Reduction of trihalomethane formation and detoxification of microcystins in tap water by ozonation

Orapin Thapsingkaew, Vilailuck Kijjanapanich and Werawan Ruangyuttikarn

ABSTRACT

The efficiency of ozonation in comparison to chlorination for removal of microcystins and production of trihalomethanes (THMs) in water was investigated. One hundred and ninety water samples of ozone and chlorine treated water were collected at a water treatment plant between August 2004 and March 2005. The level of THMs, total organic carbon and residual chlorine were determined. Protein phosphatase 2A inhibition assay was used to detect microcystins and the presence of microcystins was confirmed by HPLC. The results show that 91.5% of the THM species in treated water was chloroform and 8.5% was bromodichloromethane. The mean THM level ± standard error of mean in chlorinated water (CW) (45.1 ± 3.0 μg/L) was higher than the mean of THM level in ozonated water (OW) (18.6 ± 2.2 μg/L). In addition, no OW sample exceeded the first stage U.S. EPA maximum THM contaminant level for drinking water (80 μg/L) and only 8% of these samples exceeded the second stage level (40 μg/L). On the other hand, 3% of CW samples exceeded 80 μg/L and 68% exceeded the 40 μg/L level. The microcystin level in all water samples was below the WHO guideline value (1 μg/L) for drinking water.

Key words | chlorination, microcystins, ozonation, total organic carbon, trihalomethanes

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INTRODUCTION

Contamination of toxic substances, such as cyanobacterial toxins from toxic blue green algae bloom and disinfectant by-products (DBPs), in the water supply for residents in Thailand is a recent serious health concern. Microcystins are hepatotoxins produced by cyanobacteria, especially *Microcystis aeruginosa* (Dawson 1998; Sivonen & Jones 1999). It was the dominant toxic blue green algae blooming in many reservoirs in Thailand including Mae Kuang reservoir in Chiang Mai (Peerapornpisal *et al.* 1999).

The Mae Kuang reservoir is a major source of water supply for residents in Chiang Mai municipal area. Chlorine is used as a disinfectant for raw water and it can react with natural organic matter in the water to form trihalomethanes (THMs)(Rook 1974). THMs are classified as possible human carcinogens (IARC 1991, 1999).

Microcystins are tumor-promoting substances (An & Carmichael 1994; Bell & Codd 1994; Rudolph-Böhner *et al.* 1994; Trogen *et al.* 1996). It has been reported that a high incidence of primary liver cancer in China has been attributed to drinking water contaminated with cyanobacterial toxins (Harada *et al.* 1996). People who drink water from a pond containing low levels of microcystins have a higher mortality rate from hepatocellular carcinoma than those who drink water from a well which does not contain any microcystins (Ueno *et al.* 1996). WHO (1998) suggested a provisional guideline for microcystin-LR in drinking water of 1 μg/L and a tolerable daily intake value of 0.04 μg per kg body weight per day.

Cancer risk associated with exposure to these toxic substances in drinking water and through daily activities is our big concern. Accordingly an annual report of cancer registry from Maharaj Nakorn Chiang Mai Hospital (1999) showed mortality from liver cancer as the second most frequent cause of death among Chiang Mai cancer patients.

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Therefore, to minimize cancer risk from chemical exposure in tap water, an alternative disinfectant and detoxification of cyanobacterial bloom should be considered.

Several studies have shown that ozone effectively reduced THM formation (Owen *et al.* 1995) and also destroyed microcystins (Rositano *et al.* 1998). However, ozone has not been implemented as a tap water treatment disinfectant in Thailand. Therefore, it was selected to mitigate these problems in this study. The efficiency of ozonation in comparison to chlorination in the conventional water treatment process on removal of THMs and microcystins were investigated.

MATERIALS AND METHODS

Apparatus

The ozone generator was provided by Ozonfit® OZVa, ProMinent® company. Gas chromatography (Agilent® 6890 Series GC system, U.S.A), consisting of electron capture detector was used with an analytical column (DB-624, 25 m × 0.2 mm i.d. × 1.12 μm film thickness, J&W Scientific, U.S.A) to analyze THMs. Total organic carbon analysis system (OI analytical, U.S.A.), consisting of total organic carbon analyzer (model 1010), was used with a vial autosampler model 1051 to measure total organic carbon. Portable compact photometer (Dulcotest® DT1, Germany) was used to measure residual chlorine.

