Role of Ozone and Granular Activated Carbon in the Removal of Mutagenic Compounds

by Marie Marguerite Bourbigot,* Marie Claude Hascoet,* Yves Levi,* Francoise Erb,† and Nicole Pommery†

The identification of certain organic compounds in drinking water has led water treatment specialists to be increasingly concerned about the eventual risks of such pollutants to the health of consumers. Our experiments focused on the role of ozone and granular activated carbon in removing mutagenic compounds and precursors that become toxic after chlorination. We found that if a sufficient dose of ozone is applied, its use does not lead to the creation of mutagenic compounds in drinking water and can even eliminate the initial mutagenicity of the water. The formation of new mutagenic compounds seems to be induced by ozonation that is too weak, although these mutagens can be removed by GAC filtration. Ozone used with activated carbon can be one of the best means for eliminating the compounds contributing to the mutagenicity of water. A combined treatment of ozone and activated carbon also decreases the chlorine consumption of the treated water and consequently reduces the formation of chlorinated organic compounds.

Introduction

Although France is the birthplace of ozone disinfection (where this treatment had been used industrially to disinfect the water in Nice since 1907), its use is now very well known in many other countries. Ozone is now introduced more frequently at varying points in the treatment lines, either associated with the use of granular activated carbon (GAC) (1-3) or used in the pre-treatment process (4,5).

Loper (6) has already shown that the potential for GAC effluent to form mutagens when rechlorinated is reduced even when the GAC was used several months beyond its normal use for total organic carbon (TOC) removal. Also, recently, some widespread indications for using ozone before carbon filtration have appeared. When ozone and carbon filtration are used, the removal of biodegradable compounds can be enhanced, the chlorine demand of the treated water can be decreased, and, consequently, the formation of chlorinated compounds can be reduced.

The work presented here corresponds to studies carried out on site in a potable water production plant for the Paris area, which uses ozonation at varying stages of the treatment process. We first studied the chlorine demand of the treated water as a function of the upline ozone and activated carbon treatment. To understand how the precursors of chlorinated organic compounds are removed we measured the biodegradability of these organics.

The mutagenic properties of the organic compounds were tested using samples taken during the course of treatment before and after ozonation and after GAC filtration. The mutagenic trials were carried out on concentrates obtained by passage over XAD resins and extraction with dimethyl sulfoxide (DMSO). To determine mutagenicity, we compared the results of two tests: the V79/HGPRT system described by Huberman and Sachs (7), which measures mutations in the gene for the enzyme hypoxanthine guanine phosphoriboxyl transferase (HGPRT) (8) and the SOS Chromotest patented by the Institut Pasteur and available from Organics, Ltd.

Materials and Methods

Method for Measuring Biodegradability

A sample of 100 mL of water, sterilized previously by using 0.2-μm membrane filtration, sterilization, or pasteurization (if one is interested in the biodegradability of the particulate matter) and placed in an Erlenmeyer flask, was reseeded with 1 mL of raw water filtered through a 2-μm membrane to remove the largest particles and protozoans. The sample was incubated for
10 days to 2 months at 20°C in the dark. Dissolved organic carbon (DOC) was periodically measured, along with the number of the bacteria and the amount of \(^{3}H\)thymidine incorporated into insoluble trichloroacetic acid (TCA).

Chlorine consumption was measured before and after incubation by using the diethyl-\(p\)-phenylenediamine colorimetric titration (DPD). DOC was measured by filtering the samples through a 0.2-\(\mu\)m membrane filter and was analyzed by using a Dorhman DC 80 (COT meter by UV oxidation). Bacteria were enumerated by epifluorescence microscopy with a Leitz Dialux microscope after staining with Orange Acridine.

To determine biomass production we used the method proposed by Furhman and Azam (9). In this method, bacteria production is estimated by measuring \(^{3}H\)thymidine incorporation into DNA bacteria. The method consists of incubating a water sample for several minutes to several hours in the presence of thymidine (methyl-\(^{3}H\)) at a saturating concentration (15 nmole), then measuring the radioactivity associated with the insoluble fraction of 5% TCA (liquid scintillation with Packard Tri-Carb scintillation counter).

