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**A STUDY OF MICROBIAL REGROWTH POTENTIAL OF
WATER IN FARGO, NORTH DAKOTA AND MOORHEAD,
MINNESOTA**

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**North Dakota Water Resources Research Institute
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ABSTRACT

The microbial regrowth potential of water from different stages of the treatment trains and distribution systems of the cities of Moorhead, Minnesota and Fargo, North Dakota was studied. Assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC) were the key indicators used to justify the regrowth potential. Dissolved organic carbon (DOC), ultraviolet absorbance at 254 nm (UV_{254}), and specific ultraviolet absorbance (SUVA) were complimentary parameters examined. Water samples collected bi-weekly for the entire year of 2004 from the two water systems were analyzed for the five parameters. Raw water for both systems was high in AOC and BDOC concentrations. Both treatment systems removed substantial amounts of AOC and BDOC but the levels in their finished water were still well above the threshold concentrations for possible microbial regrowth. However, there was no significant reduction in AOC and BDOC in both distribution systems as the levels in the finished water and tap water were comparable throughout the year. The values of the other three parameters (DOC, UV_{254} , and SUVA) tended to support the AOC and BDOC results. Based on these observations, there should be minimal or no microbial regrowth in the Moorhead and Fargo distribution systems.

Background

In recent years, water quality scientists and engineers have emphasized on the biodegradability of dissolved organic matter in both raw and treated waters. This is because the biodegradable organic matter (BOM) in treated water can induce the growth or regrowth of microorganisms in the distribution system of drinking water. Residual BOM is usually the most important limiting factor responsible for bacterial regrowth in the water distribution system (Rittmann and Snoeyink, 1984). One of the most effective methods in controlling the bacterial growth in the distribution system is to limit the amount of BOM required for the growth of heterotrophic bacteria in treated water (Servais *et al.*, 1993). Water containing BOM less than a minimum concentration that supports the bacterial growth is usually biologically stable.

Biodegradable dissolved organic carbon (BDOC) has been used as one of the parameters for quantifying the amount of BOM in water. Servais *et al.* (1989) defined BDOC as the fraction of dissolved organic carbon (DOC), which can be metabolized by bacteria within a period of time. The BDOC test measures the reduction of DOC in a water sample, which is exposed to microorganisms in a period of time (Servais *et al.*, 1987 and 1989). The first BDOC measurement was introduced by Servais *et al.* (1987). It was developed as a batch procedure. A mixed microbial culture from the same environment as the sample was used as an inoculum. Incubation occurred in the dark at $20 \pm 0.5^\circ\text{C}$ for a period of 10 to 30 days and BDOC was the difference between the initial and final DOCs.

The first BDOC procedure was used specifically for testing the quality of raw water and for designing and monitoring, and optimizing operating conditions of biological activated carbon (ozonation + granular activated carbon) systems. Occasionally, it was used to examine the BDOC removal of other treatment processes such as coagulation and filtration. Interest in BDOC of finished water started to grow when BDOC was linked to the microbial proliferation in the distribution systems. As a result, BDOC is a widely used parameter in the drinking water field.

An alternative to the BDOC procedure called assimilable organic carbon (AOC), was invented by van der Kooij *et al.* (1982). AOC is the portion of the organic carbon that can be synthesized to cellular material by a single bacterial strain. In the AOC determination method, a preheated water sample is seeded with a pure strain of *Pseudomonas fluorescens* P17. The sample is incubated at 15°C , and bacterial growth is monitored daily by colony counts (spread plate techniques) until the maximum growth is reached. The incubation period (the number of days to reach the maximum yield) can be from 3 to 30 days depending on the type of the water sample. By concurrently determining the growth yield of bacteria in solutions of known acetate concentration, the maximum growth can be converted into AOC and expressed as μg of acetate-C equivalents/L.

van der Kooij (1987) and van der Kooij *et al.* (1989) included a *Spirillum* strain, NOX, into the AOC procedure as an alternative seed or a dual strain seed due to the inability of *Pseudomonas fluorescens* P17 to metabolize oxalic acid, which is one of the products frequently formed during ozonation. Unlike BDOC, AOC only accounts for the organic carbon used for cell synthesis. Since the AOC test measures cell growth of a single or dual strain, the test does not guarantee that all the assimilable carbon is measured. The inoculum may not be capable of metabolizing all

contaminants. Therefore, the reported AOC value is normally less than the reported BDOC value for the same sample. The AOC method has been widely adopted when the regrowth is a concern. One of the three threshold criteria proved useful in predicting coliform occurrences is AOC concentrations in plant effluent > 100 µg/L (Volk and LeChevallier, 2000).

BDOC has also been related to the regrowth of microorganisms. High BDOC in finished water indicates poor quality and a potential of microbial multiplication. Maintaining free chlorine residual to prevent the regrowth along the distribution system is a common solution; however, a large amount of chlorine is required. Also, chlorine residual cannot completely inactivate fixed bacteria (Le Chevallier *et al.*, 1988). Controlling microbial dynamics by limiting available substrate (BDOC) is a new and interesting approach (Rittmann and Snoeyink, 1984, Huck, 1990, and Servais *et al.*, 1993). Biologically stable water should contain less than 0.15 mg of BDOC/L. At this threshold level, microbial growth is very limited (Servais *et al.*, 1993).

In order minimize AOC and BDOC in finished water, some water treatment plants employ biological filtration in their treatment trains. The biological filtration usually consists of ozonation and filtration. The purpose of ozonation is to destruct complex organic to simpler molecules, which can be used by microorganisms in the filter. For some treatment plants, the ozonation portion of the biological filtration also serves as primary disinfection, and taste and odor control. Increases in BDOC and AOC after ozonation have been well documented (Janssens *et al.*, 1984, Servais *et al.*, 1987, and Volk *et al.*, 1993). The BDOC and AOC increases during ozonation are removed in the filter, which contains media (activated carbon or sand) covered by attached microorganisms (biofilm).

Description of the Critical State or Regional Water Problem Investigated

BDOC and AOC have been used to examine the regrowth potential of water throughout the world. Furthermore, AOC is included in *Standard Methods* (1998). However, the regrowth potential of water in Fargo, North Dakota and Moorhead, Minnesota has never been evaluated; BDOC and AOC values of water in Fargo and Moorhead have not been reported. The ultimate goal is to minimize the concentrations of these two parameters in treated water provided by the Fargo and Moorhead water treatment plants, which in turn will benefit public health by limiting the number of microorganisms in tap water. Collecting BDOC and AOC data is a first step to achieve the ultimate goal. The data will also indicate the degree of susceptibility of drinking water of Fargo and Moorhead to microbial proliferation.

It is crucial that BDOC and AOC of water in Fargo and Moorhead are studied because of the nature of high total organic concentrations in the influent and effluent and the use of ozonation at both treatment plants (Figure 1). BDOC and AOC tend to be high with these two conditions. The influent (from the Red River for the Fargo plant and the Red River blended with groundwater at 85%:15% for the Moorhead plant) and effluent total organic carbon (TOC) concentrations at both plants are sometimes as high as 8 to 10 mg/L and 1 to 2 mg/L, respectively. BDOC is a portion of total organic concentrations. Although water with high total organic concentrations does not necessarily contain large amounts of BDOC, positive linear relationships have been frequently observed between the two parameters (Servais *et al.*, 1987 and Khan *et al.* 1998b). As stated previously, ozonation of water with organics results in BDOC and AOC increases and

their removal relies on the performance of subsequent treatment, which normally is filtration. Currently, BDOC and AOC removal abilities of the filtration units at the Fargo and Moorhead plants are not known because the two parameters have not been measured.

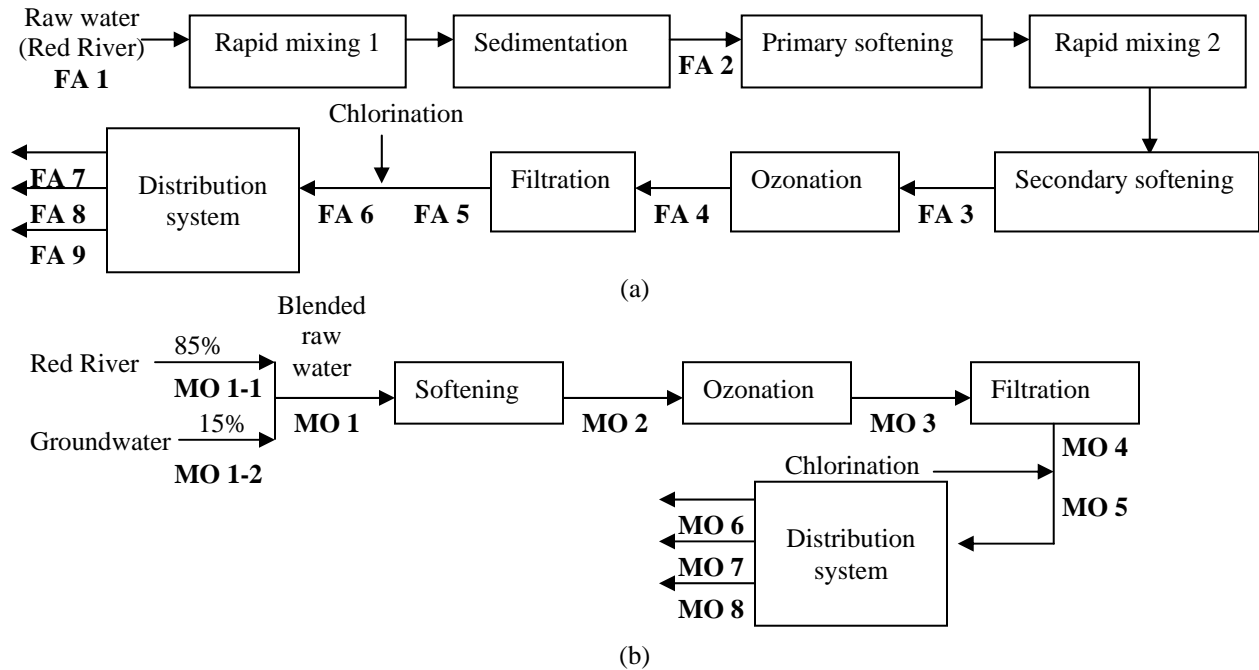


Figure 1. Simplified schematic diagrams for (a) the Fargo Water Treatment Plant and (b) the Moorhead Water Treatment Plant.

Scope of Study and Objectives

The main scope of this study was to collect BDOC and AOC data of water in Fargo and Moorhead in order to achieve the following objectives:

- 1) To evaluate the microbial regrowth potential of water especially finished water and tap water,
- 2) To predict the regrowth occurrence in the distribution systems by comparing AOC and BDOC in finished water and those in tap water, and
- 3) To compare BDOC and AOC removal of the Fargo and Moorhead plants which are different mainly in the presence and absence of the rapid mixing-sedimentation process.

Methods

Sampling

Sampling took place between January and December, 2004. Samples were collected from one treatment plant and accompanying distribution system biweekly. The plants were alternated weekly. Sampling locations are listed in Table 1. The samples were analyzed for AOC, BDOC, ultraviolet absorbance at 254 nm (UV_{254}), DOC and pH.

Table 1 Sample identification and collection locations.

