The Differences between On-Site Generated Mixed-Oxidant Solution and Sodium Hypochlorite (aka the Master Features Summary)

Wesley L. Bradford, Ph.D., *formerly* Los Alamos Technical Associates, Inc. (LATA), Los Alamos, NM, and Chief Scientist, Product Development, MIOX Corporation, Albuquerque, NM
Currently Chief Scientist, MIOX Corporation, Albuquerque, NM
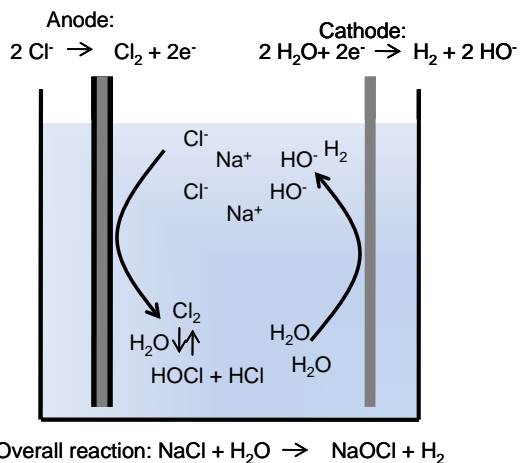Last updated 8 February 2011

Table of Contents

Background

The electrolysis of salt water to generate chlorine has been practiced commercially for more than 100 years. Electrolysis is the basic process used to manufacture nearly all chlorine-based oxidizing compounds including gas, liquid, and solid forms of chlorine. These forms of chlorine have been traditionally produced at large chlor-alkali plants, packaged in various forms and sold. Chlorine gas safety concerns and regulation have caused many water utilities to convert from gas to safer forms such as sodium hypochlorite (liquid or bleach) or calcium hypochlorite (solid); even these forms carry their own risks from spillage and contact with strong reducing agents or oils.

Within the past 40 years, several manufacturers began to produce and sell equipment that generated sodium hypochlorite on-site by the same processes used in larger chemical plants. The first on-site generators were installed at large coastal industrial plants, and used seawater as the salt brine source. Subsequently, manufacturers made the equipment smaller and water utilities began to use them as alternatives to chlorine gas.

In 1978, a number of companies in the US and Europe began to experiment with electrolytic devices that generated a gas thought to be comprised of multiple oxidants. As a class of equipment, these became known as mixed-oxidant gas generation on-site devices (MOGGODs)[1]. Use of the MOGGODs involved extraction of the oxidants generated as gases and injection of the gases into the water being treated. The Pan American Health Organization (PAHO) experimented with several MOGGOD brands in various Latin American countries as a method for rural water disinfection. In 1985, the first MIOX generator was demonstrated to the US Navy and US Army. This patented device was considered an improvement over the MOGGOD generators because it was much more robust, having fewer critical parts and not requiring salt of high purity to function effectively, and generated mixed oxidants in solution which could then be injected directly into the water. The mixed-oxidant solution injection method simplified the handling and dosing of the oxidants.

PAHO began testing MIOX generators in 1987 and many subsequent improvements made them even more robust. In 1991, LATA (the parent company of MIOX) modified the generators for automated operation and in 1992, the first MIOX generator was installed in the US. Another year of field testing and equipment modification followed and in 1994, MIOX Corporation began manufacturing and selling its products to the public. Since that date MIOX has continually improved the performance of its generation process and expanded its product line to include systems producing from a few pounds per day to several hundred pounds per day of measured Free Available Chlorine (FAC) as the mixed-oxidant solution. The schematic shows a general description of chlorine generation in a typical OSG electrolysis cell. Chloride ions are oxidized at the anode to form aqueous chlorine which manifests as hypochlorite or hypochlorous acid depending upon solution pH. At the cathode water is reduced in an overall reaction that produced hydrogen and hydroxide ions.

Anode:
$$2\ Cl^- \rightarrow Cl_2 + 2e^-$$

Cathode:
$$2\ H_2O + 2e^- \rightarrow H_2 + 2\ HO^-$$

Overall reaction: $NaCl + H_2O \rightarrow NaOCl + H_2$

MIOX generators have sometimes been considered and discussed together with on-site hypochlorite generators as just another hypochlorite generator. One reason for this perception is that solution concentration and monitoring is performed by measuring FAC. However, evidence both in laboratory and in field applications suggests that this is not the case. There are important differences in the MIOX process chemistry, the disinfection effectiveness of the mixed-oxidant solution, and other features which provide benefits in water treatment. MIOX engineering is also different. While all On-Site Generators (OSGs) and MIOX generators use salt, water and electricity to generate an oxidant solution on-site, the overall engineering and controls schemes are very different.

Chemistry

Differences between MIOX Mixed-Oxidant Solution and Sodium Hypochlorite

It should be noted initially that among the OSGs, only the manufacturers of the MOGGOD-like devices claim to produce mixed oxidants. All others claim only to produce chlorine and small amounts of oxygen (O_2) as an unavoidable product of the electrolysis of water at the anode. Early tests on prototypes of the MIOX electrolytic cell suggested the presence in the electrolyzed brine of other oxidants in quantitatively small but chemically significant concentrations in addition to the major product – chlorine – and oxygen in small concentrations but possibly exceeding brine saturation by a substantial amount. The evidence for the presence of other oxidants in the tests on prototypes led to the name "MIOX" (from MIxed OXidants), and to the characterization of the electrolyzed brine from MIOX cells as a "mixed-oxidant solution". Attempts to both chemically characterize and quantify the other oxidants in the mixed-oxidant solution continue at MIOX and in academia.

Chemical Analyses [ξ]

The MIOX mixed-oxidant solution contains chlorine (as Cl_2, HOCl, or OCl$^-$ in relative fractions depending on pH) as the primary analyzable oxidant constituent. The Free Available Chlorine (FAC) and Total Chlorine (TC) are measured routinely in testing and field application using the Hach Company (Hach) adaptation of the DPD method (Standard Methods[2] (SM) 4500-Cl G. DPD Colorimetric Method). Early attempts to measure other oxidants thought to be present – chlorine dioxide (ClO_2) (SM 4500-ClO$_2$ C. Amperometric Method I), ozone (O_3) (SM 4500-O$_3$ B. Indigo Colorimetric Method), and hydrogen peroxide (H_2O_2) using UV spectra at <230 nm (no standard method is available) and color development using Ti(IV) solution – proved problematic and inconclusive due to several difficulties, the primary one being the extremely high background matrix of chlorine[3].

With the difficulty in measuring small concentrations of other oxidants in a massive chlorine matrix, attempts were made to use UV spectrometry3. These attempts met with other difficulties. For the determination of ClO_2, there are no substantial interfering peaks from other compounds near the 360 nm peak of ClO_2 but spectral interferences are common nevertheless. Moreover, the UV absorption by ClO_2 is considered only moderate and the potential detection limit by the method is expected to be only about 1 mg/L under ideal conditions, conditions which definitely are not present in the mixed-oxidant solution. For the determination of O_3, UV spectrophotometry at 254-258 nm is notoriously insensitive, particularly in the presence of chlorine/hypochlorite which has a broad UV absorption peak beginning at about 280 nm and extending to a broad absorption plateau at about 235 nm and below. Thus, the presence of ozone would have to appear as a small shoulder peak on a broad rise; and ozone would have had to be present in substantial quantities (tens of milligrams per liter, which MIOX has never claimed) to have been detected at all by this method. For hydrogen peroxide (H_2O_2), which absorbs in the UV over a broad wavelength region similar to that of both O_3 and chlorine, the UV spectrometry analysis was obviously ineffective. It was clear from these attempts that methods other than Standard Methods2 and UV spectrometry would be required for determination of suspected other oxidants in a massive chlorine matrix.

Subsequent research by Mike Dowd[4] and Philip Singer at the University of North Carolina, using the Indigo method (SM 4500-O$_3$ B. Indigo Colorimetric Method) with chemical suppression of the chlorine, found the presence of quantitatively small (a few milligrams per liter) but chemically significant amounts of ozone. Dowd4 also demonstrated the presence of a substantial systematic excess of oxidants over chlorine alone in the mixed-oxidant solution using standard amperometric titration and sodium thiosulfate titration methods at pH 7 and at pH 2 (SM 4500-Cl D./E. Amperometric Titration Method), and a modification of the Iodometric method (SM 4500-Cl B./C. Iodometric Method I/II at pH 2 in Glacial Acetic Acid). While the nature and composition of the other oxidants could not be identified (beyond the obvious fact that they have to be chlorine and oxygen-based given the method of production), the implication of their presence in water treatment is that the other oxidants satisfy some of the oxidant demand (normally satisfied by chlorine) of water being treated and, perforce, less chlorine as mixed-oxidant solution compared to chlorine alone would be required to satisfy the oxidant demand of the raw water and

---

[ξ] Documents referenced herein that have not been published as theses, by the US Army, or as papers in journals have nevertheless been peer-reviewed internally by LATA and/or MIOX and tested by field application. Copies are available upon request to MIOX Corporation, Albuquerque, NM [www.miox.com].

leave a chlorine residual. Dowd4 demonstrated these features for the first time in laboratory test solutions containing humic acid.

Dowd's4 finding has been supported subsequently in laboratory and now countless field applications. It is reported universally by users of MIOX for disinfection of potable water, cooling tower water, and swimming pools, that the mixed-oxidant solution dosed at measured FAC concentrations equal to those dosed previously using chlorine gas, sodium hypochlorite solution, or calcium hypochlorite tablets, maintains a chlorine residual at the extremities of the distribution system whereas conventional chlorine could not. This feature is now thought to be one of many features of the mixed-oxidant solution that has enabled users to reduce chlorine doses as mixed-oxidant solution and, in many cases, to abandon chlorine booster stations en-route. Residual maintenance has been documented in laboratory by Bradford et al.[5] and in the field by Schroeder[6] and by many others to the point that superior residual maintenance is the single most common observation reported by MIOX users.

Trumm3 argued against the presence of ClO_2 in the mixed-oxidant solution because he was unable to obtain consistent results using the standard amperometric procedure involving pH adjustment (SM 4500-ClO_2 C. Amperometric Method I), but he often did detect ClO_2 in substantial concentrations (over 10 mg/L which is unlikely in the mixed-oxidant solution because such a solution would be deeply colored, which is not the case). He explained very well why this procedure is not likely to be reliable, reasons Aieta et al.[7] had expounded in 1984 – the estimate of the ClO_2 concentration is the small difference between two large numbers, an inherently inaccurate method of analysis. But Trumm failed to try Aieta's proposed new procedure (SM 4500-ClO_2 E. Amperometric Procedure II (Proposed)). In her Doctoral Dissertation work with Mark Sobsey at the University of North Carolina, Linda Venczel[8] used Aieta's procedure and found ClO_2 again in quantitatively small but chemically significant concentrations.

In addition, Venczel8 constructed a solution of mixed oxidants of chlorine (90%), ozone (5%), ClO_2 (2.5%) and H_2O_2 (2%) in concentrations determined in her and Dowd's4 analyses, and compared the effectiveness of that constructed solution to freshly-generated MIOX mixed-oxidant solution as well as hypochlorite in inactivating *Clostridium perfringens* spores*, Escherichia coli*, and MS2 Coliphage. The constructed solution achieved higher inactivation than the hypochlorite solution. In contrast, the inactivation kinetics of the constructed solution and the freshly-generated mixed-oxidant solution were nearly identical for all three test microorganisms. But the final inactivation of *Clostridium perfringens* spores and MS2 Coliphage achieved by the mixed-oxidant solution was still better than that of the constructed solution by a small amount (about 0.2 log inactivation). The implications are that the composition of Venczel's constructed solution is close to that of the MIOX mixed-oxidant solution (that is, the mixed-oxidant solution also either contains – possibly only at the point of generation, as will be discussed further below – ozone, ClO_2 and H_2O_2 as well as chlorine) but that some component, reaction, or synergy is still unaccounted for. Synergy between disinfectants in sequential additions has been observed by Kouame and Haas[9] and by Rennecker et al.[10] to name just a few.