Results thus obtained give a conversion factor of \(0.5 \times 10^{9}\) bacteria/nmole of incorporated thymidine. Knowing that the cell’s carbon content is \(0.69 \times 10^{-13} \text{g C/ bacterium (10-12)}\), we can thus determine biomass production. Using this value, we can assess the consumption of organic, knowing that the measured consumed carbon is 0.3 g of cells produced per gram of consumed carbon.

**Mutagenicity Test**

**Sampling and Concentration.** For each sample, 40 L of water were collected and concentrated by passing over 40-mL XAD-2 and XAD-7 columns. Before sampling, the packed columns were washed successively with 0.01 N hydrochloric acid (HCl), 0.01N sodium hydroxide (NaOH), ether, acetonitrile, and methanol (spectroscopic grade, Merck). To eliminate the last interfering impurities, we washed the resin with 10 L of boiling water (Milli-Q) just before using the resin. Organic compounds were adsorbed by passage of the water sample through the columns at a speed of 20 mL/min. The compounds were then eluted with 100 mL of methanol and 100 mL of methanol–ether (10:90 volume/volume). The extracts were concentrated in a vacuum concentrator at low temperature, then diluted with 10 mL of DMSO and filtered through a 0.22-\(\mu\)m membrane filter (Sartorius). The extracts contained a final concentration 4000 times greater than the starting concentration.

**Mutagenicity Test on V79 Cells.** The V79/HGPRT system described by Huberman and Sachs (7) measures the mutations in the gene for the enzyme hypoxanthine-guanine phosphoribosyl (HGPRT) (8). The mutated cells are resistant to toxic analogs of purines such as thio- guanine (6-TG), which acts as a selective agent. The predictive value of this test for detecting mutagens has been demonstrated (13,14). This system has also been adopted for identifying promoters (15) and for testing the concentrates of water (16,17). A clone of the line V79 (Chinese hamster lung) with a stable karyotype and a much lower rate of spontaneous mutations was

*FIGURE 1.* Methodology for detecting mutagenicity using V79 Chinese hamster lung cells.

*FIGURE 2.* Measurement of chlorine demand of sand-filtered water, ozonated water, and carbon-filtered water before and after incubation in the presence of bacteria (chlorine dose: 1 mg Cl\(_2\)/L).
isolated and used as target cells in the tests. The culture medium was Dulbecco's minimum essential medium (Flodo) supplemented with 10% fetal calf serum (Flodo), without antibiotics.

The following experimental protocol is summarized in Figure 1. The mutation rate and the number of resistant colonies are calculated from the cloning efficiency (CE) series.

\[
\text{Frequency of mutations} = \frac{\text{number of resistant colonies}}{\text{number of cells inoculated} \times \% \text{ CE}}
\]

In this test, if the mutation frequency was two times higher than that of the negative control, the results were considered positive.

**The SOS Chromotest.** To perform the SOS Chromotest (SOS system of E. coli activated by mutagenic damage), we used the SOS Chromotest kit (Organics, Ltd.). The SOS Chromotest bacterial strain is patented by the Institut Pasteur (French Patent 82-16316). The strain of E. coli was genetically modified by a gene fusion of lacZ gene encoding β-galactosidase under the control of the SfiA promoter. When the SOS system was activated by genotoxic assault, the enzyme was produced and was easily detected using a simple color reaction test. The toxicity analysis was performed measuring the enzymatic activity of alkaline phosphatase introduced in E. coli by genetic transformations, which is also detected by a color reaction test. The results were recorded as the ratio of the optical density (OD) value measuring the β-galactosidase activity to the OD value measuring the alkaline phosphatase activity.

**Results**

**Chlorine Demand**

After adding a chlorine (Cl₂) dose of 1 mg/L, the chlorine residual was followed in three samples during a 12-hr period before and after 40 days of incubation in the presence of bacteria. The activated carbon had been in
the chlorine if itered the filter with the was for presence down. chlorine analysis are days incubation, beginning incubation, biodegradable org. Effect of the organics after incubation of incubation of bacteria, the chlorine consumption slowed down. The biodegradable compounds were responsible for chlorine consumption. In the carbon-filtered water, the chlorine consumption before and after incubation was nearly similar and was very close to that associated with the ozonated sand-filtered water after 40 days of incubation.