Fargo water systems		Moorhead water systems	
Sample no.	Sampling location	Sample no.	Sampling location
FA 1	Raw water (Red River)	MO 1	Blended raw water
FA 2	After sedimentation	MO 1-1	Raw water (Red River)
FA 3	After secondary softening	MO 1-2	Raw water (Groundwater)
FA 4	After ozonation	MO 2	After softening
FA 5	After filtration	MO 3	After ozonation
FA 6	After chlorination (clear well)	MO 4	After filtration
FA 7	Holiday Inn Hotel	MO 5	After chlorination (clear well)
FA 8	North Dakota State University	MO 6	Busch Agricultural Resources Inc.
FA 9	Hector International Airport	MO 7	Stop and Wash Convenience Store
		MO 8	Moorhead Wastewater Plant Tap

* Sample will only be collected when utilized as a source for raw water

Analyses

pH was measured using a pH meter (Orion model SA520). UV_{254} was determined following the procedure listed in *Standard Methods* (1998), using a spectrophotometer (Thermo Spectronics model Genesys 10 UV Scanning). DOC was analyzed according to the procedure described in *Standard Methods* (1998), using a TOC analyzer (Tekmar-Dohrmann model Phoenix 8000). For DOC analysis, the samples were filtered through a glass fiber filter (Whatman, GF/F) prior to TOC determination. BDOC was evaluated in accordance with a modified protocol by Khan *et al.* (1998a), which is simpler and more accurate than the original method (Servais *et al.*, 1987). AOC was determined according to *Standard Methods* (1998).

Results and Discussion

Moorhead water treatment and distribution systems

Figures 2 to 4 show BDOC results for the Moorhead plant and distribution system. Figure 2 presents the Red river and groundwater BDOC and BDOC after softening. The Red river BDOC influenced BDOC of samples from the water treatment plant units and distribution system. This is not surprising since 85% of raw water is from the Red river. The softening process significantly reduced BDOC. Figure 3 shows an increase in BDOC after ozonation. The use of ozone oxidized complex organics into smaller more readily biodegradable molecules.

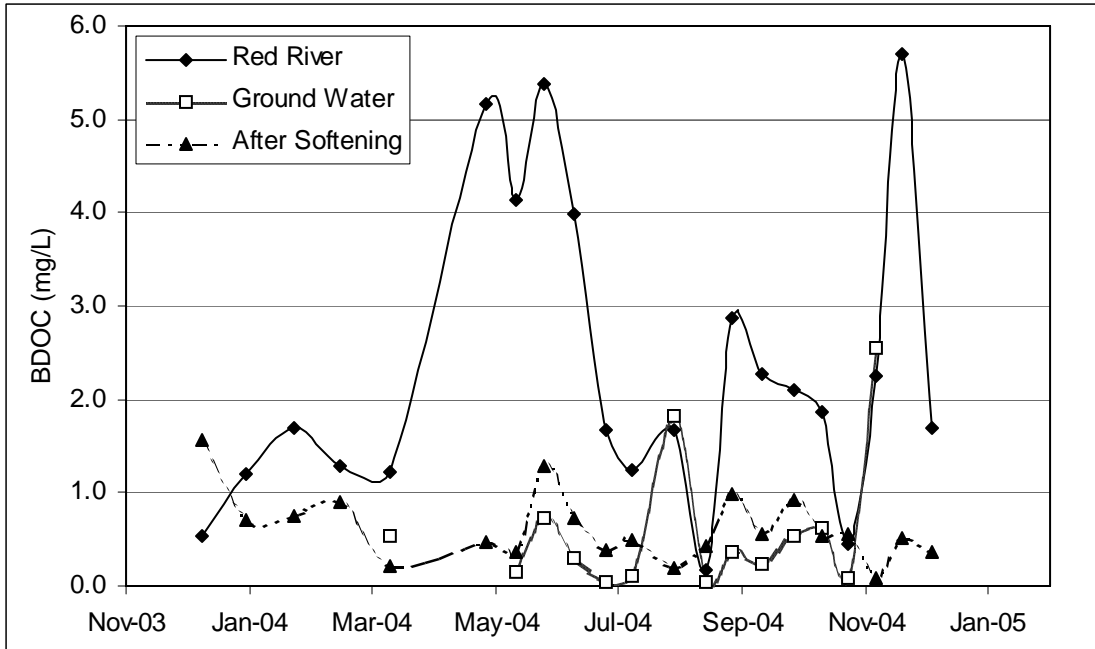


Figure 2. BDOC of the Red river, groundwater, and after softening samples from the Moorhead Water Treatment Plant.

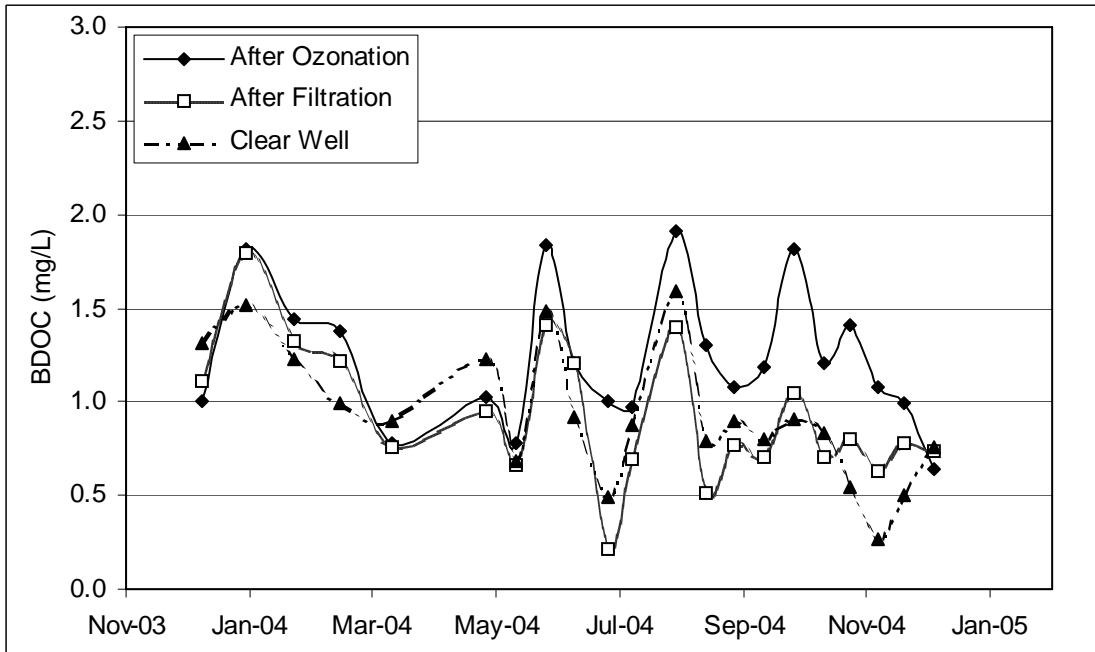


Figure 3. BDOC of after ozonation, filtration, and clear well samples from the Moorhead Water Treatment Plant.

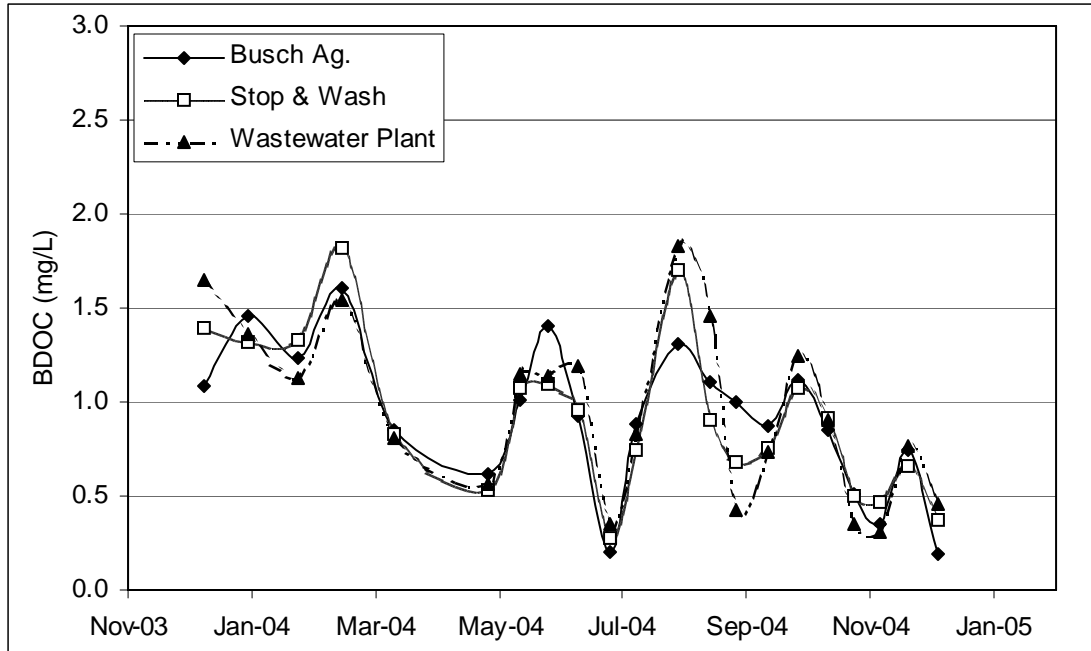


Figure 4. BDOC of samples from the Moorhead distribution network.

From June to December, the filtration system at the treatment plants was biologically active because of higher temperature of water in Summer and Fall. The system reduced a portion of the BDOC on the water but not down to the level after softening. The ineffectiveness of filtration in removing the biodegradable fractions in the water led to the potential for bacterial regrowth in the distribution system. Comparing BDOC in the distribution system samples with that in the effluent (clear well) water, it does not appear that regrowth occurred in the distribution network (Figures 3 and 4). The BDOC of the clear well samples were in most cases not much different from the BDOC values in the distribution network regardless of season. Since there were limited decreases in BDOC concentration, the regrowth was likely minimal.

Figures 5 to 7 show the DOC of the samples from the Moorhead plant and the distribution network. Figure 5 shows the variation of Red River water DOC between 7 and 15 mg/L. The groundwater had a relatively low DOC usually between 1 and 2 mg/L. The softened water had lower DOC and less variation. Figure 6 presents the DOC after ozonation, filtration, and disinfection (clear well). A decrease in DOC across the filter occurred in Summer and Fall suggesting that the filters at the treatment plant became biologically active in the warmer months. DOC in the distribution network water fluctuated between 1 and 5 mg/L with lower DOC in the summer and fall months.

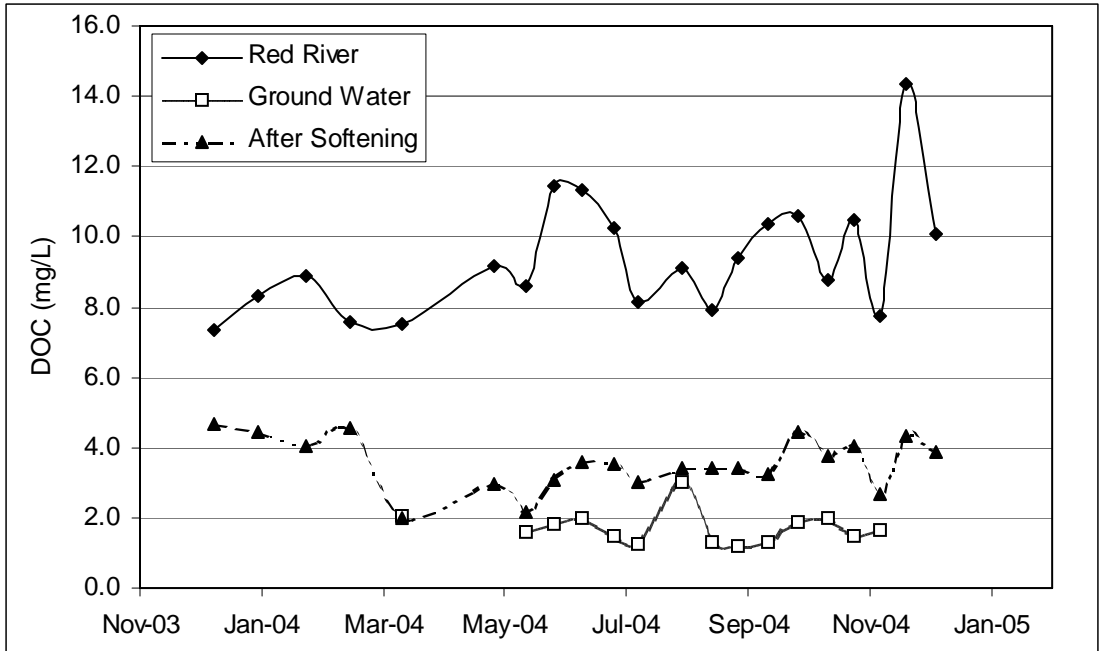


Figure 5. DOC of the Red river, groundwater, and after softening samples from the Moorhead Water Treatment Plant.

