse cells, giving rise to extracellular MC-LR, clarification should precede ozonation in most water treatment plants unless there are compelling reasons to pre-ozonate. The results also suggest that adding sufficient ozone to achieve a residual for disinfection purposes would also be adequate for removing residual extracellular MC-LR.

4.6 Clarification Following UV Pretreatments

Following UV/clarification experiments conducted on September 24th, regression analyses were conducted to examine the effects of UV_{254} pretreatments, cyanobacteria species, cell age, and clarification process on turbidity, particle counts (5 to 100 μm range), UV_{254} absorbance, and MC-LR concentration. The significant effects attributable to the multiple regression model are shown in Table 7.

Species, cell age and clarification process had a significant effect on turbidity removal, as did the two interactions with species. Greater turbidity removal was observed for *M. aeruginosa* vs. *A. flos-aquae*, younger cells, and flotation vs. settling. For UV_{254} absorbance removal, species and clarification process were significant. None of the factors were significant for particle counts removal, likely due to the size range (5 to 100 μm) selected for the counter.

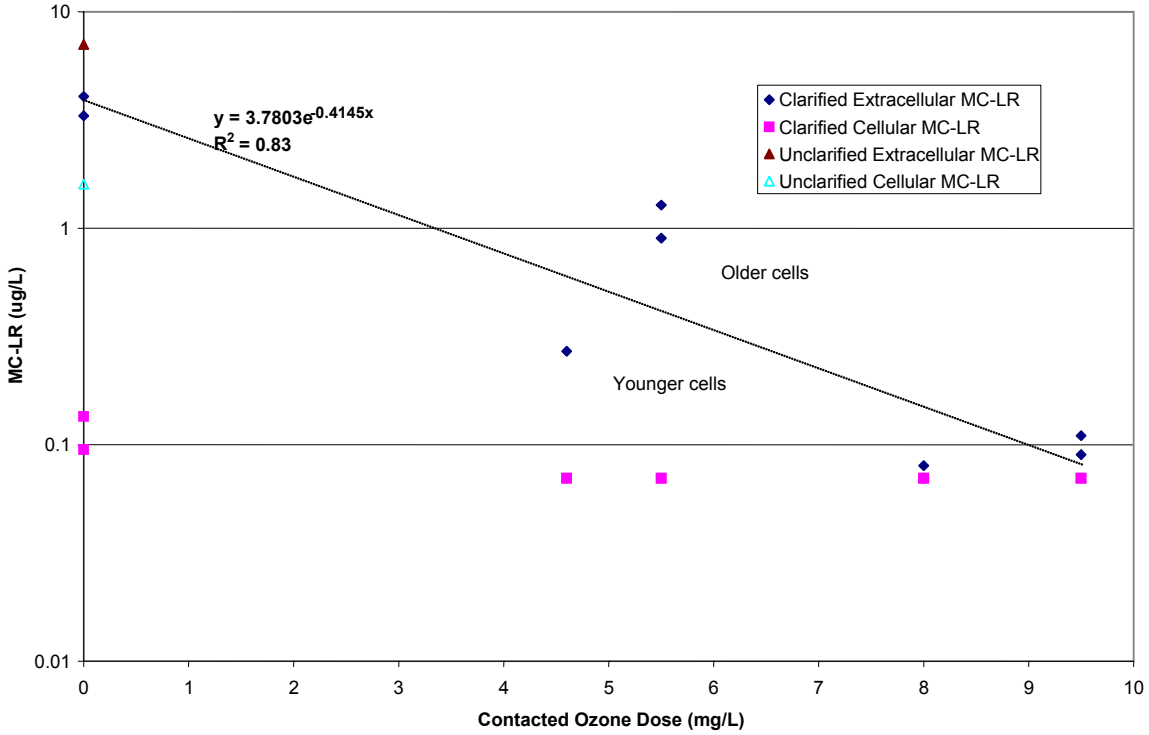

Figure 16 **Effects of ozonation on MC-LR concentrations in *M. aeruginosa.***
Note: The equation of this line was determined by regressing ozone dose on extracellular MC-LR concentrations

MC-LR was detected in all post-treatment filtrate and filter residue samples associated with *M. aeruginosa* suspensions. Due to a sampling error, no reference data were available for calculating the percentage of MC-LR removed. Instead, the concentration of MC-LR in the filtrate (extracellular) was divided by the total MC-LR concentration (filtrate plus residue) to calculate the fraction of extracellular MC-LR as a response parameter. The effects of UV fluence, cell age, and clarification process were significant with the following implications:

- Higher UV fluence decreased the fraction of extracellular MC-LR
- Suspensions with older cells had a higher fraction of extracellular MC-LR
- Flotation left a higher fraction of extracellular MC-LR compared to settling

The effect of UV fluence was unexpected because no effect on cellular or extracellular MC-LR had been observed during pretreatment only trials. The observation of a higher fraction of extracellular MC-LR associated with an older suspension *of M. aeruginosa* cells is consistent with previous results. Also, because the flotation process has been shown to remove cell-related turbidity more effectively than settling, it is not surprising that post-flotation samples would have a lower concentration of cells and, therefore, a higher fraction of extracellular MC-LR.