High performance liquid chromatography (Varian®, U.S.A.), consisting of solvent delivery system, Varian® 9012Q, Star chromatography workstation version 4.51, UV-visible absorbance detector and ProStar 310 diode array detector was used to measure microcystin level. Analytical HPLC column (Gemini 5 μm C_{18} 110A, 4.6 × 150 mm) with guard column kit C_{18} 5 μm was purchased from Phenomenex®, U.S.A.

Chemicals

Solvents used were HPLC and GC grades. Microcystin-LR standard (10 μg/mL in methanol) was purchased from Kanto chemical (Tokyo, Japan). Protein phosphatase 2A enzyme (25 units) was purchased from Promega® (Mandison, WI, U.S.A.). Other chemicals and reagents were analytical grades ordered from a local agency.

Water treatment plants

Chlorination water treatment plant

Mae Kuang water treatment plant provides treated water for all residents in the Chiang Mai municipal area, which now has a population of about 167,000 people. The plant capacity is 2,200 m^3/hr using surface water from the Mae Kuang reservoir. The water is drawn from a fixed offtake at 40 m below the full supply level. The water treatment process comprises pre-chlorination, coagulation, flocculation, sedimentation, intermediate-chlorination, filtration and post-chlorination.

Ozonation pilot plant

The ozonation pilot plant had a capacity 0.5 m^3/hr. The experiment was conducted in two laboratory units. The sedimentation tank in the first unit was a solid contact clarifier with 1.7 hours retention time and the filter was automatically backwashed. The sedimentation tank in the second unit was rectangular with 4 hours retention time and the filter backwash was operated manually. The first unit was used in the rainy season between August and December 2004. The second unit was used in the dry season between February and March 2005. The treatment process was pre-ozonation, coagulation, sedimentation, filtration and post-chlorination using the same raw water as in the conventional chlorination plant. Ozone was generated at oxidation-reduction potentials of 400, 500, 600 or 700 mV in different trials.

Water sampling

Water samples were taken weekly from the chlorination water treatment plant and the ozonation pilot plant during August to December 2004 (rainy season) and February to March 2005 (dry season). Raw, ozonated, and chlorinated water samples were collected for THMs, total organic carbon, residual chlorine and microcystin analyses.

The water samples for THM quantification were collected in 250 mL glass bottle with a TPFE-lined screw

cap and preserved with 10% sodium thiosulfate solution to eliminate any residual chlorine and to prevent additional THM formation during transportation to the laboratory.

The water samples for total organic carbon determination were collected in a 100 mL glass bottle and preserved with concentrated sulfuric acid which was added until the pH of water was less than or equal to 2.

Ten millilitres of water was collected in a beaker and determined for residual chlorine immediately at the water sampling site.

There were 105 water samples collected from both ozonation pilot and chlorination water treatment plants during August to December 2004. In addition, 85 water samples during February to March 2005 for THMs, total organic carbon and residual chlorine analyses.

The water samples for microcystin analysis were collected separately in a 1 L amber glass bottle. All water samples were kept at 4°C before analyses.

Quantification of trihalomethanes (THMs)

THM quantification was performed according to AWWA (1995) using gas chromatography-electron capture detector (GC-ECD). Thirty-five millilitres of water was extracted with 5 mL of pentane using 50 μL of 50 μg/mL 4-bromofluorobenzene as an internal standard and vigorously shaken by hand for 1 minute. Phase separation occurred within 2 minutes and the upper phase was collected into 2 mL vial with a PTFE septa screw cap. One microlitre of extracted pentane was injected into the GC-ECD. The carrier gas used was helium with a flow rate of 1.6 mL/min and the make-up gas was nitrogen. The injection technique was split/splitless. Oven temperature started with initial temperature at 75°C (15°C/min, holding time 1 min), 100°C (15°C/min, holding time 1 min), 130°C (15°C/min, holding time 1 min) and 180°C (15°C/min, holding time 1 min). Injector temperature was 225°C. Split ratio was 10:1 with detector temperature at 300°C.