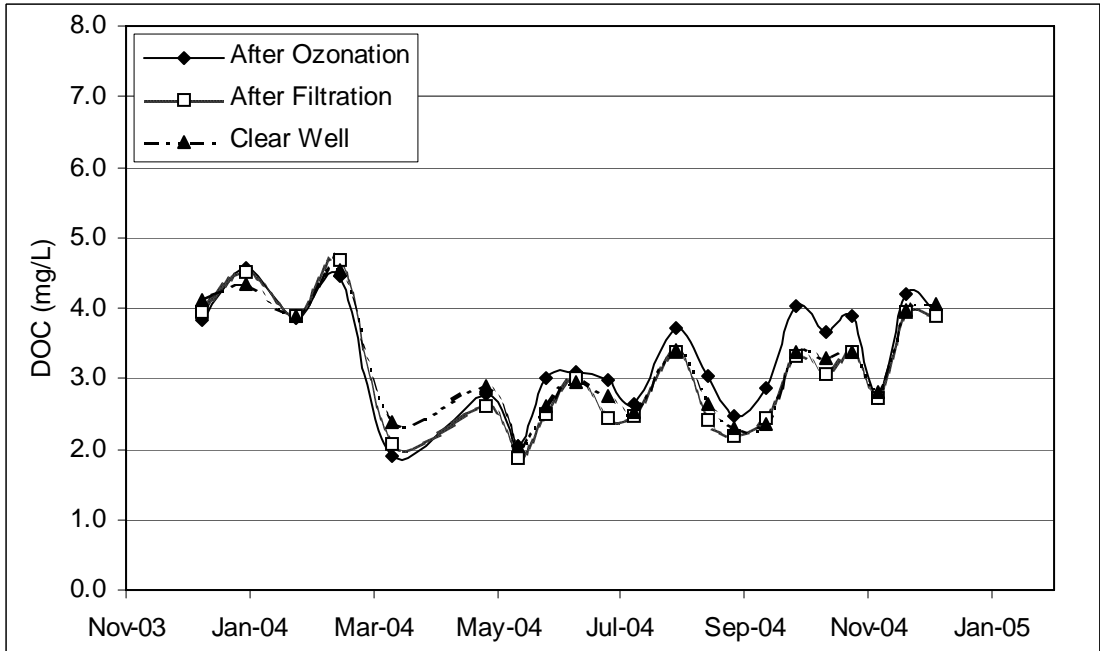


Figure 6. DOC of after ozonation, filtration, and clear well samples from the Moorhead Water Treatment Plant.

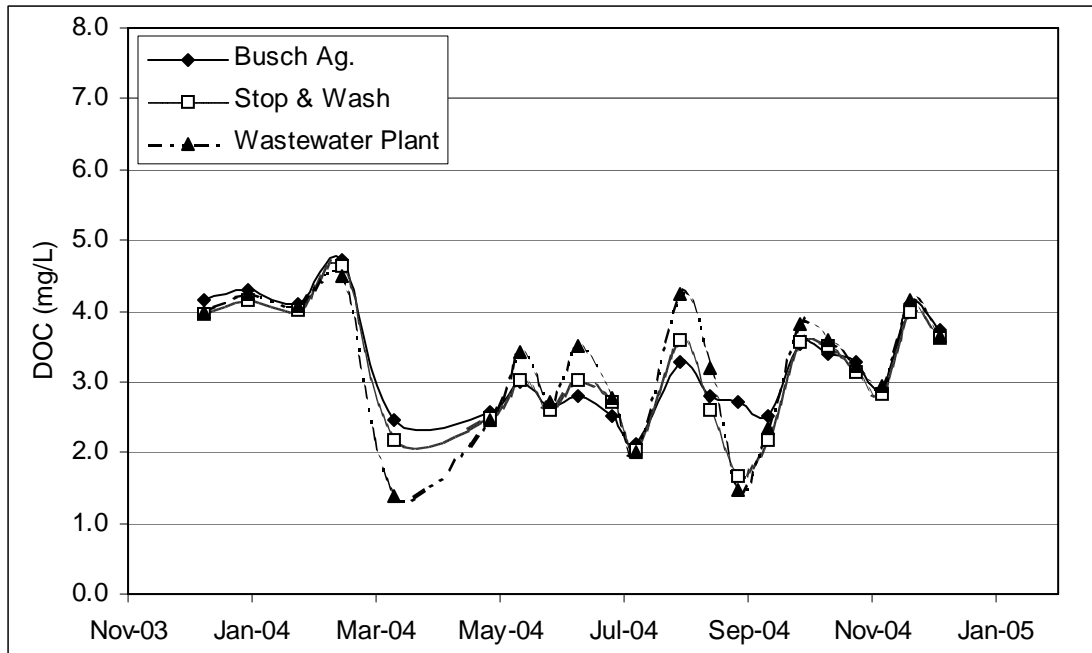


Figure 7. DOC of samples from the Moorhead distribution network.

The UV_{254} results of the samples from the Moorhead plant and the distribution network are shown in Figures 8 to 10. The Red river water had high UV_{254} indicating tremendous amounts of unsaturated and aromatic organics absorbing UV (Figure 8). After the raw water underwent softening, the majority of these complex organics was removed. Figure 9 shows less UV_{254} after ozonation suggesting that some of the UV_{254} absorbing organics were degraded during ozonation. The addition of chloramines for secondary disinfection in the clear well sample caused slight increases in UV_{254} . Figure 10 shows UV_{254} of the distribution network samples. The samples from Stop and Wash tap water had slightly more fluctuation than the samples from the other two sources. The level of UV_{254} in the distribution network samples was comparable to that of the clear well samples indicating no formation of UV_{254} absorbing compounds during the distribution.

Figures 11 to 13 present specific ultraviolet absorbance (SUVA) values of the samples from the Moorhead plant and the distribution network. The majority of the SUVA values, which are UV_{254} divided by DOC, were below 3.0 except for some raw water samples. A SUVA value below 3.0 indicates that the organics present in the water sample are biodegradable and less hydrophobic in nature. Generally, the lower the SUVA value, the more biodegradability the organics in the sample are. Organics in most of the water samples appeared to be biodegradable based on the SUVA values, which could lead to a potential for regrowth. However, significant regrowth never actually took place based on the BDOC results presented above.

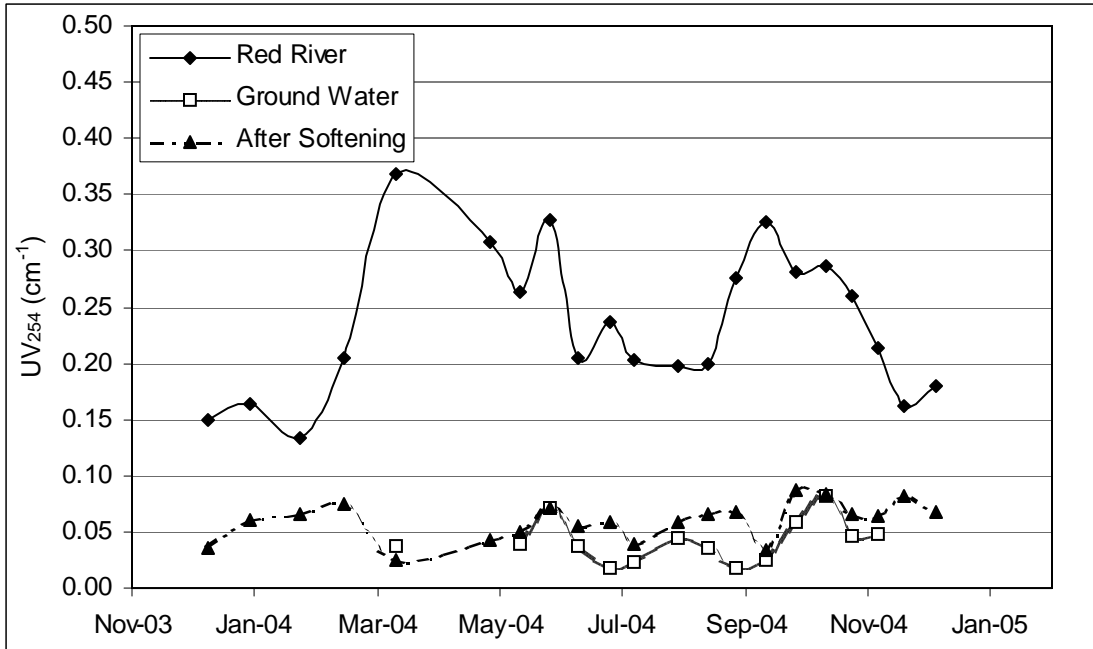


Figure 8. UV₂₅₄ of the Red river, groundwater, and after softening samples from the Moorhead Water Treatment Plant.

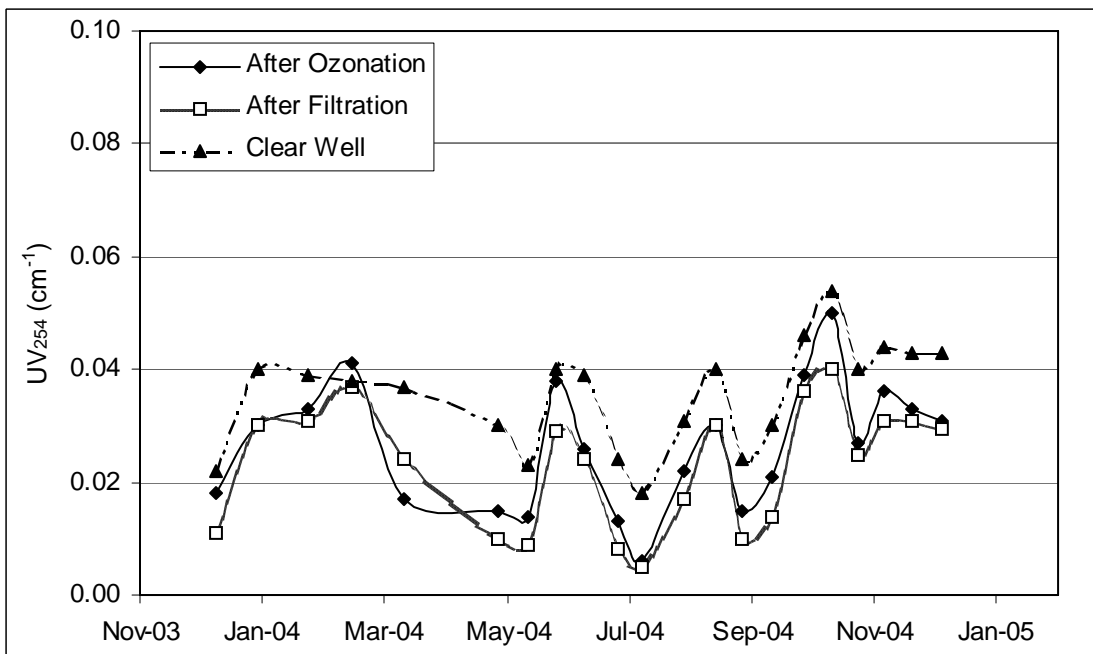


Figure 9. UV₂₅₄ of after ozonation, filtration, and clear well samples from the Moorhead Water Treatment Plant.