In the first phase of studies to chemically characterize electrolytically-generated oxidant solutions completed in May, 1998 by Gil Gordon[11] and his students and associates at Miami University, classical analytical methods were carefully and cleverly applied to the analysis of freshly-prepared MIOX mixed-oxidant solution. Gordon found no detectable (at < 0.15 mg/L) concentrations of O_3 or ClO_2 in the mixed-oxidant solution and showed further that the presence of these components in those forms together for longer than a few seconds is not possible.[‡] Gordon's studies are the most comprehensive and accurate attempts to date to characterize anolytes (solutions off the anode of the electrolytic cell) from electrolytic cells generating disinfectants from sodium chloride brines and, specifically, the mixed-oxidant solution from MIOX units. ***These studies show convincingly that the unexpected disinfection and chemical properties of the mixed-oxidant solution (unexpected, that is, compared to chlorine, the major identifiable oxidant component of the mixed-oxidant solution) cannot be ascribed to the presence of O_3 or ClO_2 as stable, analyzable components of the solution.***

Gordon's studies do not disprove the hypothesis that these components are generated at the anode and transform to other equally-effective but as yet unidentified forms; the generation of these components has been shown by the works of Bradford (cited elsewhere herein) to be thermodynamically possible. And various analyses

---

[‡]   Gordon stated in the abstract of the report that H_2O_2 also was not present. However, no attempt was made to detect or measure H_2O_2 directly as part of this work. It is likely that he assumed the absence of H_2O_2 since it is commonly used to quench FAC in solutions. However, as discussed below, that assumption may be incorrect.

conducted prior to this work (and discussed below) of the mixed-oxidant solution and the gases evolved from the generation process indicate that these components may be generated. However, the generation of these components does not in itself explain the temporal stability of the unexpected properties of the mixed-oxidant solution – the mixed-oxidant solution maintains its unexpected properties for 48 hours after generation. Clearly future investigations to identify the mechanisms for the unexpected properties, and the temporal stability of those properties, should look to factors other than the presence of O_3 or ClO_2 as stable, analyzable components of the solution.

Research on the composition of the mixed-oxidant solution continued with funding from the US Environmental Protection Agency at Miami University under the direction of Dr. Gil Gordon following the initial publication in May 1998. As of January 2004, Dr. Gordon had completed his studies but without adding to or altering the findings as discussed above.

Other Laboratory Evidence for the Presence of Oxidants Other than Chlorine

Industrial hygiene studies by Bradford and Baker[12] and by Vanden Plas[13] found both O_3 and ClO_2 in the gases generated at the anode of the MIOX cell; the measurements were made by Draeger Tubes, however, and are subject to criticism for accuracy and specificity of detection. Their presence in the gases off the anode supports both their presence (at least at the point of generation) in the mixed-oxidant solution and the prediction that they would be generated based on the electrical potential measured at the anode surface of the MIOX cell. Those measurements indicated that the potential at the anode surface was more than sufficient to make the reactions forming both O_3 and ClO_2 as well as H_2O_2 thermodynamically possible.

Oxidation-Reduction Potential (ORP) measurements also support the presence of oxidants other than chlorine in the mixed-oxidant solution. ORP measurements are subject to numerous interferences and problems in handling the probe; thus the measurements are often suspect and inferences from differences in ORP measurements of less than a few tens of millivolts must be made with caution. However, Bradford[14] found that the measured ORP of the catholyte from the MIOX cell was about 200 mV lower than predicted from theory (possibly due to a mixed-potential with dissolved hydrogen gas generated at the cathode) and the measured ORP of the mixed-oxidant solution (from the anode of the MIOX cell) was about 20-30 mV higher than predicted from theory considering the FAC/pH/Temperature/Chloride/ORP relationship in a FAC-containing NaCl solution. Theoretical considerations for the latter ORP prediction are suspect; however, the measured ORP is higher than the current theory would predict, supporting the presence of a mixed potential between chlorine and other oxidants having higher pure-phase ORPs.

For the period from first publication of the reports on prototype MIOX cells (ca 1993) until recently, MIOX was the only manufacturer of an electrolytic generator of a disinfecting solution claiming that the solution was something different from chlorine/hypochlorite. Beginning in 1999, Yen-Con Hung and colleagues at the University of Georgia, Center for Food Safety and Quality Enhancement, Griffin, GA have reported unusual microbiocidal behavior of an "electrolyzed oxidizing (EO) water" produced by electrolytic cells of similar design to the MIOX cells[15 16 17]. While these studies have not been well-controlled with respect to comparison of inactivations by FAC from a conventional source (such as bleach/hypochlorite) at the same concentrations and controlled pH and temperature in test solutions, and the arguments regarding ORP of the EO water have failed to consider adequately the FAC/pH/Temperature/Chloride/ORP relationship in a FAC-containing NaCl solution, they showed that another laboratory was seeing unusual properties from freshly-generated electrolytic solutions as well.

Beginning in August 2001, studies were undertaken at California Institute of Technology and the Jet Propulsion Laboratory in Pasadena, CA under the direction of Mitchio Okumura employing a wide variety of state-of-the-art spectroscopic methods examining both the solution and gas phases (the head space gases) of the mixed-oxidant solution for unusual chemical structures of chlorine-oxygen compounds. As of March 2003, Okumura and his post-doctoral fellows and students had completed their studies; while there were findings suggesting the presence of unusual oxidants warranting further research, no oxidant species other than chlorine (Cl_2/HOCl/OCl⁻) could be confirmed.[18] Funding for the studies was exhausted so no further research could be conducted.

In addition, Jeong, et al.[19] in investigations of the chemical species most responsible for inactivation of microorganisms in electrochemical disinfection, electrolyzed chloride-free water (to avoid the effects of chlorine) under conditions close to those used in MIOX systems. While they found that the OH• radical (among several

reactive oxygen species sought) was most responsible, they also detected O_3 in the electrolyzed solutions demonstrating that O_3 can be generated from water under the imposed conditions. The O_3 was found to be very reactive, however, and was detected (at < 0.1 mg/L) only in solutions electrolyzed at low temperatures. This finding is consistent with that of Gordon in that the mixed-oxidant solutions analyzed by Gordon were warm and analyzed several seconds following generation. Jung, et al.[20] also detected and measured $OH^\bullet$ radicals, O_3 and H_2O_2 in electrolyzed chloride-free (K_2SO_4) brines. They also reported that both bromate and chlorate were detected in electrolysis of chloride- or bromide-containing brines which they claimed provided indirect evidence for the formation of ozone and chlorine dioxide.

Weinberg et al.[21] focused on studies of mixed-oxidant solution formation of selected emerging organic contaminants in water in an effort to elucidate differences in reaction mechanisms between bleach/hypochlorite and mixed-oxidant solution. Briefly, if the mixed-oxidant solution contains oxidants other than chlorine quantifiable differences in DBP concentration and a different distribution among the DBP group species should be observable. A suite of DBPs was analyzed to investigate the effect of either chlorine or mixed-oxidant solution in source surface waters. Different analytical methods were applied for different DBPs families, namely: haloacetic acids, aldehydes, haloacetamides, carboxylic acids, trihalomethanes, haloacetonitriles, halonitromethanes and chloral hydrate. A statistically significant difference in the formation of one disinfection by-product – chloral hydrate – between mixed-oxidant solution and hypochlorite/bleach was found. ***This difference indicates the presence of other oxidants in mixed-oxidant solution (compared to hypochlorite/bleach) because chloral hydrate formation is associated with both O_3 and UV irradiation followed by chlorination***. Formaldehyde production at pH 3, where O_3 is stabilized, was higher using mixed-oxidant solution than using hypochlorite. ***This suggests that mixed-oxidant solution contains O_3 (at the point of generation) or some other constituent that behaves like O_3 which is also known for its reaction with natural organic matter (NOM) to produce aldehydes***.

Preliminary data were also generated for carboxylic acids with the same goal. Among the carboxylic acids detected in mixed-oxidant solution-treated water were acetic, glycolic, formic and oxalic acids. The concentrations detected (in the 100s µg/L) were much higher than previously reported for chlorine-treated drinking waters and suggest an oxidation process involving species in mixed-oxidant solution other than chlorine.

Rodriguez-Mozaz et al.[22] (with the Weinberg team) concluded that from a chemical standpoint, there appear to be other oxidizing species in mixed-oxidant solution as represented by DBP formation that are not present in bleach/hypochlorite-generated free chlorine solutions. Although the measurements used in this study were unable to consistently identify what these oxidizing species are, the mechanisms postulated for explaining the patterns and levels of DBPs found in mixed-oxidant solution compared to free chlorine-disinfected settled surface water are strong indicators of the higher oxidation potential of mixed-oxidant solution.
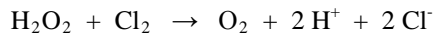
A review of the literature on reactions between FAC and H_2O_2 suggests that the apparent assumption by Gordon that H_2O_2 would be rapidly decomposed by the FAC in mixed-oxidant solution should be revisited. While the conventional wisdom is that H_2O_2 rapidly reduces FAC, it is clear that H_2O_2 is also a strong oxidizing agent and that its behavior as either a reducing or oxidizing agent depends heavily on, at least, the pH of the solution and on the form of the reaction intermediate (eg. superoxide ($O_2^{\bullet-}$), hydroperoxyl radical ($HO_2^\bullet$) or the hydroxyl radical ($OH^\bullet$)). In reviewing the chemical properties of H_2O_2 Gordon et al.[23] note that it oxidizes I^- to I_2 and Fe^{2+} to Fe^{3+}, both in acidic solution, but reduces (and dissolves) MnO_2 also in acid solution. They also remark (p. 431) that "…its reducing action is generally confined to acid solution".[§] And further (p. 432) "It is not anticipated that H_2O_2 would be used in combination with chlorine as a primary disinfectant. However, in a situation where chlorine is used as the residual, both species may be present initially. The amount of each species potentially remaining will depend on the pH, rate of reaction between H_2O_2 and free chlorine (i.e., chlorine and/or hypochlorous acid), other potential oxidation-reduction reactions, and the initial concentrations of all species". As Gordon et al. discuss (p. 430 et seq.), the reactions of H_2O_2 are complex and not well understood.

Revisiting the literature on the reaction between FAC and H_2O_2, the classic original work by Connick[24] argued that the reaction proceeded as:

[§] Oxidizing reactions of H_2O_2 always produce H_2O or OH^-. Reducing reactions always produce H_2O and O_2. The standard reduction potentials (E^o) for the reactions forming the two products are substantially different +1.77 and +0.68 (O_2 reduced to H_2O_2) respectively, both in acid solution.

$$H_2O_2 + Cl_2 \leftrightarrow HOOCl + H^+ + Cl^-$$
$$HOOCl \rightarrow O_2 + H^+ + Cl^-$$
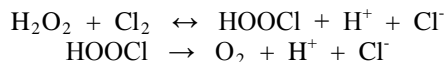
The overall reaction being:

$$H_2O_2 + Cl_2 \rightarrow O_2 + 2\,H^+ + 2\,Cl^-$$

But the rate law for the reaction varied depending on the pH of the solution. Connick recognized two rate laws: one at HCl concentrations 1 M to 10^{-4} M and a second at HCl 10^{-4} M to 10^{-6} M. He also speculated (but did not experiment) on a third rate law at higher pH (HCl $< 10^{-6}$ M).