Effect of Ozone and GAC on the Ratio of Biodegradable to Nonbiodegradable Organics

The DOC decreased rapidly during the first days of incubation, then stabilized after only 1 month of incubation. The results of the DOC measurements at the beginning and end of the experiment are shown in Figure 3. After 40 days of incubation, 16.5, 24.5, and 20% of the DOC were degraded in the sand-filtered water, the ozonated sand-filtered water, and the carbon-filtered water, respectively. The carbon filter was used after the ozonation step. The DOC residual after incubation was considered nonbiodegradable.

Biomass Production during the Incubation of Water Samples

The biomass reached higher values during the incubation of ozonated sand-filtered water than during the incubation of the two other samples, sand-filtered water and carbon-filtered water (Fig. 4).

Mutagenicity Tests on V79 Cells

The results of the mutagenic activity determined using the V79/HGPRT mammalian cell system for ozonated water at 0.75, 1.5, and 3 mg/L are presented in the histogram in Figure 5 (5 days incubation) and Table 1 (10 days incubation). The number of mutants associated with the ozonated sample at 0.75 mg/L with a final concentration of 1% and 0.5% treatment media (Table 1) was 3.5 times higher compared to that associated with the negative control after 10 days incubation. The number of mutants associated with the ozonated water at 1.5 mg/L was 2.4 times higher than that associated with the control after 10 days incubation. On the sample ozonated at 3 mg/L, no direct activity was found. It appears that a sufficient dose of ozone must be applied to destroy the mutagenic compounds in the water.

Figures 6–8 show the mutagenic activities of sand-filtered water, ozonated water, and carbon-filtered water as a function of ozone dose. The GAC-filtered
water showed no direct activity. Apparently, the activated carbon efficiently eliminates the mutagenic activity initially present in ozonated sand-filtered water.

SOS Chromotest in Bacteria

The SOS Chromotests were performed on the same concentrates as the test on V79 cells, and the results are presented in the histogram in Figure 9. Our experience with this test is limited, but we believe that a result is only very slightly positive when the OD-galactosidase-to-OD-alkaline-phosphatase ratio is about 1.5. The results confirm our previous results with the mammalian cell system. An increase in the number of mutants was detected for the ozonated water at 0.75 mg/L. The number of mutants in the ozonated water at 1.5 mg/L remained unchanged compared with sand-filtered water, but the ratio of the two results was about 1.5, and the test was thus considered only slightly positive. No mutagenic activity was noted in the sample ozonated at 3 mg/L. After activated carbon filtration, some decrease in the number of mutants was apparent, particularly when the water was previously ozonated at 0.75 mg/L.

Conclusions

The alternative treatment to chlorination, the use of ozone, does not appear to increase the mutagenicity of the water if a sufficient dose of ozone has been used. In fact, ozone can even eliminate the initial mutagenicity of the water. However, an increase in mutagenicity can be detected when low doses of ozone are used. In these cases, activated carbon filtration is a good means of eliminating the mutagenic compounds that might be produced and, therefore, the combined treatment of ozone followed by activated carbon is a better process for removing mutagenic compounds than ozone treatment used alone. We must recall that the activated carbon used in this experiment was 1.5 years old but was still able to remove some mutagenic compounds.

The removal of the initial mutagenicity is not the only effect of the combined ozone and activated carbon treatment. This process also decreases the chlorine consumption of the treated water.

In summary, our assays show that ozone increases the biodegradability of the organic matter. The ratio of biodegradable organics to nonbiodegradable organics ranges from 16.5% in the sand-filtered water to 24.5% in the ozonated sand-filtered water. These biodegradable organics consume chlorine very quickly. If we add an activated carbon filtration step, a certain amount of the biodegradable organics can be removed, and the consumption of chlorine is greatly reduced. Consequently, the formation of chlorinated organic compounds is also reduced.