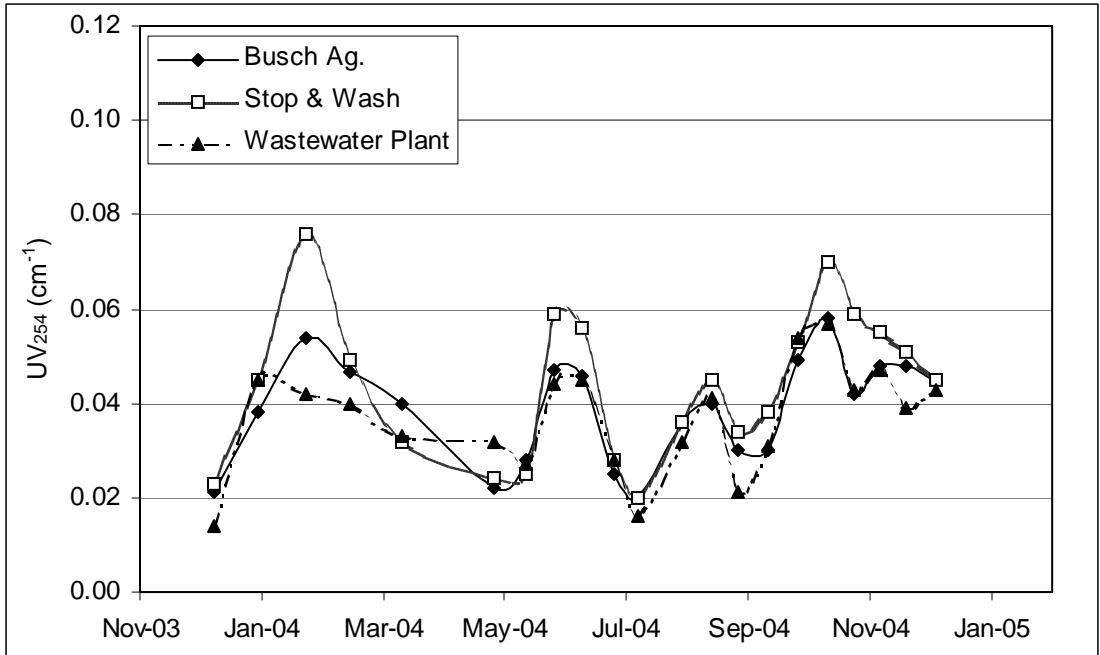


Figure 10. UV₂₅₄ of samples from the Moorhead distribution network.

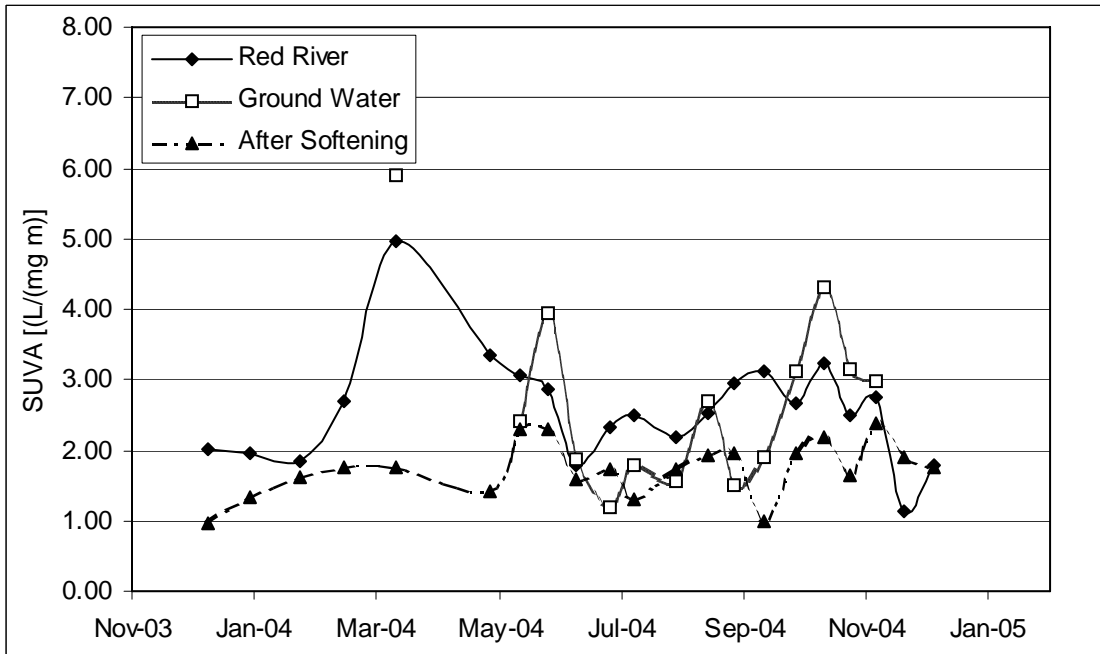


Figure 11. SUVA of the Red river, groundwater, and after softening samples from the Moorhead Water Treatment Plant.

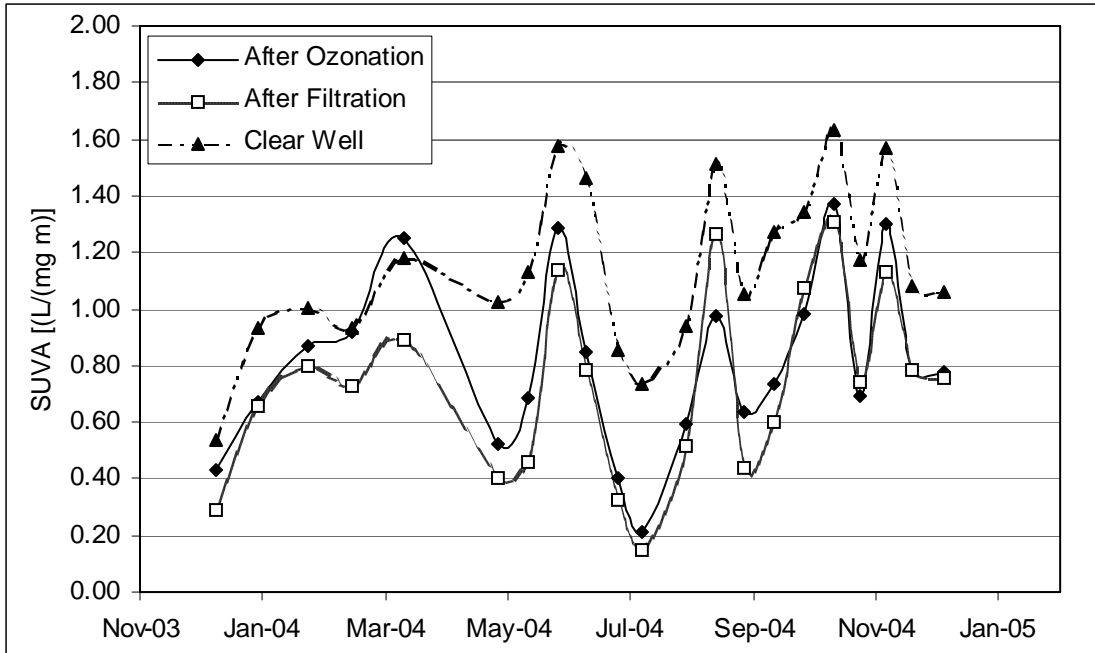


Figure 12. SUVA of after ozonation, filtration, and clear well samples from the Moorhead Water Treatment Plant.

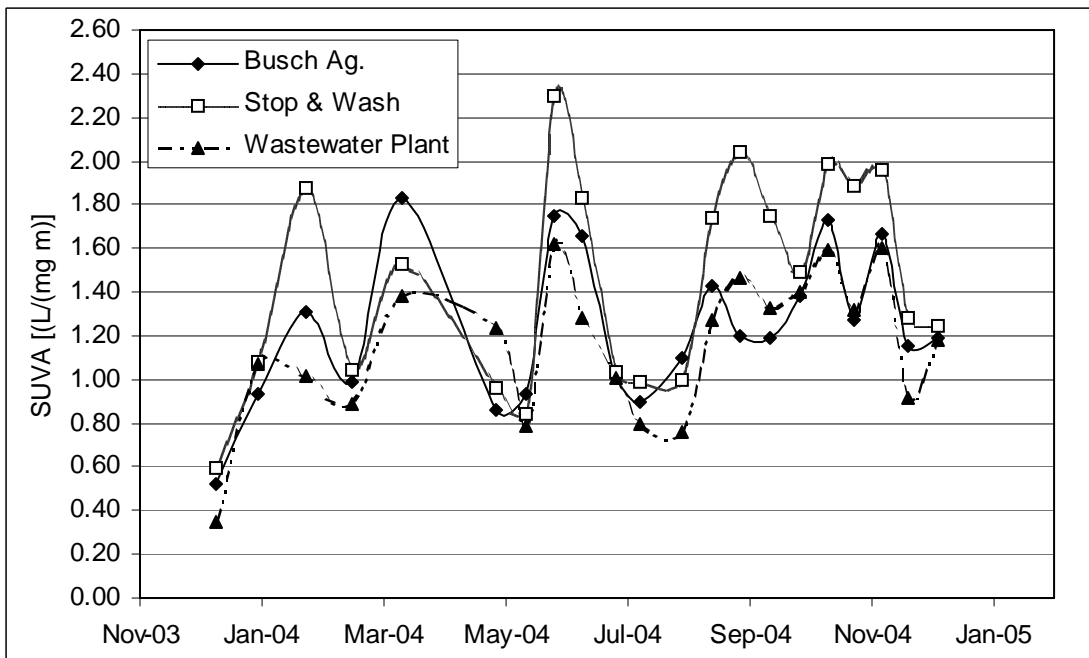


Figure 13. SUVA of samples from the Moorhead distribution network.

Figures 14 to 16 are the AOC results of the samples from the Moorhead plant and the distribution network. The AOC values represent the actual regrowth using two surrogate strains of bacteria. For the raw water samples, AOC was highly variable; the value fluctuated from 50 to 250 $\mu\text{g/L}$ (Figure 14). AOC was not detected in any of the after softening samples. This finding is unexplainable and requires further investigation. The samples might contain constituents that interfere with the AOC analysis. AOC increases were observed in only some ozonated samples (Figure 15). Filtration did not always reduce AOC; significant AOC increases after filtration occurred in some samples. These results do not agree well with the BDOC results above. Chlorination led to occasional increases in AOC. AOC in distribution network samples fluctuated dramatically but tended to be lower in Summer and Fall months (Figure 16). Although AOC in treated water frequently exceeded the threshold level of 100 $\mu\text{g/L}$, its decrease in the distribution system was not evident supporting the BDOC results on limited regrowth.

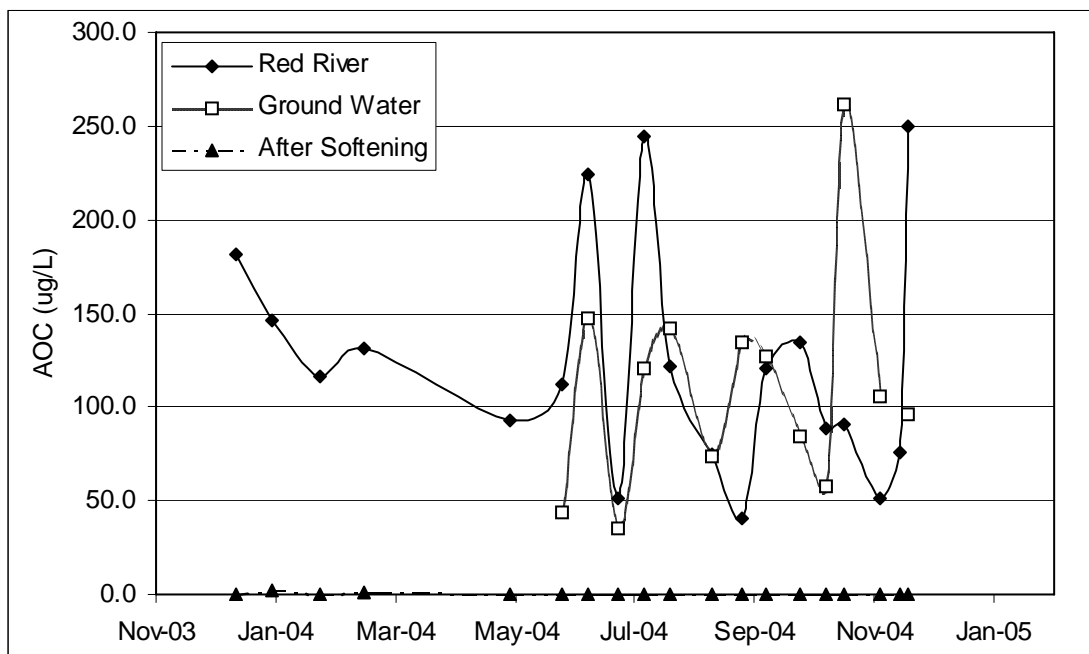


Figure 14. AOC of the Red river, groundwater, and after softening samples from the Moorhead Water Treatment Plant.