Held et al.[25] verified Connick that there are three reaction pathways all with the net reaction producing O_2 with 1:1 molar stoichiometry and recognized different reactions in each of three pH regions – acidic, intermediate (pH 3.7 to 5.0) and alkaline – each with a different rate law and rate constant.

Recapitulating and summarizing Connick versus Held et al.
- Connick's k_4 model in acid solution (Tables I, III and IV) show the rate of reaction to be very fast at pH<3 – reactions are essentially complete in 2 – 4 minutes;
- Held et al. – consistent with Connick (Table VIII) – found in the intermediate pH range where HOCl is the primary form of chlorine, a much slower reaction with reactions only ½ complete in 20 minutes. Connick and Held et al. suggest different rate laws in this pH region; and
- In the alkaline pH range, which Connick only speculated on, the rate law appears to be

$$\frac{d[H_2O_2]}{dt} = -k_3[H_2O_2][HOCl]$$

But the reaction rate is limited by the pathway

$$HO_2^- + HOCl \rightarrow O_2 + H_2O + Cl^-$$
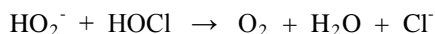
The rate constant k_b for this reaction is the one often cited – 4.4 x 10^7 M^{-1}s^{-1}. However, the rate would appear to depend on the concentration of the deprotonated H_2O_2 which has a pK_a = 11.6

Hurst et al.[26] concluded (and Espenson[27] interpreted[**]) that the rate law is:

$$\frac{d[HOCl]}{dt} = -k[HOCl][HO_2^-]$$

with k = 4.4 x 10^7 M^{-1}s^{-1}.

A few numerical evaluations using initial FAC concentrations typical of potable waters (4 mg/L) and an initial H_2O_2 concentration of 1.0 mg/L suggest that H_2O_2 might persist at concentrations <0.1 mg/L for 10s of hours at near neutral to slightly basic pH. The original work showed that FAC and H_2O_2 coexist in solution for about 48 hours at pH > 7 (the mixed-oxidant solution is pH > 9). The hypothesis of H_2O_2 being present in the mixed-oxidant solution for about two days is consistent with both original research literature on reaction mechanisms and rates and the observation that the mixed-oxidant solution is not distinguishable in chemical and biocidal properties from bleach/hypochlorite after two days following generation.

Summary

The presence of both O_3 and ClO_2 and, considering recent findings, H_2O_2 in the MIOX mixed-oxidant solution, at least at the point of generation and, in the case of H_2O_2, somewhat longer, is strongly supported by some

---

[**] In Espenson's literature review chapter, the relevant discussion is on pp.514-515.

chemical analyses, theoretical considerations, review of classic literature, and by many laboratory studies; among them Venczel's8 studies of a constructed mixed-oxidant solution; and the finding of O_3 and H_2O_2 in the products of electrolysis of chloride-free solutions by both Jeong, et al. and Jung et al.. Pending additional findings, the presence of O_3 and ClO_2, at least in the generation process, has such strong support that it is hardly in doubt. The major questions at issue are the concentrations and long-term stability of the compounds in those forms and those of the compounds that may be produced by the reactions of O_3 and ClO_2 with chlorine following generation. The presence of oxidants other than chlorine in the mixed-oxidant solution has been repeatedly established both in laboratory and in actual field practice.

> ***However, the most MIOX Corporation claims is that both O_3 and ClO_2 have the potential to be generated, have been detected in the off-gases from the cell (using Draeger tubes) and in the mixed-oxidant solution using standard methods, and that the mixed-oxidant solution behaves chemically in some applications like an O_3-containing solution and biocidally in most applications like a ClO_2-containing solution.***

Despite the potential to generate ClO_2 and O_3, the by-products commonly associated with these oxidants are not an issue in drinking water treated with mixed-oxidant solution. Chlorite (ClO_2^-), the regulated by-product commonly associated with chlorine dioxide, has never been detected in water treated with mixed-oxidant solution. In addition, bromate (BrO_3^-), the regulated by-product commonly associated with the ozonation of water containing bromide (Br^-), has not been an issue in water treated with mixed-oxidant solution. It is possible to form bromate through electrolysis if sufficient levels of bromide are present in the raw water or the salt used to make the brine for electrolysis.[28] Formation of bromate has not been an issue with on-site generation systems due to the fact that salts commonly used with these systems for potable water applications are low in bromide.

The same problems which thwarted the early attempts to quantify the composition using standard methods continued through later efforts even until the most recent work. The analyses must be able to determine quantitatively small concentrations of specific oxidant constituents in a massive oxidant (as chlorine) matrix and the existing methodology is not inherently capable of working under such conditions. Moreover, it is possible that the oxidants other than chlorine are present not as stable species recognized by current methods but as metastable intermediate reaction products not recognized by current methods.

But differences between MIOX mixed-oxidant solution and chlorine as the gas or as hypochlorite solution do exist as discussed above. In addition, the behavior of the mixed-oxidant solution in various chemical and microbiological applications in laboratory and field, as discussed briefly below, compared with the expected (or simultaneously demonstrated) behavior of chlorine alone further demonstrate substantial differences.

Chemistry-Related Differences in Behavior of Mixed-Oxidant Solution Compared to Chlorine

Superior Microorganism Inactivation in Potable Water and Processes

Microorganism inactivation of 1 to 4 logs greater than chlorine alone have been documented in laboratory and field by Shelton and Barton[29], Barton[30][31][32], Bradford[33], and Bradford and Baker[34][35] in potable water supplies, waste water and simulated cooling tower waters, and by Waldroup[36] in poultry processing lines. Subsequent studies (unpublished) by Waldroup have shown that the shelf-life of mixed-oxidant solution-treated chickens is increased by at least 3 days (or 33% longer) over that of chlorine-treated chickens using the same FAC doses in the processing line. In addition, use of MIOX-generated mixed-oxidant solution replacing chlorine in chicken processing has consistently led to large decreases in (or complete elimination of) *E. coli* and *Salmonella* detections on processed birds[37].

In laboratory studies of inactivation of four problem bacteria in the food processing industry, Russell[38] found that "MIOX [mixed-oxidant solution] was superior in terms of eliminating the four types of bacteria evaluated (*Salmonella typhimurium, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli*) when compared to bleach at equivalent concentrations of free available chlorine". A non-parametric evaluation (W.L. Bradford, 4 June 2001, unpublished) of Dr. Russell's data from the 269 paired comparisons (over practical ranges of pH and FAC doses) of inactivations using bleach and mixed-oxidant solution as the sources of disinfectant (excluding ties)

indicates that the mixed-oxidant solution was more effective than bleach by the following factors: *S. typhimurium* – 2.3; *St. aureus* – 2.5; *L. monocytogenes* – 12.0; *E. coli* – 5.0.

In studies of the mixed-oxidant solution, Son, et al.[39] reported that "…mixed oxidants are about 20-50% more efficient than free chlorine at inactivating *E. coli* and *B. subtilis* spores at certain pH conditions (pH 8.2)". In a subsequent paper – which appears to be a repeat of the "constructed solution" experiments of Venczel[8] – Son, et al.[40] reported that this same enhancement of the "efficiency" of inactivation is duplicated in a mixture made by adding small amounts of subsidiary oxidants such as O_3, ClO_2 and H_2O_2 to free available chlorine.

In a summary of several studies by students in Mark Sobsey's laboratory at the University of North Carolina, Venczel, et al.[41] reported the effectiveness of "electrochemical oxidant" (ECO) made from NaCl brine compared to free chlorine alone under various conditions – 2 or 5 mg/L, 5°C and 25°C, and pH 6, 8, and 10. They found that "[U]nder nearly all conditions, inactivation kinetics were more rapid for *E. coli*, *V. cholerae*, *C. perfringens* spore and MS2 coliphage than with free chlorine. ECO reduced *E. coli*, *V. cholerae* and MS2 by >4 $\log_{10}$ within 30 minutes and *C. perfringens* spores by >2 $\log_{10}$ within 10 minutes at pH 8 and 25°C."

Dekonenko and Bajszar[42] also found the mixed-oxidant solution to inactivate feline calicivirus on surfaces faster than bleach at the same FAC concentrations. "Considerably higher inactivation levels were achieved with mixed-oxidant solution compared to bleach when partial virus inactivation was achieved at the same FAC concentrations (50-100 mg/L) but with contact times of only 1-3 minutes."

Superior Effectiveness of Mixed-Oxidant Solution for Cryptosporidium *Inactivation*[††] [43]

Early Work Suggests Effectiveness at Practical FAC Concentrations

Inactivation of *Cryptosporidium parvum* oocysts by mixed-oxidant solution at practical FAC doses was first demonstrated by Yozwiak et al.[44] using excystation techniques for viable *C. parvum* oocyst enumeration. Subsequently, Venczel et al.[45], using neonatal mouse infectivity for terminal assays, showed at least a 3-log inactivation of *C. parvum* oocysts at an FAC dose of 5 mg/L as mixed-oxidant solution compared to nil inactivation using hypochlorite at the same FAC dose (decline in the FAC residual in the test solutions was minimal over the contact period using both mixed-oxidant solution and hypochlorite as the FAC source). Sobsey et al.[46] evaluated *C. perfringens* spores, MS2 coliphage, and *E. coli* for their responses to mixed-oxidant solution. They found that mixed-oxidant solution effectively inactivated *C. perfringens* spores in potable water and tertiary-treated waste water, with inactivations of >99.9%. *C. perfringens* spores were good indicators of *C. parvum* oocyst inactivation in water at mixed-oxidant solution doses of 4 mg/L (as FAC). In tertiary-treated waste water, *C. parvum* oocysts were efficiently inactivated (>99.9%) by mixed-oxidant solution doses of about 10-15 mg/L (as FAC)[47], sufficient to satisfy the oxidant demand of the waste water and leave an FAC residual.

Electrochemically-generated mixed-oxidant solution effectively inactivated *C. parvum* oocysts in water and treated waste water at practical doses and contact times. Further studies[48] in Sobsey's laboratory at the University of North Carolina indicated >2-log inactivation at a 3 mg/L FAC dose as mixed-oxidant solution. In addition, mixed-oxidant solution generated by a miniature electrolytic cell [aka the "Disinfection Pen", now marketed as the "MSR MIOX Purifier"], patterned after the full-size MIOX cells, has shown similar disinfecting capabilities[49].