Table 7 Significant effects (α= 0.05) in clarified, UV pretreated samples (September 24th, 2003)

Response	Factor or Interaction	Coefficient	Standard Error	Probability	Interpretation
% Turbidity Removed	Species	-20.25	1.48	0.046	Greater turbidity removal with *M. aeruginosa*
	Cell Age	-18.88	1.48	0.050	Younger cells more readily removed
	Settling/Flotation	38.53	1.48	0.024	Greater turbidity removal by flotation
	Species × Cell Age	-26.38	1.48	0.036	Effects of species and cell age are interrelated
	Species × Settling/Flotation	18.73	1.48	0.050	Effects of species and clarification technique are interrelated
% UV_{254} Removed	Species	-8.11	0.38	0.002	Greater UV_{254} absorbance removal with *M. aeruginosa*
	Settling/Flotation	1.61	0.38	0.050	Greater UV_{254} absorbance removal by flotation
Fraction Extracellular MC-LR*	UV Fluence	-0.09	0.02	0.025	Greater fraction of extracellular MC-LR with lower UV fluence
	Cell Age	0.10	0.02	0.016	Greater fraction of extracellular MC-LR with older cells
	Settling/Flotation	0.09	0.02	0.025	Greater fraction of extracellular MC-LR following flotation

* Based on *M. aeruginosa* suspensions, only

5.0 IMPLICATIONS OF RESULTS FOR TREATMENT PROCESSES

5.1 Clarification without Pretreatment

During clarification experiments, cyanobacterial suspensions were handled in ways that approximated the shearing processes that would be encountered in conventional water treatment processes. The handling included gentle agitation before treatment, rapid mixing during coagulation, and intermediate stirring during flocculation. The findings are thus expected to have direct implications for full-scale operations.

The results showed clearly that flotation was a more effective clarification process than settling for reducing turbidity and, by implication, removing cyanobacterial cells. This finding was expected because of the inherent buoyancy of cyanobacteria. Irrespective of clarification technique or growth stage, *M. aeruginosa* was generally more readily clarified, and showed a much greater response to coagulation with alum, than *A. flos-aquae*. These two species of cyanobacteria are morphologically quite different: *Microcystis* genera are composed of unicellular or coccal colonies, whereas colonies of *Anabaena* genera are filamentous and tangled (Chorus and Bartrum, 1999). The relatively small and discrete colonies of *M. aeruginosa* would be expected to behave more like particles and to exhibit a better response to coagulation. Mechanistically, this involves neutralizing charges associated with individual particles (or small colonies), causing them to move closer together and form flocs which, in turn, are more readily floated or settled. In contrast, *A. flos-aquae* would naturally be present as filamentous flocs, with little or no benefit to be gained by coagulation. The influence of colony morphology on removal efficiency was also observed in jug filtration experiments (Lawton *et al.* 1998) that resulted in approximately 60% of filamentous forms of cyanobacteria (*Oscillatoria* sp. and *Anabaena* sp.) being removed from suspension compared to only 10% of *Microcystis* sp. Based on the current observations, one might predict that adding a filter aid (pre-coagulation) would improve the removal efficiency of *Microcystis* sp. by filtration.

Although species morphology had the most profound implication for treatment processes, the effects of growth stage were also of interest. Particularly in the earlier growth stage, *A. flos-aquae* was removed to a greater degree by flotation rather than settling. The effect of increasing alum dose for removing turbidity was greater in later growth stages of both species.

The selection of appropriate treatment technologies and process settings is complicated by the diverse species, morphologies, and growth stages of cyanobacteria likely to be present during warm water months. It would be wise to monitor cyanobacterial species and growth stages regularly so that informed decisions could be made about clarification and filtration processes. Where blooms of cyanobacteria occur regularly, flotation would be the recommended method of clarification.

5.2 Pretreatments Alone

5.2.1 Potassium Permanganate

Potassium permanganate demonstrated no effect on cellular MC-LR at the doses tested in this study (up to 1.2 mg/L). Extracellular MC-LR was reduced from 0.2 µg/L to less than 0.1 µg/L when the residual $KMnO_4$ concentration was 0.4 mg/L or higher after 30 minutes of reaction time. The concentrations of cellular and extracellular MC-LR were quite low in these experiments, and further tests would be required to extend the dose-response characteristic to higher MC-LR concentrations.