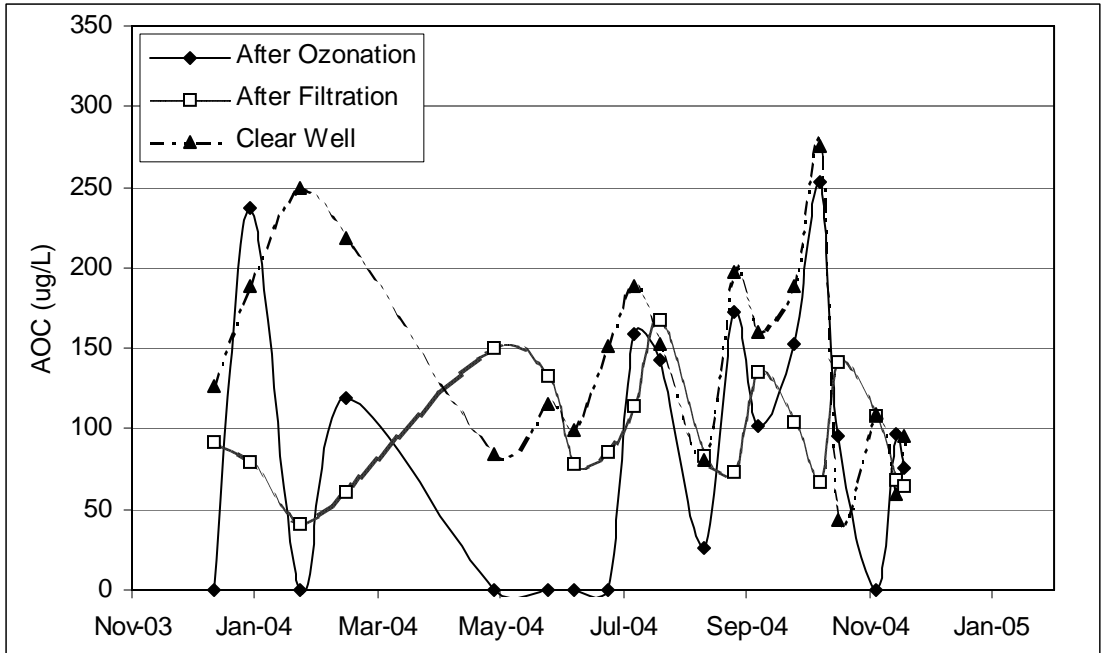


Figure 15. AOC of after ozonation, filtration, and clear well samples from the Moorhead Water Treatment Plant.

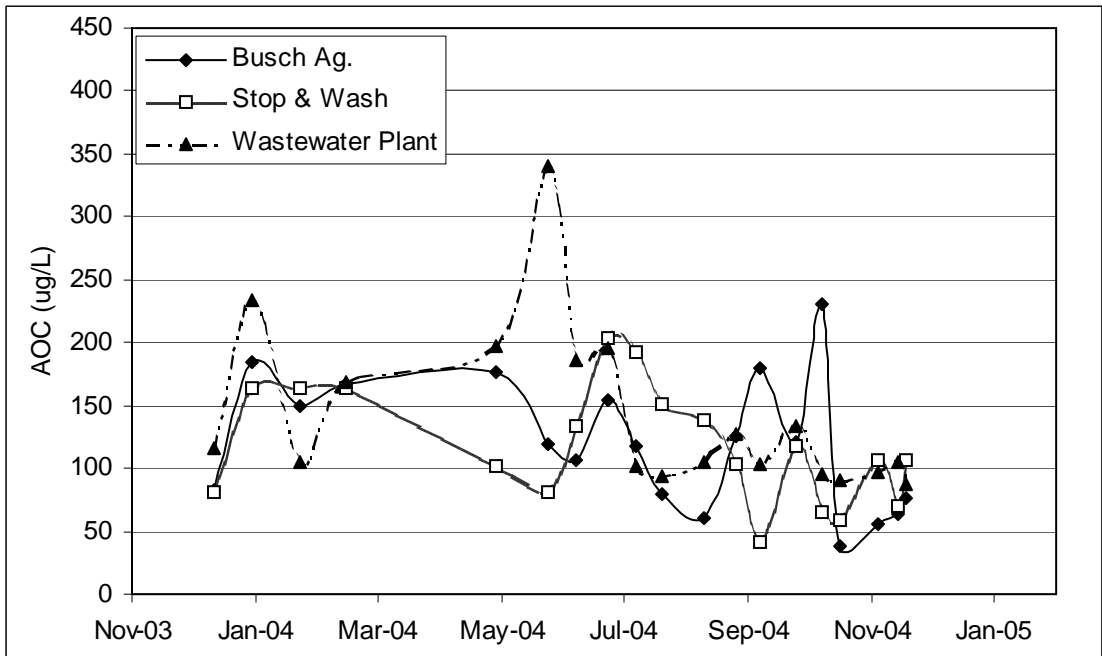


Figure 16. AOC of samples from the Moorhead distribution network.

Fargo water treatment and distribution systems

BDOC results for the Fargo plant and distribution system are presented in Figures 17 to 19. The sedimentation process did not affect BDOC (Figure 17). Except for a few sampling dates, softening reduced BDOC but not down to level achieved at the Moorhead plant (generally < 1 mg/L). Ozonation increased BDOC in most cases and slightly reduced BDOC occasionally. Filtration tended to remove more BDOC in the last six months of the year (Figure 18). After chlorination (clear well samples), BDOC increases were observed in several sampling dates. This suggests the inadequacy of the ozonation level on those dates. BDOC in Fargo tap water samples was higher than Moorhead tap water samples ranging from approximately 0.5 to 2.5 mg/L (Figure 19). Although this range of BDOC implies high microbial regrowth potentials, based on comparable levels of BDOC in clear well and distribution network samples, the regrowth was likely minimal. Season did not clearly affect the regrowth and its potential. This finding is similar to the results for the Moorhead system.

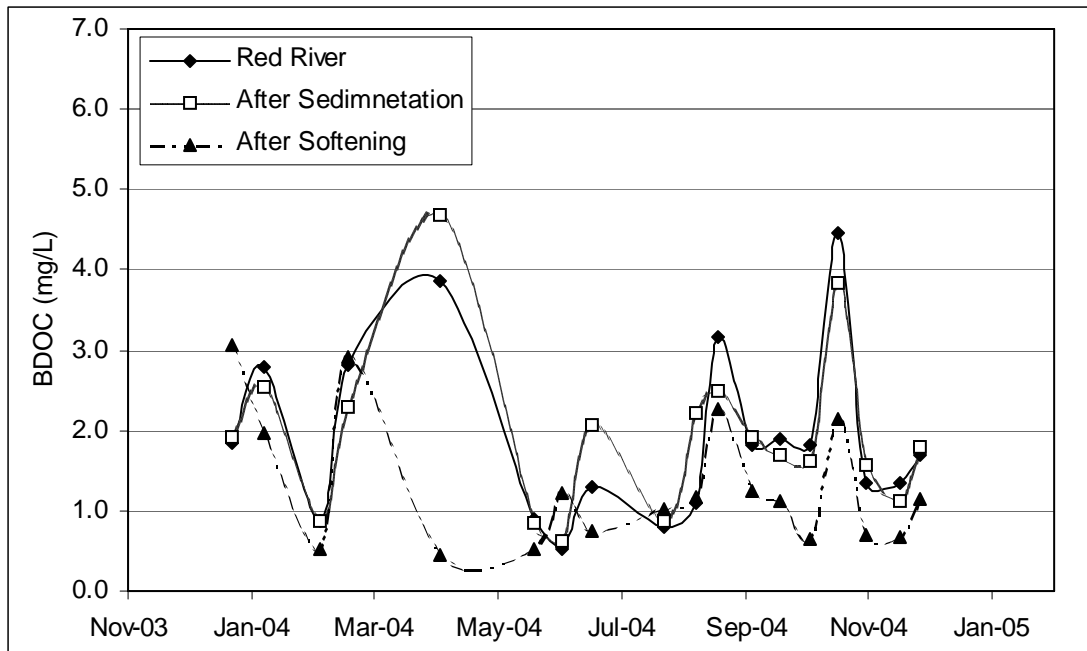


Figure 17. BDOC of the Red river, after softening and sedimentation samples from the Fargo Water Treatment Plant.

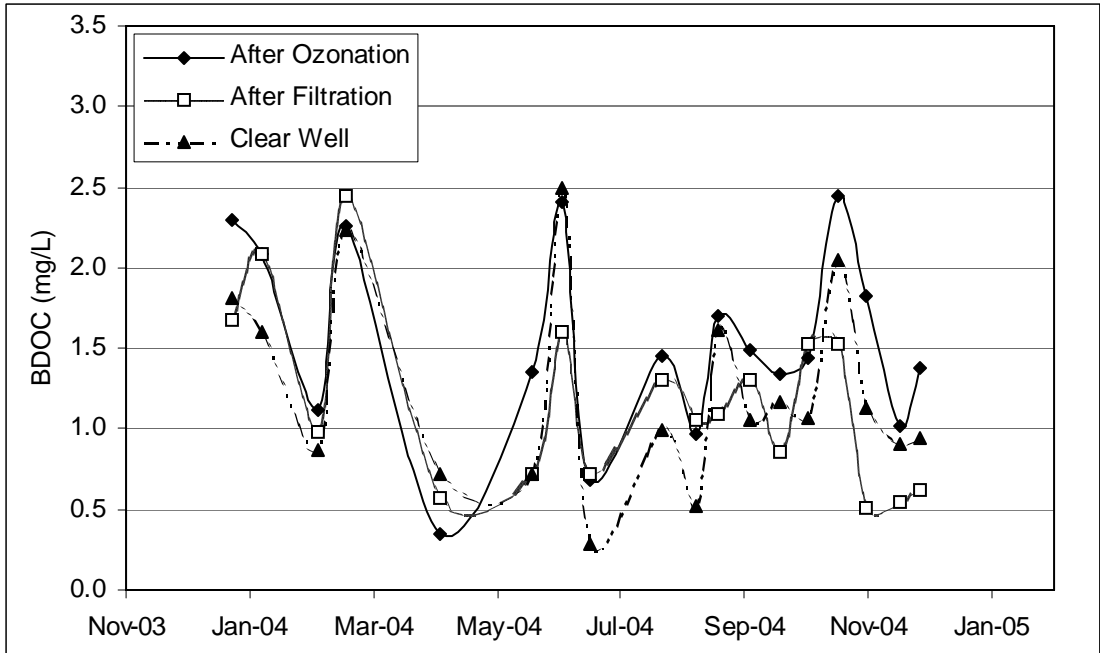


Figure 18. BDOC of after ozonation, filtration, and clear well samples from the Fargo Water Treatment Plant.