Different concentrations of trihalomethanes (10, 25, 50, 100, and 150 μg/L) were prepared in Milli-Q water and a calibration curve was constructed between THM and an internal standard, bromofluorobenzene concentration.

Total organic carbon (TOC) determination

TOC determination was performed according to the wet-oxidation method (AWWA 1995). Prior to determination of TOC, each water sample was filtered through glass microfiber filter (GF/C) and 10 mL of the filtered sample was injected into the analyzer. TOC was determined by measuring the carbon dioxide released from chemical oxidation of the organic carbon in the water sample. The water sample was acidified with phosphoric acid solution, purged to remove inorganic carbon, and oxidized with sodium persulfate. This oxidant quickly reacted with organic carbon in the water sample at 100°C to form carbon dioxide. When the oxidation was completed, the carbon dioxide was purged from the solution and carried into a nondispersive infrared detector, calibrated to directly display the mass of carbon dioxide detected. The resulting carbon mass in the form of carbon dioxide was equivalent to the mass of organic carbon originally in the sample. The TOC standard was prepared by dissolving the potassium hydrogen phthalate at concentrations of 0, 1, 2, 5, and 10 mg/L.

Residual chlorine determination

Residual chlorine was determined colorimetrically according to the *N*-*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method (AWWA 1995). Free chlorine in the water sample reacted instantly with DPD indicator to produce a red color. Water test tablet was added into 10 mL water sample, mixed vigorously and then absorbance measured by a portable compact photometer with automatic zero calibration at 528 nm. The residual chlorine in the water sample correlated positively with the concentration of the colored product.

Detection of microcystins by protein phosphatase 2A inhibition (PPi) assay

The colorimetric protein phosphatase inhibition assay was based on the method described by Heresztyn & Nicholson (2001). The *PPi* test was quantitative in the concentration range between 0.2–10 μg/L of microcystin-LR (M-LR), where the change in inhibition was approximately linear

with toxin concentration. Outside that range, results were qualitative and were expressed as greater than the quantitative maximum or less than the detection limit. Other naturally-occurring bioactive compounds can inhibit PP2A enzyme activity, so the *PPi* test is not specific for microcystins. Furthermore, the degree of inhibition of PP2A varies for different forms of microcystin. The *PPi* bioassay was calibrated, using the LR form of microcystin (M-LR). All results are reported as μg/L of M-LR equivalents. The quantity of microcystins which produced 50% inhibition of the PP2A enzyme activity (IC50) was calculated from the slope of the linear portion of the