5.2.2 Ozone

Measurable MC-LR was only found consistently at the lowest ozone dose tested, suggesting that ozone can be very effective for removing both extracellular and cellular MC-LR. The low dose corresponded to 21 mg/L of applied ozone producing a residual concentration of 0.25 mg/L (5 mg/L of ozone was actually transferred).

5.2.3 UV Light

UV treatment at the levels tested here (up to 120 mJ/cm^2) had no impact on either cellular or extracellular MC-LR in raw water. These doses are somewhat higher than might be applied for inactivation of *Cryptosporidium* spp. for example. It would be difficult to justify installing UV treatment for raw water based on these results.

5.3 Clarification after Pretreatment

5.3.1 KMnO4

Turbidity removal was greater at lower $KMnO_4$ doses while higher doses were associated with better removal of extracellular MC-LR. A significant interaction between species and $KMnO_4$ dose was found, suggesting that turbidity removal from suspensions of *A. flos-aquae* was greater at the lower $KMnO_4$ dose. Although more experiments would be required to confirm this observation, a possible explanation would be that higher oxidant applications weakened the filamentous structure associated with *A. flos-aquae* resulting in smaller floc size, and hence a lower removal efficiency. High oxidant doses are also known to disrupt cells (Lam *et al*., 1995). Other findings related to turbidity reduction were as noted previously for clarification alone: *M. aeruginosa* was more effectively removed and flotation removed more turbidity than settling from suspensions of both species.

Higher $KMnO_4$ doses resulted in more extracellular MC-LR being removed and a lower fraction of extracellular vs. cellular MC-LR. The implication is that $KMnO_4$ may be applied effectively for controlling extracellular MC-LR downstream of clarification processes.

5.3.2 Ozone

Higher ozone doses resulted in lower UV_{254} absorbance and extracellular MC-LR in clarified samples. Like $KMnO_4$, ozone could be applied effectively in a water plant for controlling extracellular MC-LR downstream of clarification processes. DOC removal was significantly affected by species and clarification process, but not by ozone dose, whereas UV_{254} absorbance removal was affected by all four treatment factors. Reductions in UV_{254} absorbance and DOC have implications for the concentration and types of by-products formed upon chlorination of water containing natural organic matter (Amy *et al*., 2001). No statistically significant interactions were noted between ozone dose and other treatment factors. Significant effects and interactions associated with turbidity reduction were consistent with those observed during clarification without preoxidation.

5.3.3 UV Light

UV pretreatments did not significantly affect turbidity reduction. Based on only one set of trials, higher UV fluence (120 mJ/cm^2) resulted in a significant reduction in the fraction of extracellular MC-LR measured in clarified samples. During trials with UV pretreatments alone, UV irradiation had not shown a reduction in MC-LR concentrations. Therefore, this observation would need to be confirmed by additional research. A confirmed result would have major implications to the water treatment industry because of the possibility of using UV irradiation as both a disinfectant and MC-LR control strategy.

6.0 STUDY CONCLUSIONS

- Removal of *M. aeruginosa* and *A. flos-aquae* cells as measured by turbidity reduction was more effective by flotation than by settling.

- The efficiency of cyanobacterial cell removal from prepared suspensions by settling or flotation was species specific and greater for *M. aeruginosa* than *A. flos-aquae*.

- Cyanobacteria of different growth stages responded differently to pretreatments and clarification. Older cells were more efficiently removed following pretreatment with $KMnO_4$.

- Optimizing the dose of $KMnO_4$ as a pre-oxidant was important for minimizing residual turbidity after clarification.

- Both $KMnO_4$ and ozone were found to be capable of oxidizing extracellular MC-LR, consistent with previous research (Hart *et al.*, 1998). Ozone was more powerful in this regard, in that shorter treatment times were effective. By maintaining a residual ozone level of >0.25 mg/L, low concentrations of both cellular and extracellular MC-LR (<10 μg/L) were reduced to below the 0.07 μg/L detection limit.

- UV irradiation at a fluence of 120 mJ/cm^2 significantly reduced the fraction of extracellular to total MC-LR during one set of trials. This observation requires confirmation by further research.

7.0 REFERENCES

Amy, G., Her, N.G., Jarusutthirak, C. 2001. Size Exclusion Chromatography (SEC) with UV, Florescence, and On-Line DOC Detection: Fingerprints of Natural (NOM), Algal (AOM), and Wastewater Effluent (EfOM) Organic Matter. In proceedings of the "Utilization of NOM Characteristics to Improve Process Selection and Performance" workshop, Berlin, October, 2001. Jointly published by CRC for Water Quality and Treatment, AWWA Research Foundation, and Vivendi Water.

An, J.S. and Carmichael, W.W. 1994. *Toxico, 32, 1495-1507.*

American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. Clesceri, LS, Greenberg, AE, and and. Eaton, AD, eds. Method 4500 – O3 B in Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA, Washington, DC. 1220 p.