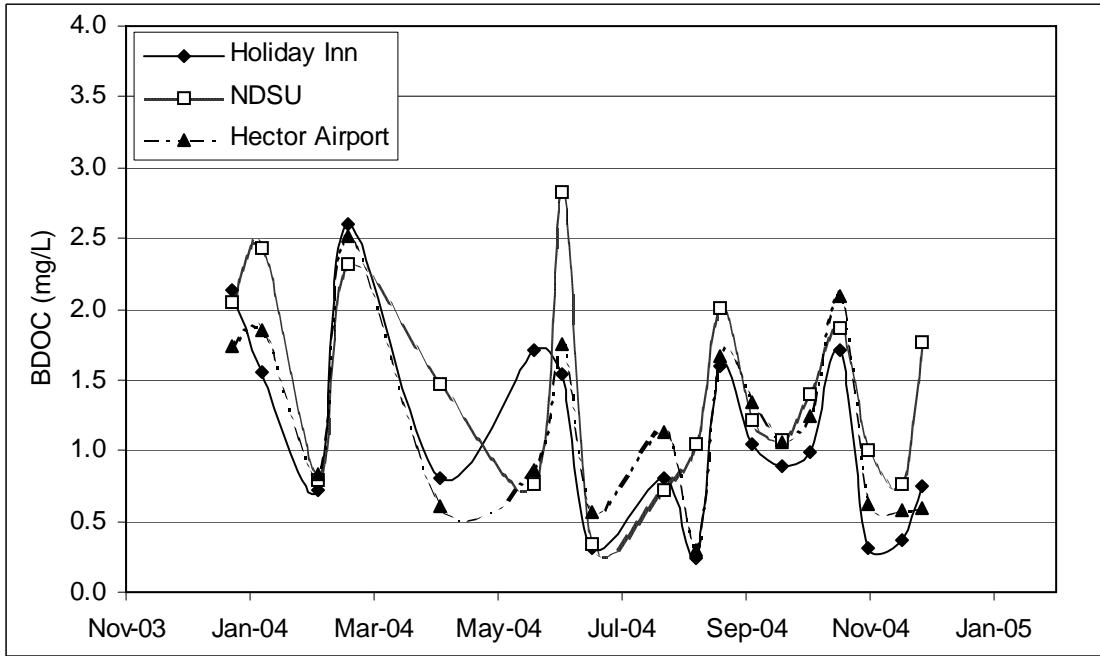


Figure 19. BDOC of samples from the Fargo distribution network.

Figures 20 to 22 illustrate the DOC of the samples from the Fargo plant and the distribution network. The results were conformable with the findings on BDOC. Sedimentation removed limited amounts of DOC while significant DOC decreases were observed after softening (Figure 20). Some DOC was eliminated during filtration (Figure 21). Most of the DOC removed was probably biodegradable (Some of the BDOC portion of DOC). There were no substantial changes in DOC starting from filtered water all the way to tap water (Figures 21 and 22). This indicates that only the DOC composition (biodegradable fraction) changed during these phases of the system but the total of amount DOC remained the same.

The UV₂₅₄ results of the Fargo plant and the distribution network are shown in Figures 23 to 25. The trends were not different from those of the Moorhead system. Tremendous amounts of UV₂₅₄ absorbing compounds were removed through softening (Figure 23). Except for chlorination, which increased UV₂₅₄ slightly ($< 0.03 \text{ cm}^{-1}$), the other subsequent treatment units and the distribution system had no dramatic effect on UV₂₅₄ (Figures 24 and 25). UV₂₅₄ of the distribution network samples ranged between 0.01 and 0.08 cm^{-1} , not much different from those of the Moorhead distribution network samples. The differences in UV₂₅₄ of the samples from the three distribution network locations were very minor in general with slightly higher values observed periodically at NDSU location.

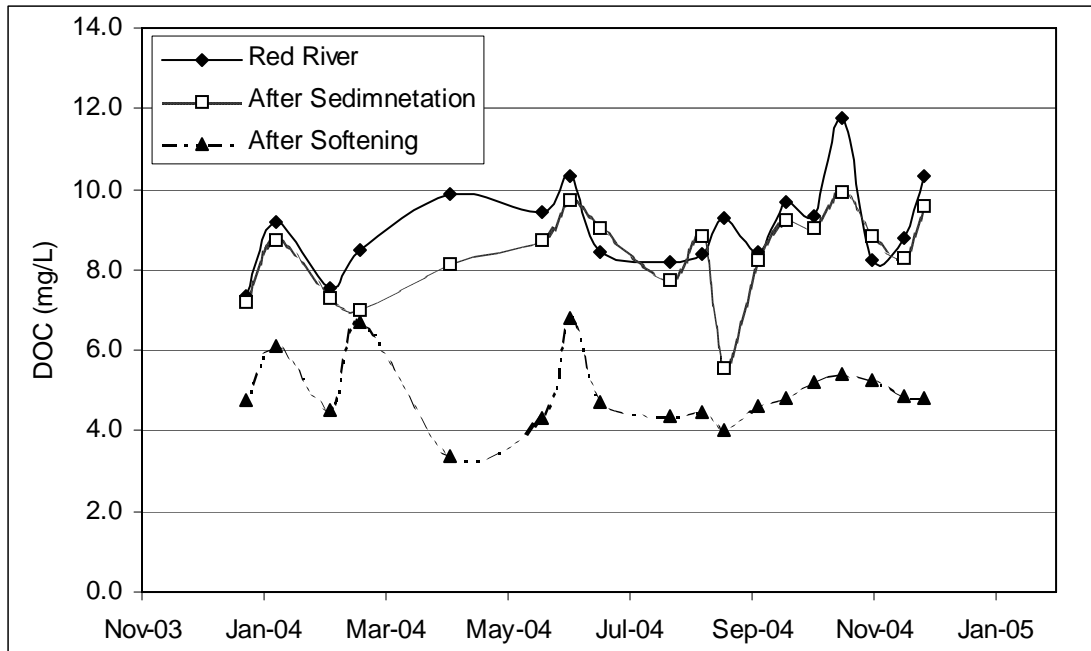


Figure 20. DOC of the Red river, after softening and sedimentation samples from the Fargo Water Treatment Plant.

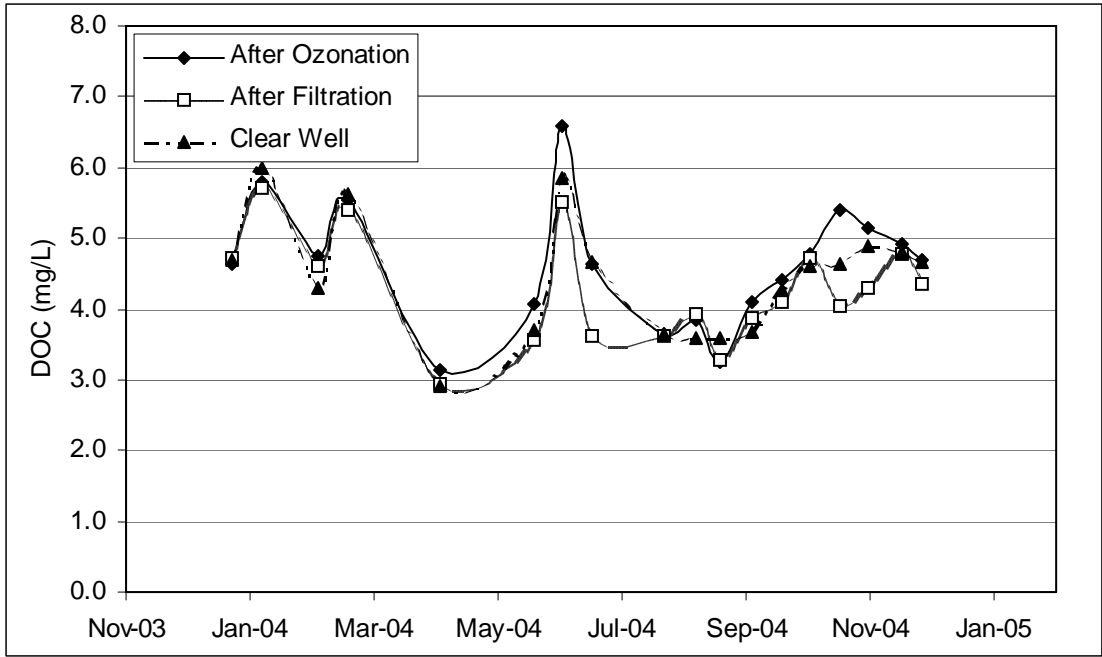


Figure 21. DOC of after ozonation, filtration, and clear well samples from the Fargo Water Treatment Plant.

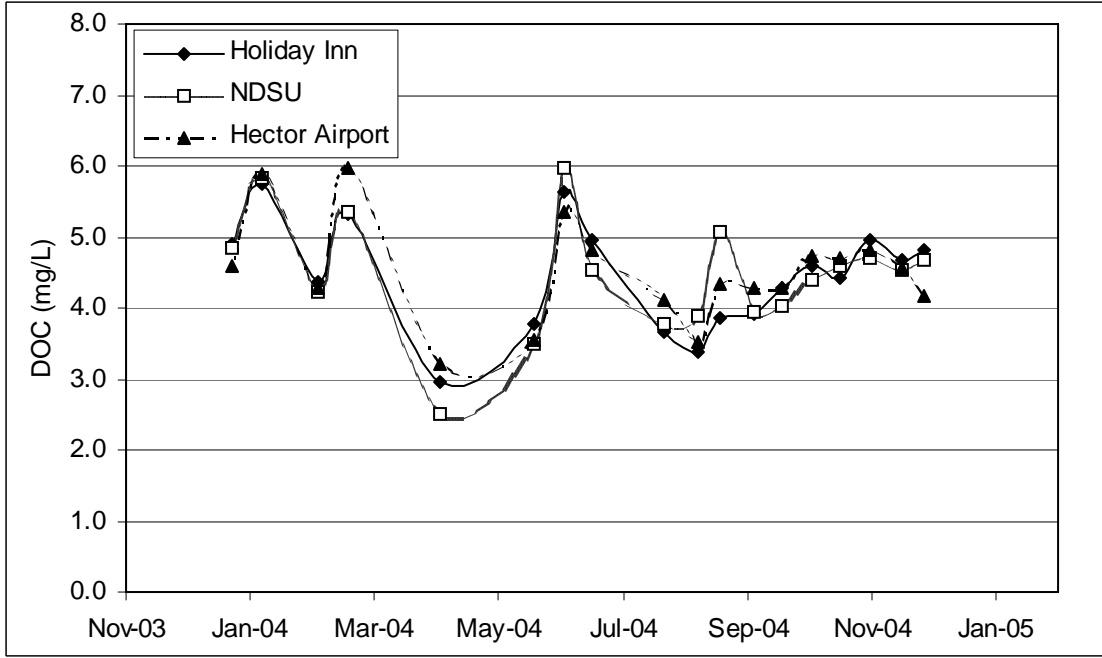


Figure 22. DOC of samples from the Fargo distribution network.

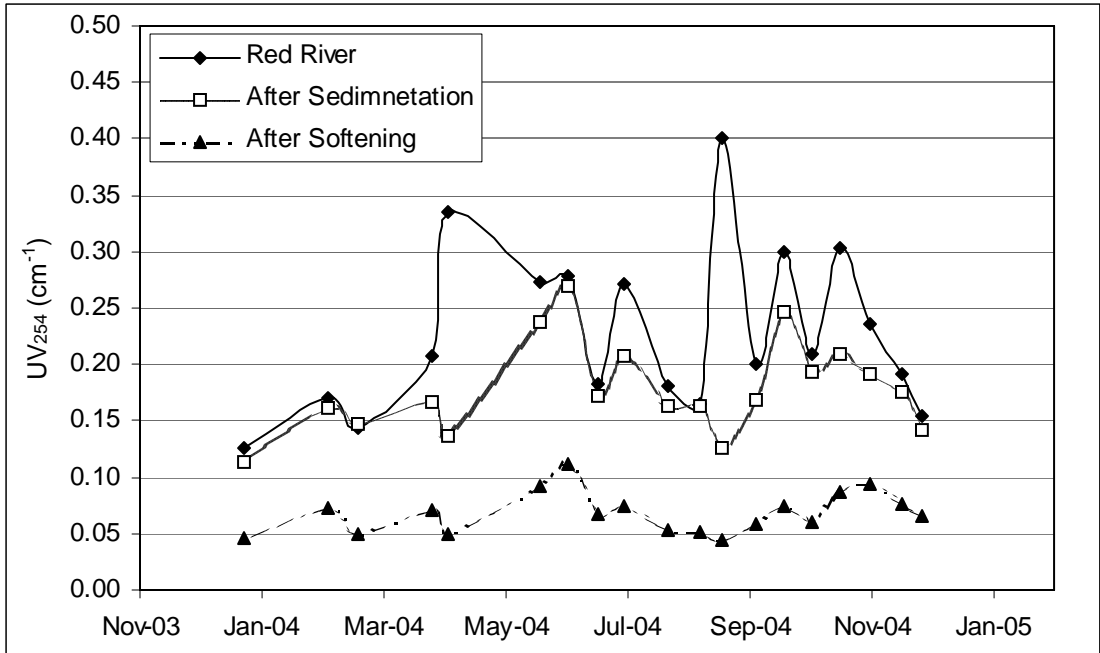


Figure 23. UV₂₅₄ of the Red river, after softening and sedimentation samples from the Fargo Water Treatment Plant.