### References

1. Brunet, R., Bourbigot, M. M., and Doré, M. The influence of the ozonation dosage on the structure and biodegradability of pollutants in water, and its effects on activated carbon filtration. Ozone Sci. Eng. 4: 15–32 (1982).
2. Bourbigot, M. M. L’ozonation dans la production d’eau potable. Eau Ind. Nuits: 72: 33–37 (1983).
3. Brunet, R., Bourbigot, M. M., Legube, B., and Doré, M. Aqua 4: 75–78 (1980).
4. Saunier, B. M., Selleck, R. E., and Trussel, R. R. Preozonation as a coagulant aid in drinking water treatment. J. Am. Water Works Assoc. 75: 239–245 (1983).
5. Pascal, O., Bablon, G., Ducauze, C., and Feinberg, M. Chemometric control for a coupled preozonation/coagulation process. Paper presented at 10A Montreal Workshop, 1984.
6. Loper, J. C., Tabor, M. W., Rosenberg, L., and DeMarco, J. Continuous removal of both mutagens and mutagen forming potential by an experimental full-scale granular activated carbon treatment system. Environ. Sci. Technol. 19: 383–389 (1985).
7. Huberman, E., and Sachs, L. Cell-mediated mutagenicity of mammalian cells with chemical carcinogens. Int. J. Cancer 33: 326–333 (1984).
8. Caskey, C. T., and Kruh, G. G. The HPRT locus. Cell. 10: 1–9 (1979).
9. Furman, J. A., and Azam, F. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters. Mar. Biol. 66: 109–120 (1982).
10. Billen, G., Joiris, C., Wijnants, J., and Gillain, G. Concentration and microbiological utilization of small organic molecules in the Scheldt estuary. East Coast. Mar. Sci. 11: 279–294 (1980).
11. Billen, G. Heterotrophic utilization and regeneration of nitrogen. In: Heterotrophic Activity in the Sea (J. E. Hobbie and P. S. Williams, Eds.), Plenum Press, New York, 1984, pp. 313–355.
12. Billen, G., Servais, P., and Fontigny, A. Action des Populations Bactériennes vis-à-vis de l’Elimination des Matières Organiques dans les Filtres Biologiques. Cie Gen. des Eaux report, 1985.
13. Kuroki, T. A., Abbondandalo, A., Drevon, C., Huberman, E., and Laval, F. Mutagenesis assays with mammalian cells. In: IARC Long-Term and Short-Term Screening Assays for Carcinogens. IARC Monographs, Supplement 2, Lyon, 1980, pp. 107–133.
14. Bartsch, H., Malaville, C., and Camus, A. M. Bacterial and mammalian mutagenicity tests, validation and comparative studies on 180 chemicals. In: Molecular and Cellular Aspects of Carcinogen Screening Tests (H. Montesano, H. Bartsch, and L. Tomatis, Eds.), IARC Publ. 27, Lyon, 1979, pp. 179–242.
15. Lankas, G. R., Baxter, C., and Christian, R. T. Effect of tumor promoting agents of mutation frequencies in cultured V79 Chinese hamster cells. Mutat. Res. 95: 133–140 (1977).
16. Gruener, N. Mutagenicity of ozonated, recycled water. Bull. Environ. Contam. Toxicol. 20: 522–526 (1978).
17. Gruener, N., and Lockwood, M. P. Mutagenic activity in drinking water. Am. J. Publ. Health. 70: 276–278 (1980).

### Table 1. Direct mutagenic activity of ozonated sand-filtered water versus ozone doses (incubation time = 10 days).

| Ozone dose, mg/L | Concentration, % | Control | Mutations/10⁶ viable cells |
|-----------------|-----------------|---------|--------------------------|
| 0.75            | 0.1             | negative 18.19 | 27.25 |
|                 | 0.5             | positive 72.77 | 3.04  |
| 1.5             | 0.1             | negative 20.80 | 49.11*|
|                 | 0.5             | negative 91.70 | 4.89  |
| 3               | 0.1             | negative 42.63 | 33.74 |
|                 | 0.5             | positive 87.92 | 11.50 |

* Positive results (mutation frequency is at least two times higher than that of the negative control.)