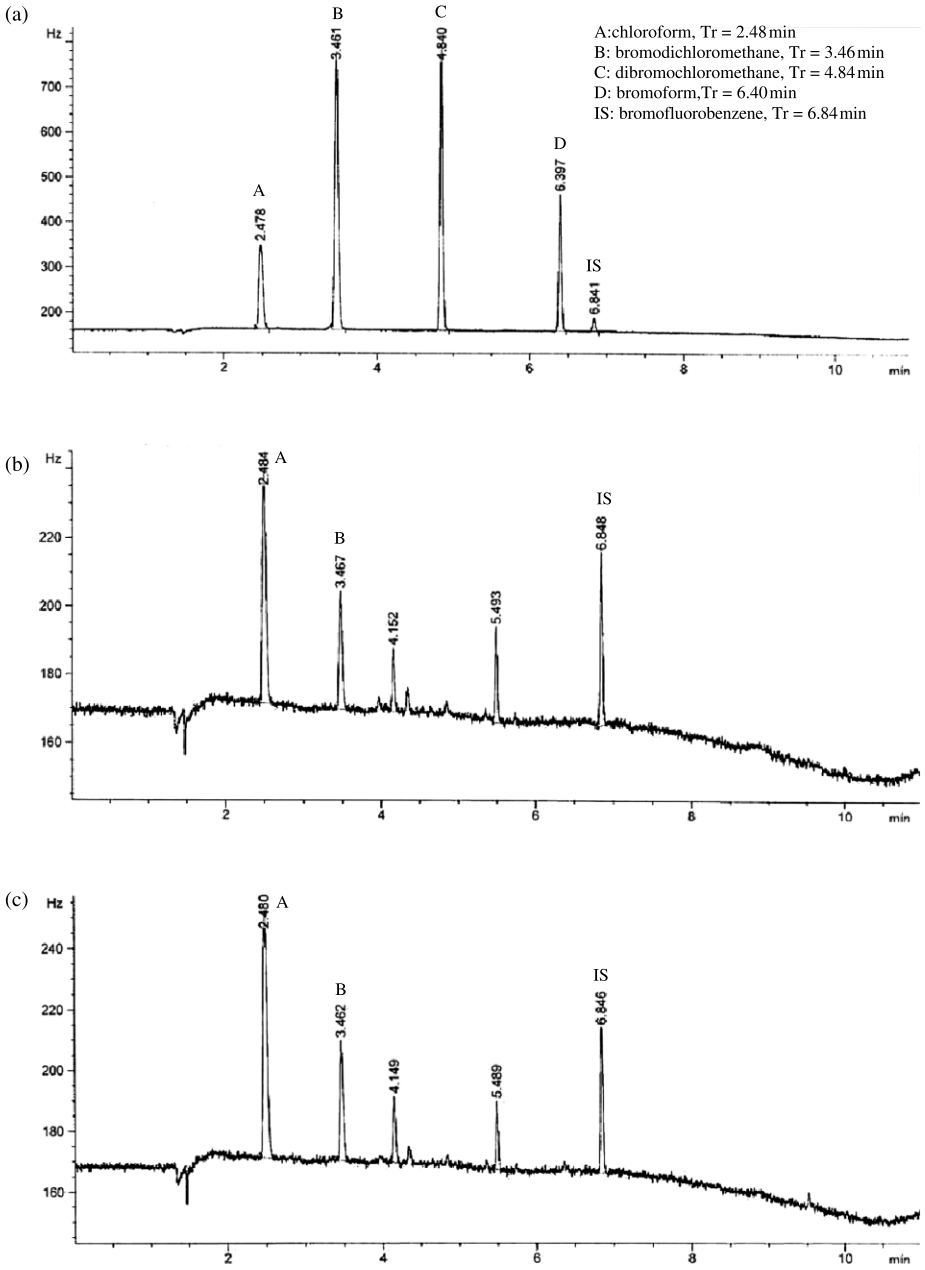

Figure 1 | Gas chromatogram of mixed THM standard (a) at the concentration of 150 μg/L showing the peaks of chloroform (A), bromodichloromethane (B), dibromochloromethane (C), bromoform (D) and 4-bromofluorobenzene (internal standard, IS) at the retention time of 2.48, 3.46, 4.84, 6.40 and 6.84 minutes, respectively. In ozonated water (b) and chlorinated water (c) showing only the peaks of chloroform and bromodichloromethane and two other unidentified compounds.

inhibition curve. The absorbance of each sample was corrected, using a reagent blank (PP2A enzyme omitted), which compensated for color in the sample and for any naturally-occurring phosphatase enzyme in the sample. Each sample was analyzed in duplicate. Samples in which microcystin-LR equivalent exceeded 0.40 μg/L were confirmed by HPLC.

Analysis of microcystins by high performance liquid chromatography (HPLC)

100 mL of water sample was frozen and thawed 3 times and filtered using Whatman filter No. 4. The sample was concentrated using a Sep-Pak C_{18} cartridge. Prior to introducing the sample, the cartridge was activated with 5 mL of 100% methanol and washed with 10 mL of Milli-Q water. Microcystins were eluted from the cartridge with 2 mL of 100% methanol and evaporated to dryness in a nitrogen gas stream. The sample was redissolved in 30 μL of 100% methanol and 20 μL of sample was injected onto the HPLC. Microcystin-LR was analyzed using a linear gradient elution of 30–70% acetonitrile with 0.05% trifluoroacetic acid in 40 minutes, and a flow rate of 1 mL/min at the wavelength of 238 nm. The calibration curve was produced by plotting peak area versus microcystin-LR concentration using 0.1, 0.2, 0.4, 0.8 and 1.6 μg/mL standards.