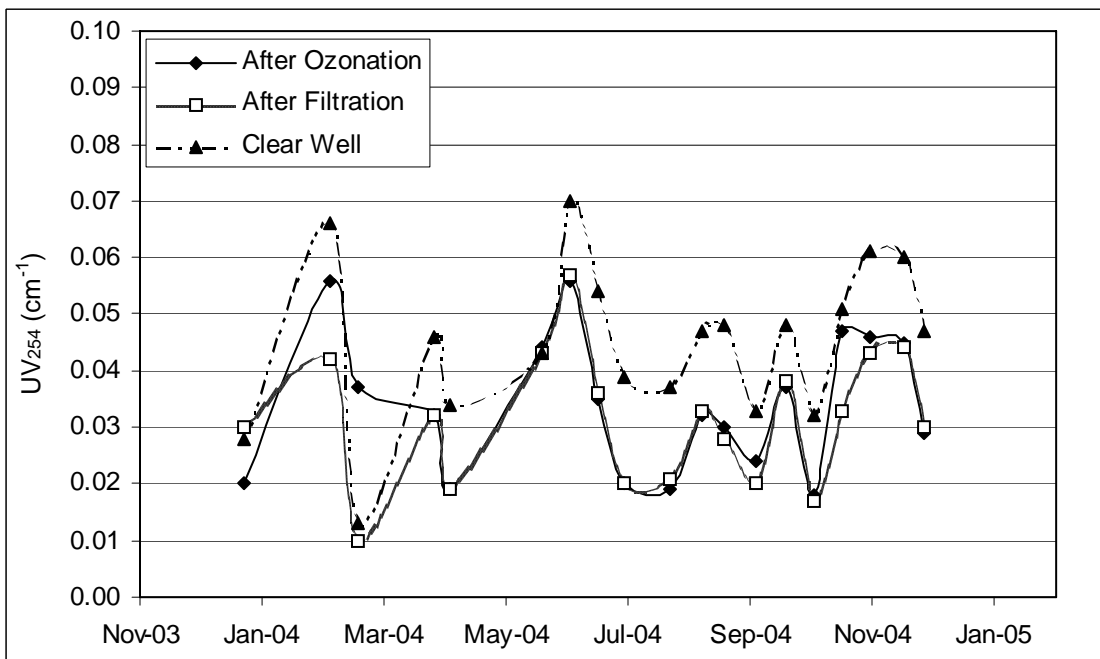


Figure 24. UV₂₅₄ of after ozonation, filtration, and clear well samples from the Fargo Water Treatment Plant.

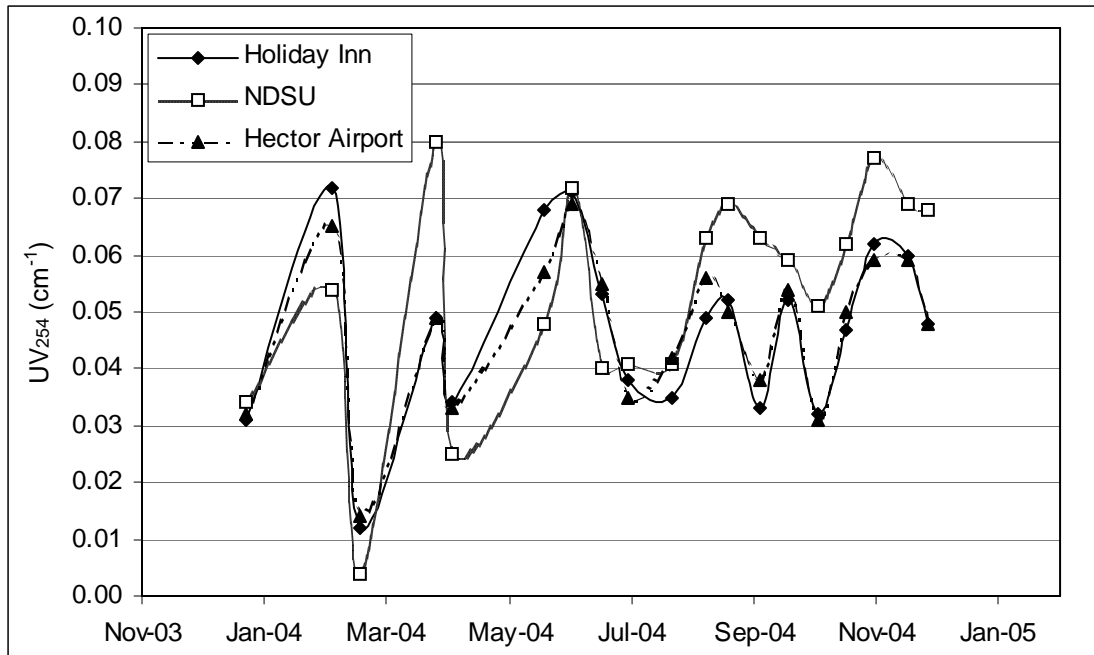


Figure 25. UV₂₅₄ of samples from the Fargo distribution network.

Figures 26 to 28 show the SUVA values of the samples from the Fargo plant and the distribution network. The SUVA results resembled the results of the Moorhead plant and followed the UV₂₅₄ trends above. Note that SUVA is a ratio of UV₂₅₄ and DOC. In this case, SUVA was dominated by the UV₂₅₄ values. Softening removed less biodegradable and more hydrophobic compounds (Figure 26). After softening, the water contained more biodegradable fractions as SUVA was always below 3.0 (Figures 27 and 28). Again, even though the low SUVA values indicate that sizable fractions of organics in treated water were biodegradable, there were limited changes in the values across the distribution system suggesting minimal or no regrowth.

The AOC values of the samples from the Fargo plant and the distribution network are presented in Figures 29 to 31. The raw water (Red River) samples had AOC between 50 and 250 µg/L confirming the range of values observed for the Red River samples of the Moorhead (Figure 29). The sedimentation process did not reduce AOC. It is unclear why it even increased AOC in some cases. On the contrary, AOC was removed substantially through the softening process. No AOC was detected after softening on a few sampling dates. This observation is important to the finding of no AOC left after softening at the Moorhead plant at all sampling dates. Softening might be a process that can be used to effectively remove AOC or might produce interference(s) to the AOC test. If the former is true, combining with the above BDOC results of the two systems, AOC is more susceptible to softening than BDOC. These are potential topics for future studies. AOC increases through ozonation and decreases after filtration were not always observed as expected (Figure 30). Chlorination increased AOC in some cases. Based on similar levels of AOC in clear well water samples and tap water samples (Figure 31), the regrowth was not evident.

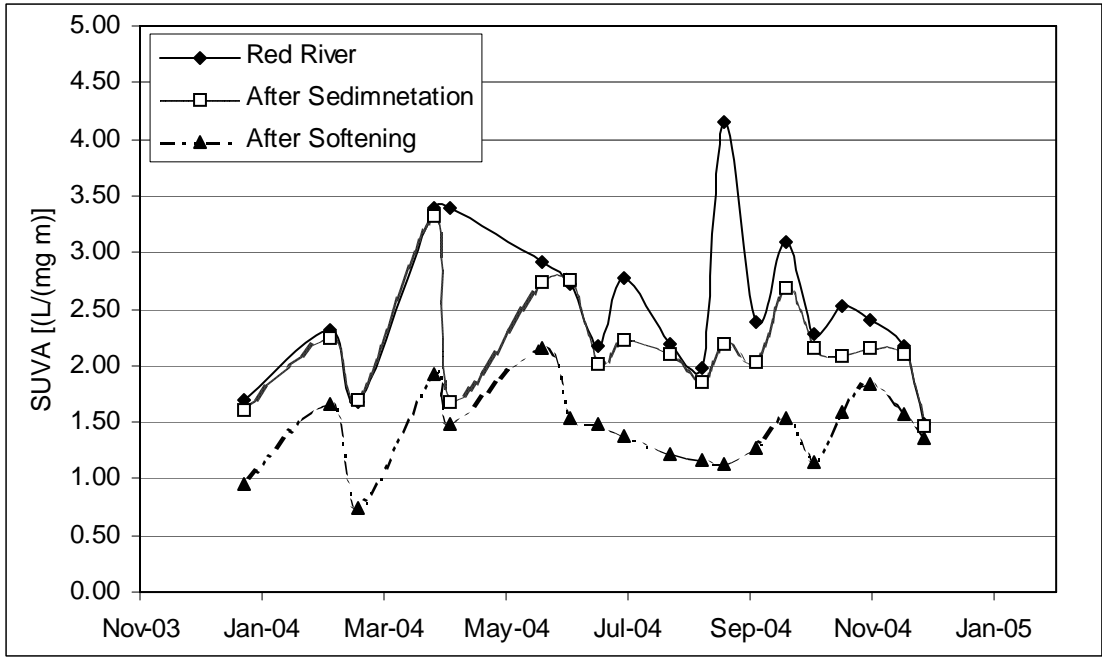


Figure 26. SUVA of the Red river, after softening and sedimentation samples from the Fargo Water Treatment Plant.

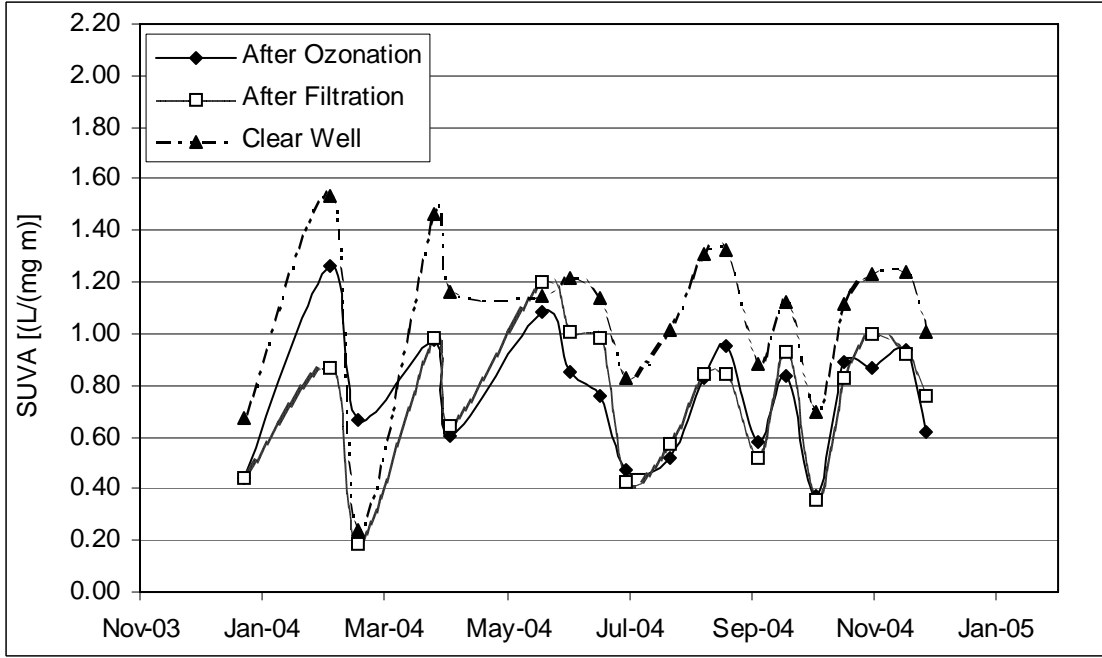


Figure 27. SUVA of after ozonation, filtration, and clear well samples from the Fargo Water Treatment Plant.