A series of studies on the Disinfection Pen and other MIOX systems, conducted by Bajszar[50] using a newly-developed method of terminal analysis, have shown up to 3.3-log inactivation of *C. parvum* oocysts in standard tests of the mixed-oxidant solution – a 5 mg/L FAC dose (as mixed-oxidant solution), at room temperature,

---

[††] This report identifies actual operating experience in the field and laboratory. From a regulatory standpoint, the concentration times time (Ct) values for mixed-oxidant solution have not yet been established. The data herein does not constitute development of Ct values for mixed-oxidant solution. The MIOX Corporation has always maintained that the Ct values for chlorine shall be used in calculation of Ct values for mixed-oxidant solution treated water. Indeed, the US EPA listed on-site generated oxidants (mixed oxidants) as a compliance technology for drinking water[43], and included a footnote to the data specifying that chlorine shall be used as the basis for calculation of Ct values for on-site oxidant (mixed-oxidant) treated water until research pending on mixed-oxidant Ct values is complete.

with a pH 7.0 maintained by a 0.01M phosphate buffer, in oxidant demand-free water, at 90 minutes contact time (with FAC residual showing minimal decline). The terminal analysis uses polymerase chain reaction (PCR) amplification of a fluorescent-labeled heat-shock gene, presumed to be activated only in viable oocysts. A bleach/hypochlorite comparison showed about 0.9-log inactivation.

As of January 2002 and continuing to the present, protocols for *C. parvum* oocyst inactivation studies were, the subject of much discussion within the microbiological research community. Both the sources and the methods of isolation, purification, and storage for oocysts, as well as the methods for terminal assays of viable oocysts, have been under discussion in the technical literature. For example, Chauret et al.[51] found a 15-fold difference in the Ct values for 2.0-log inactivation of *Cryptosporidium* oocysts by ClO_2, depending on the supplier (and presumably the methods of preparing the oocysts used by each) of oocysts used in testing. **Nevertheless, as of the 17 November 2006 update of this document, the MIOX mixed-oxidant solution had been tested in direct comparison with bleach/hypochlorite (at FAC concentrations that are practical for potable water) in three different laboratories using four different methods of terminal assay and found to inactivate *C. parvum* oocysts by 2-4-logs; whereas inactivation by bleach/hypochlorite as the source of disinfectant has been nil** (with the single exception of Bajszar's studies discussed above, that showed 0.9-log inactivation by bleach/hypochlorite). To our knowledge, no other generator or supplier of chlorine-based disinfectants can make a similar claim.

Difficulties in Repeating the Early Observations

However, beginning in June 2001, the consistent laboratory findings of mixed-oxidant solution being able to inactivate 2- 4 logs more *C. parvum* oocysts than FAC as bleach began to change for reasons that have yet to be elucidated. Instead of the high level of inactivation, the latest research discussed further below indicates a level of superiority in inactivation of *C. parvum* oocysts much reduced – approximately 0.5-log inactivation over that of FAC as bleach.

Venczel, et al., who had also investigated *C. parvum* oocysts under the same conditions discussed in the previous section, noted that "Contrary to previous results, however, *C. parvum* oocysts were not inactivated by ECO, and the reasons for this difference are uncertain". And later they stated that "…the effectiveness of such oxidants for inactivating *C. parvum* oocysts was variable and sometimes ineffective." However, Sasahara, et al.[52] found that "Exposure to the mixed-oxidant solution at 2 and 5 mg/L (residual chlorine) [at pH 7.2 in PBS] yielded a significant inactivation of infectivity in the dose and exposure time-dependent manner, while exposure to 5 mg/L (residual chlorine) of sodium hypochlorite for contact times of up to 4 hours produced no measurable inactivation of infectivity". [Brackets added by Bradford]. Morphological examination also revealed oocyst degeneration after exposure to 5 mg/L of the mixed-oxidant solution but not with sodium hypochlorite at the same exposure level. Bajszar and Dekonenko[53] believe that the high level of inactivation observed by Sasahara et al. was due to the manner in which the investigators applied the neonatal mouse infectivity model which estimates high.

Tests performed at BioVir Laboratories, Benecia, CA in April 2003[54] using mixed-oxidant solutions generated by the Disinfection Pen suggested that prior testing protocols had used contact times (90 minutes maximum) that were not long enough to cause inactivation of *C. parvum* oocysts. With revision of the standard testing protocol to extend contact times to 240 minutes, Bajszar[55] (using US EPA Type I water (Albuquerque tap water)) found that mixed-oxidant solution at an initial FAC concentration of 5 mg/L inactivated >2 log at the 2-hour mark, >3 log at the 3-hour mark, and >4 log at the 4-hour mark, with a final FAC residual 2.1 – 2.5 mg/L. By contrast, FAC (as bleach) at an initial concentration of 5 mg/L inactivated only ~1 log at the 4-hour mark, with a final FAC residual of 3.6 mg/L. Subsequently, Bajszar[56] verified this same general pattern of *C. parvum* oocyst inactivation and also showed that FAC as mixed-oxidant solution was actually superior to chlorine dioxide (ClO_2). In retrospect, these results have been called into question.

The only other known test reflecting an inability of MIOX mixed-oxidant solution to inactivate *C. parvum* oocyst superior to that of FAC as bleach was conducted in Australia by Keegan, et al.[57] The research appears to be flawed, however:

- Under the Materials and Methods section, no mention is made of a cleanup step to isolate oocysts from the shipping/storage medium. In the Sobsey studies, a high oxidant demand was found in the oocyst stock solutions as received, necessitating a cleanup step which resulted in nil oxidant demand being added to the test solutions with the oocyst challenge.

- The experimenters used 5 mL of the stock oocysts solution, diluted with phosphate buffer, mixed-oxidant solution, and other liquids to make a 10 mL test solution. The (putatively) already-dirty stock solution had been spiked with two antibiotics – a standard practice for shipping and storage. The oxidant demand of the stock solution and, thus, of the test solutions as well would be expected to be fairly high.

- No measurements were made of the Free Available Chlorine (FAC) in the test solutions. Instead, the experimenters based their conclusions on FAC **dose** alone, rather than a measured residual, for both mixed-oxidant solution and hypochlorite.

- Lacking FAC measurements (or any measurement of a chlorine residual) during the test, it is unlikely that an FAC residual was maintained for any part of the test, a probable explanation for the inability to achieve inactivation with mixed-oxidant solution.

The Keegan et al. study appears to have been conducted with no evidence for a disinfectant residual being maintained through the contact period; thus, the later and better-controlled studies at BioVir Laboratories are more valid.

Recent Studies Suggest Mixed-Oxidant Solution is Superior but Not Effective at Practical FAC Concentrations

The tests by Sasahara, et al. and Bajszar suggested that the problem with inactivation of *C. parvum* oocysts using mixed-oxidant solution that arose first in Sobsey's laboratory in June 2001 (and persisted through 2004, prompting the comment by Venczel, et al. noted above that the effectiveness of ECO was variable and sometimes ineffective) may have been caused by increased resistance to chlorine disinfection in the strains of *C. parvum* oocysts supplied for testing, requiring longer contact times for inactivation. Nevertheless, it seemed clear at that point in time that: 1) FAC as mixed-oxidant solution is superior to FAC as bleach/hypochlorite in *C. parvum* oocyst inactivation; 2) significant inactivation DOES occur at practical doses of FAC as mixed-oxidant solution; but 3) the effectiveness of mixed-oxidant solution, as well as the differences between FAC as mixed-oxidant solution and FAC as bleach/ hypochlorite, can be seen only after contact times of 2 hours or greater.

The most recent work by Bajszar and Dekonenko, however, concludes that mixed-oxidant solution "exhibits a higher efficacy in [*C. parvum*] oocyst inactivation than hypochlorite", but only ~0.5 log superiority at practical FAC doses and residuals. The superiority of mixed-oxidant solution over hypochlorite becomes larger at higher FAC doses and residuals but at doses and residuals practical for potable water, a large superiority is not apparent. As of the current update to this document (February 2011), this is the position of the MIOX Corporation.

Oxidation of Manganese (Mn(II)) and Removal in Classical Treatment

In studies of mixed-oxidant solution pretreatment at a small classical treatment plant in Greenfield, IA, Bradford[58] and Herrington and Bradford[59] reported oxidation and subsequent removal in classical coagulation/flocculation/filtration treatment of Mn^{2+} under water quality conditions that defy conventional understanding[60] – the FAC concentration at the clarifier was only 0.1 mg/L (1/5th that typically required), the pH of the raw water was too low (by at least 1 unit), and the water was too cold (by about 10°C). Moreover, the oxidation reaction was completed in 20 minutes and the Total Trihalomethane (TTHM) concentration produced by dosing with mixed-oxidant solution in pretreatment was only 16 μg/L. Both chlorine (at all temperatures) and $KMnO_4$ (in cold waters) treatments had failed to remove the Mn^{2+} in prior trials. Subsequently in January, 1999, the plant operator reported that Mn^{2+} oxidation was being accomplished with even less FAC (0.05 mg/L at the clarifier) as mixed-oxidant solution and a shorter contact time (22 seconds) between mixed-oxidant solution dosing and clarification. Mn^{2+} in raw water was 0.075 mg/L and was reduced to 0.015 mg/L in finished water.

Evidence for the Microflocculation Effect Similar to that of Ozone

In the studies at Greenfield, IA reported by Bradford[58] and Herrington and Bradford[59], pretreatment by mixed-oxidant solution to an FAC dose of 0.75 - 1.0 mg/L caused a reduction in coagulant demand of 39.7% over a 4-month period of the test. This effect was consistent with the definition of "microflocculation" by Becker and O'Melia[61] and quantitatively identical to their findings using ozone alone for pretreatment, as well as with those of

other researchers[62] [63] [64] [65] [66] who had tried both chlorine and ozone and found the microflocculation effect only with ozone. The microflocculation effect using mixed-oxidant solution was observed and put to effective use at Santa Fe, NM as discussed below and at Clinton, MA[67]; in the latter, direct comparisons were made between mixed-oxidant solution, which showed a significant microflocculation effect, and sodium hypochlorite, which showed no effect.

Jar tests conducted subsequently at the Santa Fe, NM treatment plant showed a 25% reduction in coagulant demand as well as a dramatic improvement in both the rate of formation and the texture of the floc at an FAC dose of 1.0 mg/L as mixed-oxidant solution in pretreatment. In full-scale operation at Santa Fe, pretreatment by MIOX mixed-oxidant solution reduced the alum demand by about 40%, and improved turbidity removal at the clarifier. Pretreatment using the mixed-oxidant solution followed by final disinfection also using mixed-oxidant solution resulted in a reduction in total chlorine dose (pretreatment plus final disinfection) of about 30% compared to normal practice using chlorine gas alone for final disinfection, maintenance of a satisfactory residual in the distribution system without further boosting, and TTHM concentrations of < 25 μg/L (as of May 27, 1998). The mixed-oxidant solution used in pretreatment caused the microflocculation effect in both respects discussed by Becker and O'Melia[61] – both a reduction in alum demand and a reduction in finished water turbidity – allowing also a plant startup time in April, 1998 of 2 days compared to a normal startup time of 2 weeks, and a doubling of the water production rate in full operation through the summer of 1998. Moreover, TTHM concentrations in distribution system water were about 50% lower compared with the previous year when chlorine gas alone was used[68].

Testing of the microflocculation effect by the mixed-oxidant solution to date indicates that the effect has the following features: (1) the composition of raw waters in which the effect has been observed meet Becker and O'Melia's[61] qualitative criteria for the occurrence of the microflocculation effect using only ozone; and, as shown by cessation of the effect at Greenfield when one of those criteria were violated by a seasonal change in the DOC concentration in the raw water, the occurrence of the effect appears to adhere strictly to the Becker and O'Melia criteria; (2) the effect occurs at FAC doses as mixed-oxidant solution that are equal to or less than ozone doses that the literature indicates would be required to cause the same effect (ozone doses typically ≤ 0.5 mg/mg DOC versus FAC as mixed-oxidant solution ≤ 0.3 mg/mg DOC); (3) the reduction in coagulant demand by mixed-oxidant solution used in pretreatment exceeds the best reduction reported in the literature using ozone (ozone 30% reduction; mixed-oxidant solution 40% at two operating locations); and (4) an increase in turbidity removal also occurs using the mixed-oxidant solution whereas an increase in turbidity removal using ozone has not always been reported.