RESULTS

THMs, TOC and residual chlorine levels in treated water

The levels of THMs, TOC and residual chlorine in ozonated water were lower than those found in chlorinated water. Figure 1 shows a typical gas chromatogram of THM mixed standard (a), ozonated water extract (b) and chlorinated water extract (c). The average speciation of the THMs species was 92% chloroform and 8% bromodichloromethane by mass (Table 1). Other species like dibromochloromethane and bromoform were not found. The levels of THMs, TOC and residual chlorine in treated water are shown in Table 2. THM levels in chlorinated water ranged between 3.5–97.0 μg/L whereas the THM levels in ozonated treated water ranged between 0–51.6 μg/L. No ozonated water sample exceeded the first stage of U.S. EPA maximum THM contaminant level (MCL) in drinking water of 80 μg/L and only 8% of these samples exceeded the second stage U.S. EPA MCL in drinking water of 40 μg/L. 3% of chlorinated water samples exceeded the first stage U.S. EPA MCL and 68% of these samples exceeded the second stage U.S. EPA MCL. The levels of THMs, TOC and residual chlorine in ozonated water at each ORP value were not significantly different.

Table 1 | The THM species in ozonated and chlorinated water

Sample	Chloroform (Mean ± SE)	BDCM (Mean ± SE)	Total THMs (μg/L)
August–December 2004			
Ozonated water	11.85 ± 2.43	0.89 ± 0.57	12.74 ± 2.90
Chlorinated water	49.37 ± 3.86	3.25 ± 1.08	52.62 ± 4.84
February–March 2005			
Ozonated water	22.50 ± 2.30	3.41 ± 0.26	25.91 ± 2.56
Chlorinated water	31.56 ± 2.61	4.24 ± 0.36	35.80 ± 2.97

THMs: Trihalomethanes; BDCM: Bromodichloromethane; SE: Standard error of mean.

Microcystin levels in treated water detected by PPi assay

The level of microcystins in all water samples was below the method detection limit of 0.54 μg/L and the WHO provisional drinking water guideline of 1 μg/L. Therefore, the efficiency of the ozonation process on the removal of microcystins was difficult to evaluate because the level of microcystins in raw water was too low.

Microcystin levels in treated water analyzed by HPLC

Twenty seven water samples which had microcystin concentrations over 0.40 μg/L detected by the *PPi* assay were selected to confirm whether it was microcystin-LR by the HPLC-UV visible detection. The microcystin-LR in raw water was very low and only 2 raw water samples were detected at the concentrations of 0.04 and 0.03 μg/mL. The detection limit was 2 ng on column.

Table 2 | The levels of THMs, TOC and residual chlorine (mean ± SE) in ozonated and chlorinated water

		Chlorination TOC (mg/L) in				Ozonation TOC (mg/L) in		
Sampling time	**THMs in chlorinated water (μg/L)**	**Raw water**	**Chlorinated water**	**Residual chlorine in chlorinated water (mg/L)**	**THMs in ozonated water (μg/L)**	**Raw water**	**Ozonated water**	**Residual chlorine in ozonated water (mg/L)**
August–December 2004 at								
ORP 400	50.65 ± 8.64	1.62 ± 0.01	1.65 ± 0.06	1.62 ± 0.27	16.15 ± 2.05*	1.62 ± 0.01	1.23 ± 0.05†	2.18 ± 1.66
ORP 500	56.18 ± 5.74	2.29 ± 0.01	1.85 ± 0.14	1.53 ± 0.28	9.45 ± 4.30*	2.29 ± 0.01	1.59 ± 0.04†	0.55 ± 0.15*
ORP 600	63.06 ± 11.72	1.85 ± 0.18	1.23 ± 0.11	2.59 ± 0.26	24.40 ± 8.01*	1.85 ± 0.18	1.14 ± 0.15†	2.56 ± 0.91
ORP 700	42.10 ± 8.97	2.00 ± 0.16	1.66 ± 0.06	2.06 ± 0.03	7.54 ± 4.06*	2.00 ± 0.16	1.15 ± 0.13†	1.68 ± 0.90
February–March 2005 at								
ORP 400	42.27 ± 5.49	1.65 ± 0.10	1.61 ± 0.05	2.10 ± 0.28	36.37 ± 7.61	1.65 ± 0.10	1.46 ± 0.12	0.34 ± 0.10*
ORP 500	26.88 ± 6.26	1.61 ± 0.15	1.48 ± 0.05	0.89 ± 0.33	19.79 ± 3.33	1.61 ± 0.15	1.40 ± 0.02†	0.30 ± 0.05*
ORP 600	32.99 ± 4.41	1.60 ± 0.06	1.48 ± 0.06	1.17 ± 0.31	23.18 ± 3.59	1.60 ± 0.06	1.45 ± 0.04	0.34 ± 0.08*
ORP 700	45.61 ± 2.42	1.76 ± 0.01	1.49 ± 0.01†	1.26 ± 0.22	28.64 ± 5.04*	1.76 ± 0.01	1.46 ± 0.08	0.23 ± 0.01*