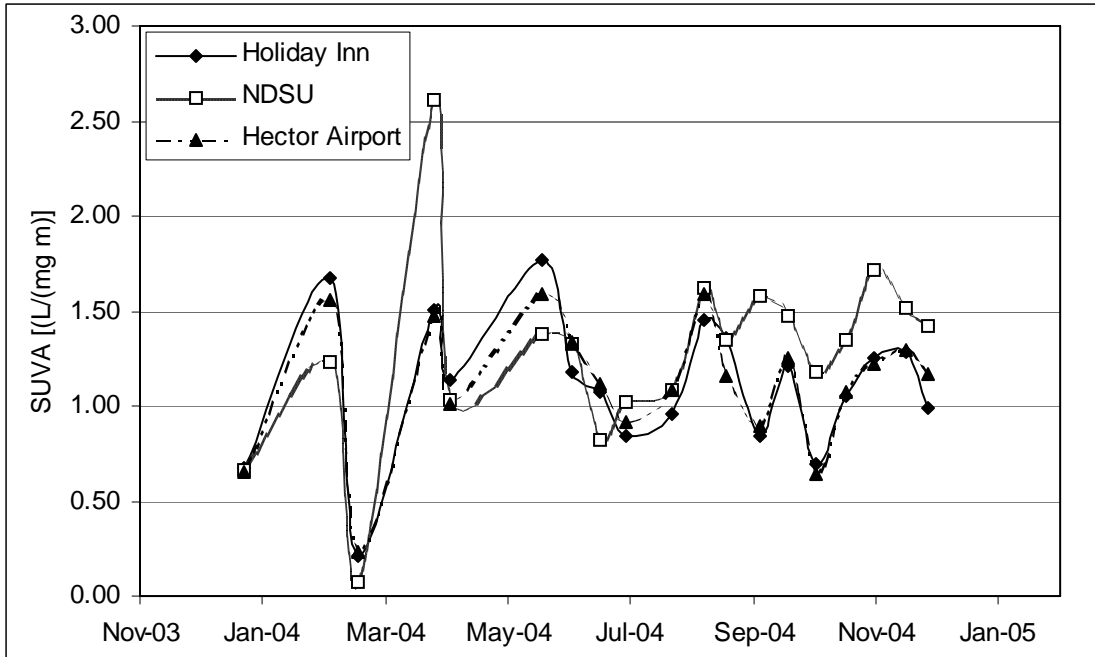


Figure 28. SUVA of samples from the Fargo distribution network.

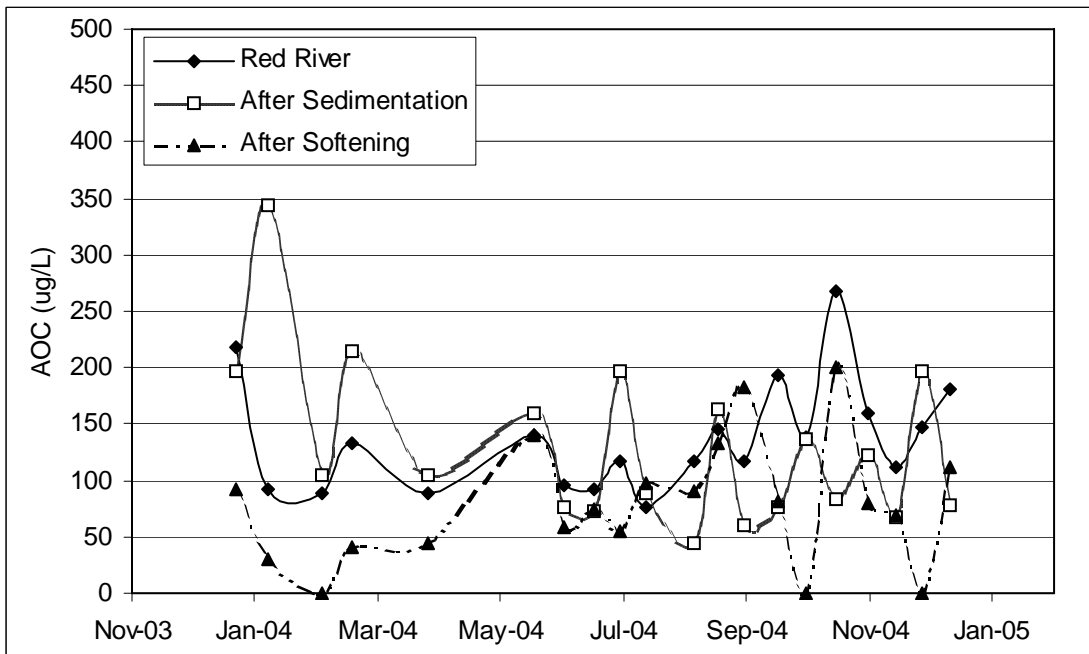


Figure 29. AOC of the Red river, after softening and sedimentation samples from the Fargo Water Treatment Plant.

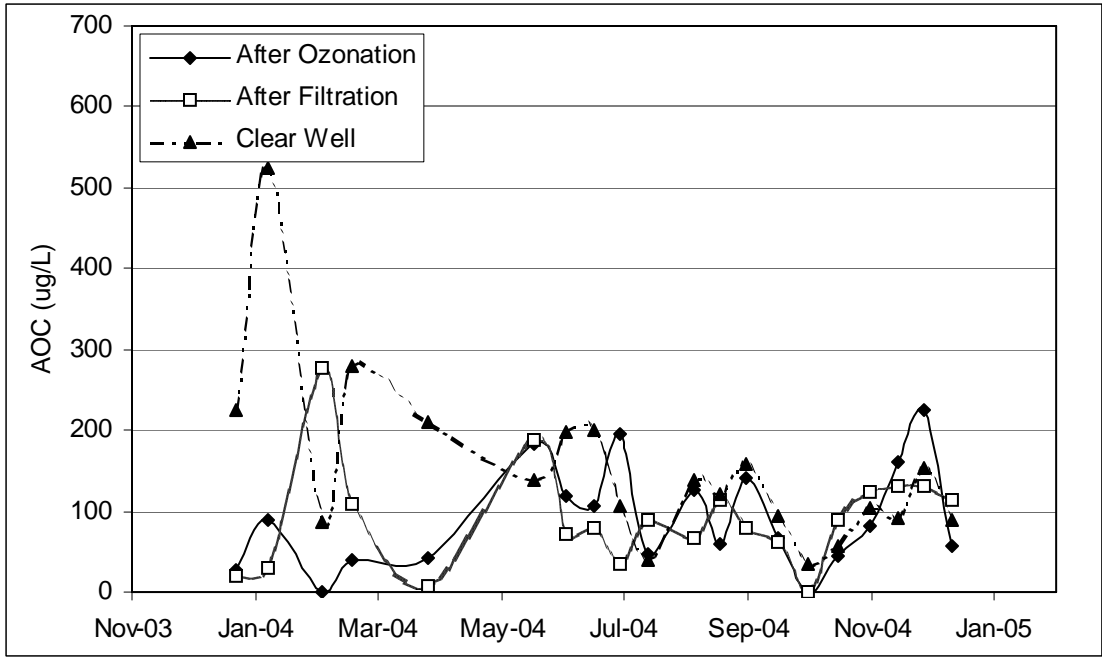


Figure 30. AOC of after ozonation, filtration, and clear well samples from the Fargo Water Treatment Plant.

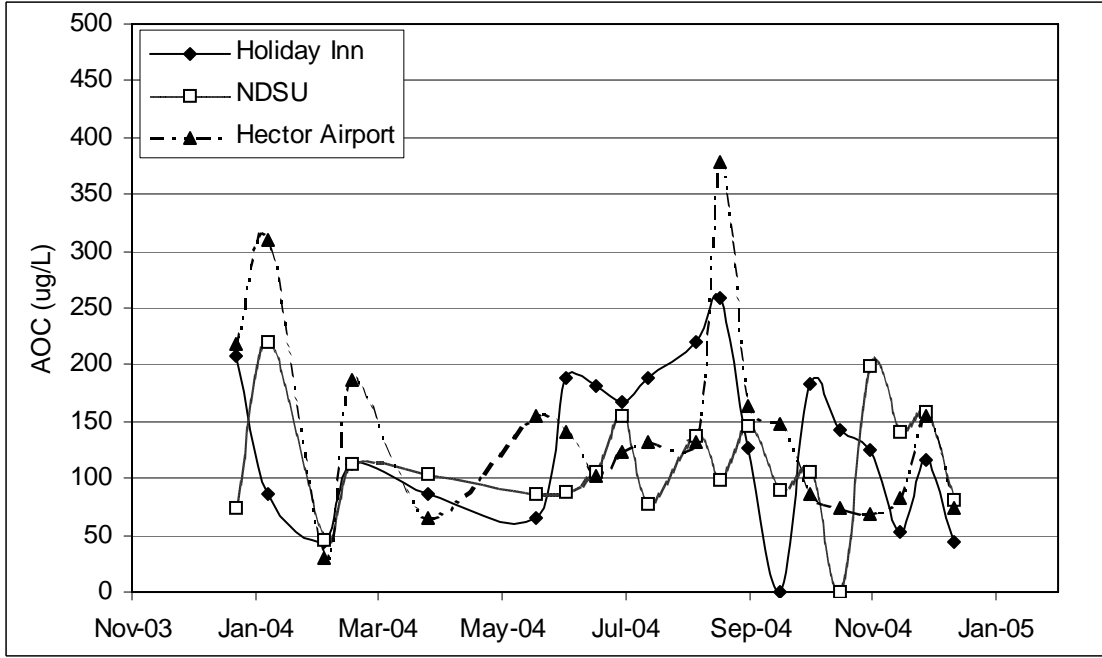


Figure 31. AOC of samples from the Fargo distribution network.

Conclusions

The microbial regrowth potential through the Moorhead and Fargo water systems were investigated using BDOC and AOC as the main indicators. DOC, UV₂₅₄, and SUVA of the water samples collected bi-weekly from the two systems were also determined. The sampling occurred for the entire year of 2004. For the Moorhead water system, BDOC in the Red river raw water was high. Softening was very effective in reducing BDOC significantly. Ozone enhanced BDOC but filtration removed only some of the increases (not down to the levels in softened samples). Chlorination slightly increased BDOC, which remained relatively unchanged in distribution system. AOC was not detected in all after softening samples. This observation is not explainable. Other than that, the AOC results were inconsistent and therefore were not as useful as the BDOC results. The results of the other three parameters supported the BDOC results. The results for the Fargo system were similar to those for the Moorhead system. The rapid mixing and sedimentation processes at the Fargo plant had very minimal effects on BDOC and AOC. The finished water of the Fargo plant had higher regrowth potential (BDOC) than that of the Moorhead plant.

The filters at the two plants tended to be more biologically active during the Summer and Fall months. The finished waters of the Moorhead and Fargo systems had high regrowth potential evidenced by their BDOC and AOC concentrations, which were very much higher than the threshold levels. However, BDOC and AOC at comparable levels to those in finished waters were observed in all tap water samples regardless of season. Since BDOC and AOC were not depleting, it was likely that no actual significant regrowth in the distribution systems took place.

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