At Rend Lake, IL[69] mixed-oxidant solution added in pretreatment in FAC concentrations sufficient to satisfy only the immediate oxidant demand of the raw water resulted in a decrease in turbidity at the top of the Claricone from 2.0 ntu without pretreatment to 0.4 ntu with pretreatment, and an increase in TOC removal from 5% without pretreatment to 15% with pretreatment. This pretreatment strategy enabled significant improvement in water treatment with a production of TTHMs of only 16 μg/L at this step. Hypochlorite solution used in pretreatment had shown no effects at all. Other benefits that resulted from use of mixed-oxidant solution in pretreatment included elimination of pin-point floc, substantially faster stabilization of the clarification process, and elimination of algae that had formed in the Claricone in prior use. Calculations indicated that the FAC as mixed-oxidant solution dose to TOC concentration was only about 0.1 mg/mg – substantially less than the oxidant dose/TOC ratio typically required to cause microflocculation using O_3 in pretreatment (0.3 - 0.5 mg/mg).

There are strong suggestions from potable water treatment plant operators successfully using mixed-oxidant solution in pretreatment for the microflocculation effect that disinfection by-product (DBP) production is also reduced where the microflocculation effect occurs. At West Point, GA (as reported by the operator, Leon Wessinger, November 2003), mixed-oxidant solution was being added at the rapid-mix sufficient to leave a barely-detectable FAC residual at the slow-mix stage of classical alum coagulation treatment. A Georgia state regulator visited the plant in June 2003 and inadvertently caused performance of an experiment at full-scale operation. Because the State of Georgia forbids chlorination pre-clarification due to DBP formation, the regulator required Mr. Wessinger to cease the mixed-oxidant solution dose pre-coagulation. Within a period of days, both the TTHM and the haloacetic acid (HAA5) concentrations increased in samples taken in distribution. When these results were reported, Mr. Wessinger was permitted to reinstate injecting mixed-oxidant solution pre-coagulation. Within a few days, the DBP concentrations in distribution declined. The ranges of DBP concentrations in distribution reported with and without mixed-oxidant solution dosed in pretreatment are as follows:

DBP Concentration Range	Mixed-oxidant Solution Dosed in Pretreatment	
	No	Yes
TTHM (µg/L)	80 - > 100	< 60
HAA5 (µg/L)	70 - 80	< 50

The most likely cause for this effect is that microflocculation also improves the removal of the DBP precursor organic materials – humic and fulvic acids – into the floc, thus removing them from the water prior to final disinfection. Ghadimkhani et al.[70] noted improved TOC removal by pre-ozonation and pre-chlorination before coagulation in laboratory studies of potable water treatment providing support for this hypothesis.

The microflocculation effect using mixed-oxidant solution in pretreatment had been demonstrated by jar testing at the Lajas Filtration Plant in Puerto Rico (letter to David Velazquez, Engineer, from Carlos Gonzalez, New Technology Systems, 1/18/01), indicating a reduction in alum demand by the pretreatment of at least 35%. Subsequently (2/20/02 – 3/12/02), the microflocculation effect was demonstrated in actual operation at the Coto Laurel Treatment Plant, Ponce, PR by switching gradually over a period of days between chlorine gas and mixed-oxidant solution dosed in pretreatment (prior to coagulant addition at rapid mix). With mixed-oxidant solution being dosed, the alum consumption declined by ~50%. During the times that mixed-oxidant solution was being used, TOC removal calculations also indicated improved removal. In addition, customers called the plant to remark on the excellent taste of the water which, at that time of year, tended to have a fishy taste and odor apparently associated with anaerobic conditions in the raw water source – a lake – coupled with high manganese concentrations in the raw water (e-mail from Carlos Gonzales 2/24/06).

As of the current update of this document (8 February 2011), the microflocculation effect caused by adding mixed-oxidant solution in pretreatment had been reported anecdotally or had been demonstrated in jar tests or in actual plant operations in waters at or from 30 potable water treatment plants. The effect has also been demonstrated in potable water treatment in Japan.

Biofilm Removals in Distribution Systems

Early Observations Suggest Unusual Behavior against Biofilms

The effects of the mixed-oxidant solution on biofilms had been reported initially by Bradford and Baker, but more controlled studies by Crayton et al.[71] [72] at a KOA campground in Great Falls, MT showed dramatic effects of the mixed-oxidant solution compared to hypochlorite solution, the standard treatment at the site prior to use of MIOX[‡‡]. The campground owner reported apparently complete sloughing of distribution system biofilms within 2 weeks of startup of the campground water system for the summer, and an entire summer of operation with no Total Coliform violations in distribution system samples, no loss of pressure in the distribution system, and no biofilms in showers, all of which had commonly occurred previously. Moreover, no slime growths occurred on surfaces in the water park and swimming pool at the campground, which enabled him to eliminate the usual biocidal treatment, and the water had an excellent taste at all locations in the distribution system.

Petersen and Bradford[73] observed elimination of small occurrences of biofilm scales from cooling surfaces of cooling towers within a few days of replacing the organic disinfectant (hydantoin) then in use with mixed-oxidant solution. And with continued use of mixed-oxidant solution (at FAC residuals about 0.2 - 0.3 mg/L in the cooling water) the biofilm scales did not return. In addition, the occurrence of detectable planktonic aerobic bacteria in the cooling water basin was reduced to nil (weekly test slides showed below detection (< 1000/mL) aerobic bacteria counts consistently). Within the cooling tower industry, elimination of biofilms and elimination of aerobic bacteria detections in this fashion is considered unusual.

[‡‡] It should be noted that the practice of the campground owner prior to use of mixed-oxidant solution was to slug-dose the system with bleach. According to conventional wisdom, this practice should have been more effective at removing biofilm accumulations than would steady disinfectant dosing. The fact that this practice was not effective at biofilm removal is instructive in itself, and defies the conventional wisdom.

Charles T. Smith (Quality Water Technology, Wilkes Barre, PA, personal communication, 15 January 2001) reported being able to remove heavy accumulations of biofilms (described as "leathery" and acidic, causing serious corrosion of the cooling surfaces) from a cooling unit above a bakery using mixed-oxidant solution. Significantly, the biofilms did not return for 3 weeks following the treatment and even then were not heavy enough to require cleaning – i.e. the "leathery" structure of the biofilm did not return. Using bleach for cleaning in prior experience, "leathery" biofilms returned in 10 days to an extent that cleaning was required.

In a dramatic demonstration, in December 2002, the owners of Ainono Hot Springs in Japan video-taped the removal of biofilms by mixed-oxidant solution from 4-inch pipe conveying the spring water to their facility pools and in the recirculation piping. Prior disinfection practice involved dosing at 1.5 mg/L FAC (as NaOCl), which resulted in less than a 0.2 mg/L residual. Video tape of the piping interior before switching to mixed-oxidant solution showed heavy deposits of a leathery-appearing gelatinous mass of biofilms. Mixed-oxidant solution was then dosed at 0.6 - 1.0 mg/L FAC, resulting in a 0.4 - 0.7 mg/L residual. During the next 6 days, gelatinous masses discharged from the piping into the facility pools; the masses were removed manually. After 6 days, the boroscope camera was reinserted into the piping and showed substantial removal of the gelatinous masses of biofilm. At 22 days, the boroscope camera revealed that the biofilm deposits were virtually completely removed from the piping.

As reported 22 March 2002, in an inadvertent demonstration of the ability of mixed-oxidant solution to control algae, the operator of the Embassy Suites, Honolulu, HI indoor swimming pool converted his 2 MIOX generators (SAL80s) to produce hypochlorite instead of mixed-oxidant solution. Over a period of a few days, while still maintaining a typical FAC residual in the pool water, black and brown algae deposits formed on the sides and bottom of the pool. At the suggestion of MIOX staff, he then converted one of the two generators back to its original configuration, producing mixed-oxidant solution. Within a few days, the algae deposits disappeared. A similar report was received in December 2003 from the operator of the YMCA pool in Valdosta, GA. When mixed-oxidant solution was substituted for bleach as the disinfectant source, a black algae deposit which had accumulated beneath a water slide, sloughed off within a few days of starting mixed-oxidant solution use.

Experience as of 2001 was summarized as follows (W.L. Bradford, letter to Dr. Don Phipps, Orange County Water District, Fountain Valley, CA 9 July 2001) [author's additions, definitions, and examples in brackets]: "But what we have seen amongst many (indeed most) new users of MIOX systems (replacing chlorine with MOS [mixed-oxidant solution]) is a response from the treatment and distribution system that ranges from massive release of biofilms (the stuff comes out in great gobs, seen at some locations in portions of the treatment plant [as at Las Vegas, NM] and in distribution [as at the KOA Campground in Great Falls, MT]) to behaviors that can be interpreted using a biofilm-release conceptual model [as at Farmington and Lower Valley, NM] – e.g. some hits of coliform bacteria in the presence of a substantial FAC residual at points in the distribution system (these last a few weeks to several months depending, we think, on how vigorously the utility flushes its lines after starting MOS) and a gradual increase in the FAC or TC residual in distribution with time, allowing the utility to reduce the FAC/TC residual leaving the plant. The latter is normally accompanied by a reduction in disinfection by-product formation in distribution [as at Greenfield, IA]". The formation of disinfection by-products by biofilms in distribution systems, the subject of speculation for several years, was identified in 2001 by Rossman et al.[74].

A clue as to the mechanisms by which the mixed-oxidant solution affects biofilm deposits in such a remarkable fashion was reported by Phipps and Rodriguez[75] by their observations of the condition of a cellulose acetate substrate following removal of a single-culture biofilm by mixed-oxidant solution. The surface was reported as "sterilized" to the point that DAPI staining showed no intact DNA remaining – Dr. Phipps had never seen such a condition before in several years of research on biofilm removals using chlorine primarily. The latest models of biofilm attachment have an initial deposition of extra-cellular polysaccharide (also referred to as extracellular polymeric substances [EPS] in the literature)[76] occurring, which renders the surface more suitable for further attachment and protects the depositing organisms from attack by disinfectants. The extra-cellular material continues to be produced and forms the organic "glue" holding the assemblage of organisms living and dead, iron and manganese oxide deposits, corrosion products, and inorganic particles that happen to come by, together in the amorphous mass we call biofilms. According to Phipps' remarks, it appears that the mixed-oxidant solution attacks the polysaccharide substrate [or EPS] in a way that chlorine does not. The surface is sterilized and rendered unsuitable for regrowth/recolonization, requiring any biofilm-forming microorganisms to "start over", re-conditioning the surface for attachment.

In summarizing 4 years of experience (since installation in October 2001) with a MIOX system, which replaced bleach for disinfection at the Tachikawa Citizen Swimming Pool, Tachikawa, Japan, Sanmiya[77] reported several observations beneficial to pool operation that are related to biofilm removal: 1) elimination of biofilms from piping and the filter sump, and elimination of *L. pneumophila* detections in 3 weeks after startup; 2) slimes said to be caused by low FAC residuals and/or inhomogeneous FAC residuals were eliminated from the bottom of the pool; 3) dirty filter media, scheduled for replacement at the time of installation of the MIOX system, actually recovered and continued to be used long after the normal replacement date; 4) filter back-flushing frequency was reduced to ~$1/3^{rd}$ of the previous frequency and solids removal was improved; and 5) "scale" was removed from the heat exchangers resulting in lower energy consumption to heat the pool water and a faster heating of replacement water.