THMs: Trihalomethanes; TOC: Total organic carbon; ORP: Oxidation-reduction potential; SE: Standard error of mean.
*$P < 0.05$: Significantly different from chlorinated water.
†$P < 0.05$: Significantly different from raw water.

DISCUSSION

Provincial water authority in Thailand is seeking an alternative disinfectant to replace chlorine in water treatment. This study confirms other studies (Glaze *et al.* 1982; Owen *et al.* 1995; Rositano *et al.* 1998), showing that ozone is effective in reducing THM formation and destroying microcystins. Muttamara *et al.* (1995) also reported that ozone appeared to be the most potent and promising of the currently used drinking water disinfectants and, at the same time, it is a strong oxidant with fewer carcinogenic by-product formation. One limitation of using ozonation is its cost which is about twice higher than that of chlorination.

Variable values of THMs in drinking water in different countries have been reported. The THM levels in Japan, Egypt, Korea and Saudi Arabia ranged between 12.5–37.5, 18.3–67.3, 55.0–102.6 and 0.03–41.7 μg/L, respectively (Kagino & Yaki 1980; Fayad 1993; Hassan *et al.* 1996; Kim *et al.* 2002). Current study in Thailand reported the mean value of THMs from Aung-Kaew and Mae-Hia water works in Chiang Mai province of 60 and 62.5 μg/L, respectively. The predominant THM species detected was also reported to be chloroform (Wattanachira *et al.* 2004).

It has also been reported that pre-ozonation could reduce the concentration of dissolved organic carbon, which could react with chlorine to form THMs, resulting in the reduction of THM formation (Amy *et al.* 1990; Chang *et al.* 2002). Similar to several previous studies, the level of THMs in ozonated water tended to be lower than those of the THMs in chlorinated water in this study.

That the brominated THMs such as dibromochloromethane and bromoform were not detected may be due to the low presence of bromide in the raw water. Bromide is converted to hypobromous acid which can react with natural organic matter in surface water to form brominated THMs.

Similar to Pekkoh's study (2002), the concentration of microcystins contaminated in Mae Kuang reservoir was very low. This study showed that the concentrations of microcystins in all water samples, which were detected by *PPi* assay, were low and below the method detection limit (IC10 was 0.54 μg/L) and the provisional guideline in drinking water (1 μg/L).

CONCLUSION

In conclusion, this study suggests that the ozonation pilot plant is successful in reducing THM contamination during the water treatment process. The THM level in water samples from both plants was lower than the U.S. MCL of 80 μg/L in drinking water. Chloroform was the major THM species in treated water. In addition, the concentration of microcystins in Mae Kuang reservoir was very low and the level was below the provisional guideline in drinking water of 1 μg/L. The optimum ORP for treating Mae Kuang raw water by pre-ozonation was 500 mV. At this ORP, microcystins in raw water appeared to be reduced. Moreover, the efficiency of TOC removal by ozonation pilot plant was higher than those of TOC removal by chlorination water treatment plant. Therefore, ozonation is an alternative process which can be utilized in the production of drinking water because it significantly reduces the formation of THMs. The efficiency of the ozonation process on the removal of microcystins was difficult to evaluate because the level of microcystins in raw water was too low.

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