Belov, A. P. 1998. A Model of Phycotoxin Release by Cyanobacterial Cells. *Ecological Modeling 110: 105-107.*

Box, G., Hunter, W, and Hunter, J. 1978. "Factorial Designs at Two Levels", Ch. 10 in Statistics for Experimenters. John Wiley & Sons, New York. 653 p.

Carlyle, P.R. 1994. Further Studies to Investigate Microcystin-LR and Anatoxin-A Removal from Water. Report No. FR0458, Foundation for Water Research, Marlow, UK. http://www.fwr.org/waterq/fr0458.htm.

Chorus, I. and Bartrum, J. (Eds.). 1999. Toxic Cyanobacteria in Water, a Guide to Their Public Health Consequences, Monitoring, and Management. E & FN Spon, London.

Cousins I. T., Bealing, D.J., James, H. A. and Sutton, A. 1996. Biodegradation of Microcystin-LR by Indigenous Mixed Bacterial Populations. *Water Research 30(2): 481-485.*

EC Engineering, 2003. DAF Batch Tester Details: http://www.ecengineering.net/html/dbt_details.html

Hart, J., Fawell, J. K. and Croll, B. 1998. The Fate of Intra- and Extracellular Toxins during Drinking Water Treatment. *Water Supply 16(1/2): 611-616.*

Health Canada. 2003. Summary of Guidelines for Canadian Drinking Water Quality (04/03), Prepared by the Federal-Provincial-Territorial Committee on Drinking Water.

Hudson, HE and Wagner, EG. 1981. Conduct and Use of Jar Tests. *J. AWWA 73:pp. 218-223.*

James, W. and Skinner, F. 1994. Accelerated Ozone Removal from Cold Lake Water Using UV Radiation: A Pilot Study. Alberta Environmental Centre, Internal publication.

Kenefik, S. L., Hrudey, S. E., Peterson, H. G. and Prepas, E. E. 1993. Toxin Release from *Microcystis aeruginosa* After Chemical Treatment. *Water Science and Technology 27(3/4): 433-440.*

Lahti K., Rapala, J., Fardig, M., Niemela M. and Sivonen, K. 1997. Persistence of Cyanobacterial Hepatoxin, Microcystin-LR in Particulate Material and Dissolved in Lake Water. *Water Research 31(5): 1005-1012.*

Lam, A. E., Prepas, E. E., Spink, D. and Hrudey, S. E. 1995. Control of Hepatoxic Phytoplankton Blooms: Implications for Human Health. *Water Research 29: 1845-*1854.

Lawton, L., Cornish, B., and MacDonald, A. 1998. Removal of Cyanobacterial Toxins (Microcystins) and Cyanobacterial Cells from Drinking Water Using Domestic Water Filters. *Water Research Vol. 32, pp. 633-638.*

Metcalf, J.S. and Codd, G.A. 2004. Cyanobacterial Toxins in the Water Environment – A Review of Current Knowledge. Foundation for Water Research., University of Dundee, U.K.

Mole J., Chow, C., Drikas, M., Burch, M. 1997. The Influence of Culture Media on Growth and Toxin Production of the Cyanobacteria Microcystis aeruginosa Kutz Emend Elenkin. Presented at the 13th Annual Conference of the Australian Society of Phycology and Aquatic Botany, Hobart, January 1997.

NCSS. 1996. Number Cruncher Statistical System. Version 6.0.22, NCSS, Kaysville, UT.

Nicholson, B. C., Rositano, J. and Burch, M.D. 1994. Destruction of Cyanobacterial Peptide Toxins by Chlorine and Chloramine. *Water Research 28(6): 1297-1303.*

Pietsch J., Bornmann K., and Schmidt W. 2001. "Relevance of Intra- and Extracellular Cyanotoxins for Drinking Water Treatment". Presented at the annual meeting of the Water Chemistry Division in the German Chemical Society, Bad Wildungen, Germany, May 2001.

Rashash, D. M., Hoehn, R. C., Dietrich, A. M., Grizzard, T. J. and Parker B.C. 1996. Identification and Control of Odorous Algal Metabolites. American Waterworks Association Research Foundation.

Shawwa, A.R. and Smith, D.W. 2001. Kinetics of Microcystin-LR Oxidation by Ozone. Ozone *Science & Engineering, Vol. 23, pp.161-170.*

Westrick, J., 2003. Everything a Manager Should Know about Algal Toxins But Was Afraid to Ask. *J. AWWA 95:9 pp. 26-34.*

World Health Organization. 1999. Toxic Cyanobacteria in Water. I. Chorus and J. Bartram eds. E & FN Spon. London.

Zurawell, R. 2002. An Initial Assessment of Microcystin in Raw and Treated Municipal Drinking Water Derived from Eutrophic Water in Alberta. Alberta Environment Publication T672.