The mixed-oxidant solution was applied to remove biofilms from the water distribution system in a hospital building in Derby, England, UK (Waterman Environmental Services Ltd., Sutton Coldfield, UK, e-mail report with data, 2 March 2006). The official water cleaning and normal disinfection protocol in the UK – approved by the National Health Service – is treatment with chlorine dioxide. However, Waterman Environmental Services had been unable to pass inspection in initial distribution system cleaning using the approved protocol. Over a 2-week period (9 February through 24 February 2006) they dosed mixed-oxidant solution at 50 mg/L FAC into the hospital building distribution system (unfortunately, FAC residuals were not monitored at the sampling taps). *Pseudomonas* sp. and *Pseudomonas aeruginosa*, as well as *Legionella pneumophila*, were detected at the taps, indicating biofilm sloughing. Major *Pseudomonas* sp. sloughing appeared to be complete in about 3 days but *L. pneumophila* continued to be detected sporadically through the 10th day of treatment (last detected on 21 February). The construction contractor for the hospital (Skanska of Sweden) remarked that they were "…totally impressed with the speed at which we (Waterman) have sterilized the entire building (some 3500 outlets)."

In the case of biofilm removals from distribution systems and other surfaces, few peer-reviewed publications have been produced. But MIOX Corporation has received numerous anecdotal reports from new users of MIOX systems, so much so that it has become common practice to caution new MIOX system users to expect biofilm sloughing, even if no biofilms are thought to be present, and to prepare for an active, but hopefully brief, distribution system flushing program. There are also cases, documented by photographs and anecdotal reports, of removal of old, encrusted biofilm-like materials, which also contained iron and manganese oxides, by mixed-oxidant solution from pipes at Diana, TX and Crossville, TN; the deposits had developed in the presence of a well-maintained FAC residual using chlorine (gas or hypochlorite) as the disinfectant.

Experiments with Laboratory Biofilm Models

Demonstrations of superior biofilm removal by mixed-oxidant solution using laboratory models have been less convincing than the reports noted above. Indeed, the dramatic biofilm releases at the KOA campground reported by Crayton et al.[71][72] in 1997 had been preceded by experiments with laboratory models which showed no difference between mixed-oxidant solution and bleach/hypochlorite. Additional more carefully designed experiments using up-to-date laboratory models were conducted at the Montana State University, Center for Biofilm Engineering (MSU/CBE) over an 18-month period in 2006 – 2008. The objective was to study the efficacy of disinfectants (sodium hypochlorite versus mixed oxidant solution from a MIOX system) on the removal of biofilm that had been grown under low disinfectant concentrations but relatively rich nutrient conditions, followed by treatment using both mixed-oxidant solution and bleach/hypochlorite.

Four experiments were performed; two with the treatment step at breakpoint chlorination (one with and one without additional ammonia-N) and two with the treatment step at below breakpoint (one with and one without additional ammonia-N). For all five (one experiment was repeated) experiments, five biofilm growth reactors were utilized with one reactor acting as a non-disinfected control, two with biofilm grown under low mixed oxidant conditions and the final two with biofilm grown under low chlorination by bleach/hypochlorite. The reactors were allowed to operate until a steady-state biofilm had accumulated. During this time, effluent and biofilm cell counts were taken periodically. Upon treatment, where disinfectant concentrations were increased, one of the two reactors having biofilm grown with mixed-oxidant solution received chlorine treatment while one of the two reactors with biofilm grown with sodium hypochlorite received mixed-oxidant solution treatment. An intensive sampling scheme was employed to monitor the transient events in the effluent water and biofilm over 24 hours during the treatment step.[78]

The data were evaluated for patterns in the treatment step contrasting treatment using mixed-oxidant solution and treatment using bleach/hypochlorite. The parameters evaluated and the results were as follows:

- Treatment FAC – the FAC concentrations in the two solutions supplying the treatment necessary to achieve the target FAC or Total Chlorine (TC) in the effluent. A higher FAC concentration was required in all mixed-oxidant solution treatment indicating that the mixed-oxidant solution reacts with components – probably organic components – that bleach/hypochlorite does not. This is a feature of mixed-oxidant solution often observed by MIOX customers in startup of a MIOX system replacing mixed-oxidant solution for bleach/hypochlorite;
- Effluent Δ(TC – FAC) – the difference between the TC measurement and the FAC measurement indicates the concentration of "combined chlorine", the inorganic and organic chloramines present in the effluent. A smaller value indicates more complete reaction of the disinfectant with ammonia and organic nitrogen compounds. In all cases where the target concentration in the effluent was FAC ≥ 1.0, mixed-oxidant solution treatment showed more complete reaction with nitrogen-containing components of the reactors;
- Viable Cell Counts (HPC)-Reactor Effluent – indicates completeness of the inactivation of the sloughed biofilm cells. In addition, the times into the treatment step when the Viable Cell Counts in the effluent were less than those in the influent to the reactors – indicating completeness of the biofilm sloughing – were recorded. No clear pattern between mixed-oxidant solution and chlorine could be seen; and
- Viable Cell Counts (HPC)-Biofilm on Coupons – indicates the extent of biofilm sloughing. In most cases, the mixed-oxidant solution treatment was clearly superior to the bleach treatment; in one case, no difference could be seen. In addition, the reductions in biofilm densities compared to the controls observed in the treatments were quite high at 4 – 6 logs. The typical reduction observed in testing of this kind by the MSU/CBE is only 2 logs.

Complete removal of the biofilms – the most dramatic feature of mixed-oxidant solution treatment observed by some MIOX clients in startup and seen also in the field trials of the original studies done by CBE [71][72] – was not observed. ***However, in these experiments, mixed-oxidant solution treatment was often superior to bleach treatment by a considerable degree whereas bleach treatment was never superior to mixed-oxidant solution treatment. These results indicate that mixed-oxidant solution is superior to bleach for biofilm removal and control over a broad range of water treatment and disinfection practices of potable water systems.***[79]

Recent Indirect Evidence of Biofilm Removal from Distribution Systems

Direct evidence of biofilm removal from a potable water distribution system is notoriously difficult to obtain. However, in a 2-year study at the Lake Murray Water Treatment Plant (LMWTP) in West Columbia, SC, six lines of indirect evidence for biofilm removal were developed.

In April 2007, the LMWTP discontinued the use of chloramines and switched entirely to FAC as mixed-oxidant solution being sent to distribution. Over the 2-year study period the following features, which are consistent with a biofilm removal model, were observed:[80]

1. A gradual decrease to nil by November 2007 – six months after FAC as mixed-oxidant solution began being sent to distribution – in chlorinous odors at residences in the Direct Customer service area of the LMWTP; this observation is consistent with the recent findings of Li and Blatchley[81] that chlorinous odors (primarily from NCl_3) are formed by reactions between FAC and organic–N compounds which would be present in biofilms;
2. Substantial decreases between the summer of 2007 and the summer of 2008 in the formation of TTHMs between the Clearwell and the Farthest Point in the Direct Customer distribution system; this observation is consistent with the finding of Rossman et al. that TTHMs are formed in reactions between FAC and biofilms;
3. A dramatic decrease in the total FAC doses in treatment since use of mixed-oxidant solution began, while maintaining a satisfactory residual in distribution; biofilms consume FAC; thus removal of biofilms from distribution permits lower FAC doses and residuals entering the distribution system;
4. Stability of the low FAC residual in distribution; the absence of FAC–consuming biofilms would be expected to reduce the variability of the FAC in distribution;
5. A substantial decrease in the TTHM concentrations in the Clearwell sample and in the distribution samples from October through 18 December 2007 which cannot be attributed to decreases in temperature or

decreases in residual in distribution (the mean residuals in distribution were higher in November and December); and

6. Decreases in the TTHM concentrations in the Clearwells between 23 October and 18 December 2007 that cannot be accounted by either a decrease in temperature or a decrease in mixed-oxidant solution dosing between those two dates (indeed the mean FAC residual in the Clearwells was higher in December than in October which, all else being unchanged between the two months, would tend to make *higher* TTHMs in December).

Ammonia Oxidation at Sub-Breakpoint Chlorine Doses as Mixed-Oxidant Solution and Apparent Superior Fragmentation of Organic Nitrogen Compounds Typically Found in Human Body Fluids

Studies by Bradford and Cisneros[82] and operational experience reported by Daniel[83], all summarized by Bradford[84], indicate that the mixed-oxidant solution causes oxidation of ammonia in ammonia- and chloramine-containing waters at FAC doses well below those needed for oxidation by classical breakpoint chlorination. Neither nitrites nor nitrates were produced by mixed-oxidant solution; thus the loss of ammonia (including ammonia as chloramines) occurred as nitrogen (N_2) gas. The effect supports the presence of oxidants other than chlorine in the mixed-oxidant solution, as originally demonstrated convincingly by Dowd[4]. One practical application of this feature is in the maintenance of disinfection residual in chloraminated water poised at breakpoint using minimal doses of mixed-oxidant solution compared to chlorine, as shown by Daniel. Another was demonstrated at Fonda, IA[85] where a FAC/NH_3-N (ammonia-nitrogen) mass dose (as mixed-oxidant solution) ratio of 8.4:1 without correction for other oxidant-demanding materials was used operationally to remove ammonia (NH_3) from raw ground water (a ratio of 10 is normally required using chlorine alone); a ratio of as low as 5.3:1 had been observed in pilot studies. Moreover, the mixed-oxidant solution appears to initiate the breakpoint reaction at near stoichiometric FAC doses. This feature contrasts with the behavior of FAC as bleach in many breakpoint applications. For example, at Fonda where the raw ground water contained 3.0 mg/L NH_3–N, FAC doses as high as 50 mg/L as bleach/hypochlorite solution were required to initiate the breakpoint reaction, whereas an FAC dose of only 25.2 mg/L as mixed-oxidant solution was required to initiate the reaction and drive it to completion.

Experience with use of the mixed-oxidant solution compared to previous use of chlorine alone (as gas or bleach), as reported anecdotally by MIOX system users, suggests that the mixed-oxidant solution has capabilities superior to that of chlorine alone in decomposing the organic nitrogenous components of human body fluids. Swimming pool operators who have switched from bleach (or chlorine gas) as the source of disinfectant to the mixed-oxidant solution report a cessation of chlorinous odors and burning eye complaints from swimmers, and a much reduced (often to nil) need to "shock" the water with excess oxidant to remove the odors[86]. It is worth noting also that these reports come from operators of both indoor and outdoor swimming pools; thus UV from sunlight appears not to be a determining factor.

One working hypothesis explaining these observations is that disinfection with chlorine alone causes slow reactions with organic nitrogenous compounds, creating substantial concentrations of volatile and odorous dichlor- and trichloramines[87] and even cyanogen chloride[88] as reaction rate intermediates en route to complete breakpoint, whereas disinfection with mixed-oxidant solution causes the same reactions to occur faster, allowing only small concentrations of these reaction intermediates to be produced. An alternative working hypothesis is that the other-oxidant components (other than chlorine) of mixed-oxidant solution react with the organic nitrogenous components in mechanisms that do not form substantial reaction intermediates. In either case, a breakpoint-like reaction and decomposition of the organic nitrogen compounds seems to be the likely mechanism, and the mixed-oxidant solution is causing those reactions to proceed either faster or with a different stoichiometry than occurs using chlorine alone.

During a period of several months in 2003, Dempsey [§§]reported similar observations with considerable quantitative detail added, plus additional observations, in his clients' pools when they replaced mixed-oxidant

---

[§§] Personal communications between the author and Mr. Rick Dempsey, founder and President of Simply Water, LLC, Houston, TX, consultant on chemistry and operations to the commercial pool industry and equipment supplier for large commercial and municipal swimming pools (with over 18 years experience).

solution for bleach/hypochlorite for disinfection. These discussions resulted in a revised ***working hypothesis*** described briefly as follows: (details are provided in Bradford): ***MIOX mixed-oxidant solution in swimming pool water causes steady oxidation of organic chloramines (and cyanogen chloride (CNCl)) and rapid completion of the breakpoint reaction on nitrogen (-N-) fragments from that oxidation rather than allowing accumulation of them (including volatile dichloramine ($NHCl_2$), trichloramine (NCl_3), and CNCl) in the pool water, as is likely the case using chlorine (to wit, the common swimmer's complaints of "chlorinous" odors and burning eyes when bleach/hypochlorite is used for disinfection). This steady removal of organic nitrogen and rapid completion of the breakpoint reaction would be expected to cause the following beneficial effects, as have been noted by swimmers in, and operators of, several pools using mixed-oxidant solution as a replacement for chlorine for disinfection:***

- Maintenance of an acceptable disinfection residual at lower doses of FAC as mixed-oxidant solution than required using chlorine;
- Nil production of chlorinous odors in air overlying the pool water and no burning eyes among swimmers;
- The "nil chlorinous odor" feature has the associated benefit to swimmers that dramatically lower concentrations of the chloramines are inhaled; as a result, swimmers report feeling refreshed rather than "groggy" by their swimming exercise and, indeed, that the water feels "faster"; [this feature should also be welcomed by asthmatics as indicated in one of a number of studies in Europe[89]].
- The need to "shock" the water with excessive bleach/hypochlorite and/or persulfate to remove combined chlorine (the sum of chlorine as chloramines and organic chloramines) is dramatically reduced or eliminated[ψ];
- Elimination of shocking, as well as of draining and replacing pool water, for management of combined chlorine concentrations causes a reduction in the rate of accumulation of Total Dissolved Solids (TDS) in the water and a reduction in costs associated with draining and replacing.
- Better disinfection both because the mixed-oxidant solution is a better disinfectant than chlorine alone and the bulk of the disinfection residual in the pool water would be present as FAC not the combined chlorine; and
- Improved clarity of the water both because the organic amine substrate would not be present to stimulate bacterial growth and disinfection activity is increased.

Possibly related to improved water clarity, as well as to reduced chlorinous odors, is swimmers' reports that they no longer "feel the need to shower after swimming"; rather they feel clean and refreshed by their swim. Weaver et al.[90] recently reported the presence of several volatile organic compounds as the by-products of chlorine disinfection in the air of indoor swimming pools including $NHCl_2$, and NCl_3 and CNCl.

At the Tachikawa Citizen Swimming Pool, Tachikawa, Japan, introduced in the previous section, Sanmiya noted: 1) measured chlorine concentrations in the air inside the swimming pool building were 1/3rd of the previous concentrations and of similar pools using bleach; 2) chlorinous odors no longer occurred; 3) the pool expected to experience huge savings in reduced corrosion (due to reduced chloramines in the air) of the building ventilation system; 4) the water was judged to be safe for even people with frail skin (again, due to the low concentrations of chloramines in the water; and 5) the rate of water replacement was decreased by ~50% (water replacement is often used as a Combined Chlorine control strategy; the pool operators found such a strategy to be unnecessary). Measured chlorine concentrations in the air of the Tachikawa pool and others were presented by Sanmiya as shown below.

[ψ] The experience of pool operators using mixed-oxidant solution (replacing bleach/hypochlorite for disinfection) to date is that the need for "shocking" has been eliminated completely and that the combined chlorine accumulated in a day of heavy swimmer load is removed from the water overnight so long as the FAC as mixed-oxidant solution residual is ≥ 3.0 mg/L. In Mr. Dempsey's experience (over 20 years in managing commercial swimming pools), this feature has never been observed in swimming pools using chlorine (as gas or bleach) for disinfection. In order to achieve removal of combined chlorine overnight, typically the FAC as chlorine gas or bleach must be raised to 8 - 10 mg/L by superchlorination, or persulfate must be added in large doses.

Name of Facility	Disinfection method	Before MIOX installed	After MIOX installed
Tachikawa Pool	Mixed oxidant	0.01 ppm maximum	0.0060 ppm
A city pool	Sodium Hypo + Ozone	0.0146 ppm	
B city pool	Sodium Hypo	0.030 ppm	0.0095 ppm

Bradford and Dempsey[91] conducted further evaluation of the experience with mixed-oxidant solution in commercial swimming pools and of analyses of water samples collected at selected pools. Their findings are summarized as follows:

1. Nitrate expressed as nitrogen (NO_3^--N) concentrations do NOT accumulate in the mixed-oxidant solution-treated pool waters, in contrast to literature reports on bleach-treated pools.[92] This observation suggests a chemical mechanism for the degradation of organic nitrogen compounds by mixed-oxidant solution that is different from that of bleach;
2. The accumulated Total Organic Carbon (TOC) concentrations from the Body Fluid Analog (BFA) added continuously by bathers are MUCH lower than those reported in the literature for bleach-treated pools and much lower than shown in laboratory models; and
3. The cause for persistent positive biases in the Combined Chlorine (CC) measurements, which are observed in some mixed-oxidant solution-treated pools, is most likely slow degradation of some organic nitrogen (organic–N) components of the BFA. This results in small concentrations being observed (in the Total Kjeldahl Nitrogen analysis) at any time, and formation of organic chloramines – a persistent CC measurement arising from this mechanism is equivalent to a "*nuisance residual*".

The causes for these remarkable features of the mixed-oxidant solution are being investigated in academic laboratories. However, these features suggest mechanisms for the oxidation of ammonia and organic nitrogen-containing compounds that are different from those of chlorine alone (as bleach or chlorine gas). Specifically, the absence of chlorinous odors and the lack of NO_3^-–N being formed may be related through the reaction scheme of Jafvert and Valentine.[93] Rapid completion of the breakpoint reaction by mixed-oxidant solution minimizes the accumulation of $NHCl_2$ thus minimizing both chlorinous odors from both $NHCl_2$ and NCl_3 and formation of NO_3^-–N in mixed-oxidant solution–treated swimming pool waters. Lower than predicted TOC concentrations and lower than expected Total Kjeldahl Nitrogen concentrations are also consistent with the absence of chlorinous odors from, specifically, NCl_3. Li and Blatchley showed formation of NCl_3 by reaction of FAC with organic-N components of the BFA. More detailed discussion is available in Bradford.[94]

Reduced Concentrations of Total Trihalomethanes but Increased Production of Brominated Forms

Users of MIOX mixed-oxidant solution for potable water treatment uniformly experience, at worst, no difference in the production of Total Trihalomethanes (TTHMs) and other disinfection by-products compared to chlorine (as gas or as hypochlorite). More commonly, however, users of mixed-oxidant solution experience a 30-50% reduction in TTHMs (with occasionally a truly dramatic reduction being reported, like 90% in one case). The causes for these reductions have not been fully explained, but are known to be due to several factors in the potable water treatment and distribution process. The experience of MIOX users as of 2002 in TTHM and other disinfection by-product reductions was summarized by Hamm[95].

The lack of consistency in the experience of potable water treatment plants vis-à-vis reductions in disinfection by-product (DBP) concentrations in samples from the distribution system by using mixed-oxidant solution is not fully explained. However, the current *working hypothesis* is that the mixed-oxidant solution offers three opportunities, or mechanisms, for such reductions to occur: 1) the other-oxidant (other than chlorine) component(s) of the mixed-oxidant solution satisfies some of the oxidant demand of the water, allowing achievement of satisfactory FAC residual at lower FAC doses as mixed-oxidant solution, and the reduced dosage results in less reaction with DBP precursors; 2) the other-oxidant component(s) react preferentially with precursor organic compounds (humic and fulvic acids) without forming DBPs; and 3) removal of biofilms from distribution systems by mixed-oxidant solution both stabilizes the FAC residual in the water in-transit and reduces the formation of DBPs in distribution as described by Rossman et al.[74]. Operation of the first two mechanisms may be observed in a Simulated Distribution System (SDS) test (Standard Methods[2], 1998, Method 5710C and Method 5710D).

Operation of the third mechanism can be observed only by actual trial of mixed-oxidant solution for final disinfection by the potable water treatment plant; it is often observed that, over a period of weeks following introduction of the mixed-oxidant solution for final disinfection, the TTHM and HAA5 concentrations generated within the distribution system decline.

Experience to date (2009) indicates that reductions in DBP production using mixed-oxidant solution can be observed in the SDS test in about half the waters tested. However, reductions in DBP production within the distribution system (coupled with stabilization of the FAC residual in the water in-transit) using mixed-oxidant solution for final disinfection are almost universally observed.

The best-studied example of the effects of mixed-oxidant solution on total FAC dosing and DBP concentrations in distribution is at the Lake Murray Water Treatment Plant (LMWTP) in West Columbia, SC discussed earlier regarding evidence of biofilm removal. The LMWTP converted from chloramines to FAC as a mixed-oxidant solution in water sent to distribution at midnight on 24 April 2007. DBP concentrations as expected soon increased in the plant and in distribution. Over the following months, plant management and personnel optimized in-plant operations using mixed-oxidant solution. Results through April 2009 were: 1) reduced FAC total dose by a factor of two while maintaining satisfactory FAC residuals in distribution; 2) reduced and stabilized distribution residuals from 2.7 mg/L (as Total Chlorine) at the start of the project to 0.5 mg/L (as FAC) in April 2009, and 3) since September 2007, with one exception, all DBP concentrations in distribution have been well below the MCLs. Moreover, substantial decreases in the formations of DBPs *in distribution* occurred between the summers of 2007 and 2008. The LMWTP has been able to remain in compliance with the MCL regulations through the date of this update (February 2011).

In a direct comparison of various features of the performance of chlorine gas, MIOX, and an on-site chlorine/hypochlorite generator (the ELCAT manufactured by Electrocatalytic, Inc.), both the MIOX mixed-oxidant solution and the solution from the ELCAT unit were shown to produce lower concentrations of TTHMs than chlorine; but the MIOX mixed-oxidant solution produced a higher percentage of the brominated forms of the THMs in the bromide (Br⁻)-containing raw waters compared to either chlorine or the ELCAT unit solution[96]. The production of a higher percentage of brominated forms of the THMs in waters containing bromide would be expected if oxidants stronger than chlorine – that is, ozone-like oxidants – were present in the disinfectant[97].

Stoichiometric Oxidation of Sulfides to Elemental Sulfur in Waters at Normal pH and Rapid Oxidation to Sulfate

White[98] remarks (p.492-497) that chlorine alone is effective in oxidizing sulfides, but 1) the "natural chlorine demand" of the water must be satisfied before sulfide oxidation can occur (that is, an excess of chlorine is required for the oxidation of sulfides to occur); and 2) a high pH is required for completion of the reaction. Other features of the reaction with chlorine alone include a tendency for chlorine to cause a "burnt match odor" identified with sulfites (SO_3^{2-}), a product of extended reaction of sulfides and sulfur with excess chlorine.

The performance of the mixed-oxidant solution in oxidation of sulfides in water is in sharp contrast to that of chlorine alone: 1) the "natural chlorine demand" of the water usually does not need to be satisfied *first* for sulfide oxidation to occur; indeed in many cases, particularly in the absence of NH_3-N, the sulfides can be quantitatively and stoichiometrically removed leaving no detectable sulfides and no detectable chlorine in the final treated water using a stoichiometric amount of chlorine as mixed-oxidant solution required for oxidation only to elemental sulfur; 2) a high pH is not required for the reaction to proceed to completion; and 3) there is no "burnt match odor" in sulfide-containing waters treated with mixed-oxidant solution; that is, the reaction does not proceed beyond elemental sulfur because an excess of chlorine need not be present for the reaction[99].

These features of the mixed-oxidant solution have been used for sulfide odor removal in drinking waters and control of odors in sewage mains[100]. To date it has been observed that only ammonia in very high concentrations as in anaerobic sewage and very high concentrations (about 1 g/L) of oxidant-demanding organic material can compete successfully with the sulfide-oxidizing reaction. Under these conditions, an excess of FAC as mixed-oxidant solution is required to complete the sulfide oxidation reaction.

Barrett[101] presents a different, but equally important and useful, distinguisher between mixed-oxidant solution and chlorine in the oxidation of sulfides. The water treatment plant, using sulfide-containing ground water as the raw source, had long-standing problems with burnt-match odors and loss of disinfection residual in distribution using chlorine gas alone in treatment. Use of mixed-oxidant solution in pilot eliminated the burnt-match odor soon after startup, and within 2 weeks, a stable FAC residual was observed at locations in distribution where a residual had been difficult to maintain previously, allowing the dose of mixed-oxidant solution to be reduced at the plant. When the pilot MIOX system was removed and chlorine gas substituted, the burnt-match odors returned to the treated water as soon as the ground storage tank was emptied of the mixed-oxidant solution-treated water. The poor residual maintenance in distribution problem returned over a period of a few weeks. The rapid removal of the burnt-match odor (due to SO_3^{2-}) when mixed-oxidant solution was used is believed to have been caused by rapid oxidation of the sulfides to sulfate (SO_4^{2-}) *at the treatment plant* rather than in distribution as had clearly been the case using chlorine gas. This, in turn, improved FAC residual maintenance in distribution because no further reaction between FAC and sulfides (or SO_3^{2-}) was occurring. Over a 2-week period with continued use of mixed-oxidant solution, biofilms were removed from the distribution system, eliminating that source of oxidant demand, and allowing FAC residual to be maintained throughout the distribution system.

Both features of the mixed-oxidant solution in oxidation of sulfides have been observed – rapid, selective and stoichiometric oxidation to elemental sulfur (S^o) and rapid, probably stoichiometric, oxidation to sulfate (SO_4^{2-}). The conditions under which the two different reactions occur have not been identified or quantified; however, both reactions lead to benefits over chlorine alone in the treatment process.

Water Treatment Summary

When compared with chlorine as gas or as hypochlorite, mixed-oxidant solution has been shown consistently to achieve a greater inactivation of microbial contamination at faster rates in a wider range of water (pH and temperature) conditions. Mixed-oxidant disinfection has been researched and studied for more than twenty years. Researchers at a number of universities, public health institutions, government laboratories, private testing laboratories, engineering firms, and public and private water utilities have conducted laboratory and field studies. Compared with chlorine, the results have consistently shown mixed oxidants to yield superior disinfection results and to achieve inactivation similar to chlorine dioxide.

In addition, the unusual chemical behaviors of the mixed-oxidant solution (compared to that of chlorine alone) as discussed in summary (with available references) herein, not only offer benefits over chlorine alone in the treatment of potable and industrial waters, but also leave little doubt that the mixed-oxidant solution is fundamentally *different* from chlorine or hypochlorite (bleach) alone, containing one or more oxidants, in addition to chlorine, whose identities have yet to be determined. The regulated by-products commonly associated with other strong oxidants such as ozone and chlorine dioxide are not an issue in mixed-oxidant solution-treated water.

The publications, reports, presentations and anecdotal discussions cited herein are proof that the water industry's research, operational, and regulatory community has thoroughly compared MIOX mixed-oxidant solution disinfection with chlorine and found it to be superior. The details of these reports will show a reader that MIOX mixed-oxidant solution is a more effective disinfectant. The research also compares mixed-oxidant solution and chlorine inactivation rates with easier-to-disinfect bacteria and viruses – but in all cases where the test microorganisms have been sufficiently resistant to be incompletely inactivated (thus providing a basis for discrimination), MIOX mixed-oxidant solution has been shown to be more effective than chlorine.

MIOX backs its claims of superior disinfection with peer-reviewed research and field-testing. Laboratory disinfection results have been replicated at customer sites using production model MIOX equipment. **MIOX-treated water provides rapid, thorough microbial inactivation similar to that of chlorine dioxide, but in the same process, maintains a durable chlorine residual in the distribution system, as required by the EPA, to protect treated water from recontamination.**

Conclusions

Mixed-oxidant generators manufactured by MIOX Corporation produce a disinfectant with properties that are demonstrably different from those of sodium hypochlorite. The differences are clear and well documented. As a result of these differences, MIOX mixed-oxidant solution can be used to address water quality issues that chlorine gas or hypochlorite, and indeed other oxidant disinfectants as well, simply cannot.

A BRIEF HISTORY OF THIS DOCUMENT

25 November 1997 – seven features of the mixed-oxidant solution, considered to be significantly different from a chlorine-only solution, as portrayed by the technical literature, were summarized in a 2-page document for discussion with US EPA, RREL, Cincinnati, OH.

24 April 1998 – the number of identified unique features of the mixed-oxidant solution was expanded to eight, a discussion of the results of attempts to characterize the mixed-oxidant solution chemically was added, and a marketing discussion was attached to the end by the first, and at that time President of MIOX, W. Michael Robson – Text length 5.3 pages, 38 references, total length 7.5 pages.

30 May 1998 – discussion expanded due to additional field and laboratory evidence in the areas of the microflocculation effect and biofilm removal from distribution systems – Text length 6.5 pages, 38 references, total length 9.2 pages.

22 September 1998 – the uses of the document for both succinct presentation of findings to the water treatment research community and for marketing increased rapidly. This update included expanded discussions of attempts at chemical characterization including the work of Dr. Gil Gordon, and a summary of more recent publications on *Clostridium perfringens* spores and *Cryptosporidium parvum* oocyst inactivation studies at the University of North Carolina – Text length 8.3 pages, 41 references, total length 11.2 pages.

15 January 1999 – added further laboratory and process-monitoring findings of superior microorganism inactivation in chicken processing, and observations on the rapid rate of Mn^{2+} oxidation at Greenfield, IA – Text length 8.4 pages, 43 references, total length 11.5 pages.

28 April 2000 – added further publications from Dr. Mark Sobsey's laboratory and students on *C. parvum* oocyst inactivation, further observations on microflocculation (at Clinton, MA), and superior ammonia oxidation at Fonda, IA – Text length 8.6 pages, 48 references, total length 11.9 pages.

30 January 2002 – cosmetic changes included removal of W. Michael Robson as coauthor, and severe editing of the marketing section at the end of the document, deferring this discussion to continually-updated MIOX marketing literature and MIOX system specifications. Minor additions made to the ***Chemical Analysis*** section [acknowledging the appearance of Electrolyzed Oxidizing water in the literature and further characterization work underway at CalTech and JPL]. Substantial additions were made to: ***Microorganism Inactivation*** [adding Scott Russell's findings]; ***Cryptosporidium* Inactivation** [adding George Bajszar's studies]; ***Microflocculation*** [adding the Rend Lake studies]; ***Biofilm Removal*** [adding reduction in TTHMs at Greenfield, IA and elsewhere and Don Phipps' findings with biofilm lawn removal in laboratory]; ***Ammonia Oxidation*** [adding the recent swimming pool experience]; and ***Sulfide Oxidation*** [adding Walter Barrett's experience with sulfide oxidation in ground waters in Florida] – Text length 10.5 pages, 66 references, total length 15.1 pages.

09 January 2004 -- updates to the status of research by Dr. Gil Gordon and Dr. Mitchio Okumura in the **Chemistry - *Summary*** section; added minor clarification on FAC residuals in test solutions in *Cryptosporidium parvum* oocyst inactivation studies. Major additions of anecdotal (unpublished) reports from MIOX users including: evidence from West Point, GA on increased removal of DBP precursors into the floc by the microflocculation effect; the reports from the Embassy Suites, Honolulu, HI pool and the Valdosta, GA YMCA pool on removal of black algae, and the video tape of biofilm removal from the Ainono Hot Springs, Japan piping; retitled the ***Ammonia Oxidation at Sub-Breakpoint Chlorine Doses*** section and added the latest observations and ***working hypothesis*** for mechanisms of mixed-oxidant solution removal of chlorinous odors from swimming pools; and added a note on the three

opportunities, or mechanisms, for mixed-oxidant solution reduction of DBPs in potable water -- Text length 13.2 pages, 66 references, total length 18.8 pages.

17 November 2006 – added Jeong et al. (2006) observation of O_3 in chloride-free water electrolyzed under conditions similar to those of MIOX cells; expanded the discussions of *Superior Microorganism Inactivation in Potable Water and Processes* and *Practical Effectiveness of Mixed-Oxidant Solution for* **Cryptosporidium** *Inactivation* to include papers by Son et al., Venczel et al., Sasahara et al., and Bajszar; added discussions of the microflocculation experiences in Puerto Rico, and a summary of observations on microflocculation to date to *Evidence for the Microflocculation Effect Similar to that of Ozone*; added the experience of Waterman at the Derby Hospital in the UK and Sanmiya's experience at the Tachikawa Citizen Swimming Pool in Japan to *Biofilm Removals in Distribution Systems*; and added Bradford and Dempsey's (2005) observations and those of Sanmiya (2005) on elimination of chlorinous odors from swimming pools and the recent paper by Bernard et al. on the relation between asthma and attendance at indoor swimming pools to the *Ammonia Oxidation at Sub-Breakpoint Chlorine Doses*… section – Text length 16.3 pages, 78 references; total length 22.7 pages.

26 August 2009 – added recently-developed evidence by the Weinberg group for the presence of other oxidants to *Other Laboratory Evidence for the Presence of Oxidants Other than Chlorine*; revised, with subheadings to assist the reader in following the chronology of investigations, the section *Practical* (now *Superior) Effectiveness of Mixed-Oxidant Solution for* **Cryptosporidium** *Inactivation* to include both the history of research in this area as well as the latest findings by Bajszar and Dekonenko that the mixed-oxidant solution is superior to bleach but not as effective as earlier efforts had indicated; expanded with subheadings to assist the reader in following the chronology of investigations, the section *Biofilm Removals in Distribution Systems* to include the findings of laboratory model studies at Montana State University, Center for Biofilm Engineering and from the 2-year study at the Lake Murray Water Treatment Plant in West Columbia, SC., expanded *Ammonia Oxidation at Sub-Breakpoint Chlorine Doses as Mixed-Oxidant Solution and Apparent Superior Fragmentation of Organic Nitrogen Compounds Typically Found in Human Body Fluids* to include the work of the Blatchley group on volatile chlorinated byproducts of reactions with organic nitrogen components of the BFA, the relationship of the behavior of mixed-oxidant solution in swimming pools to the work of Jafvert and Valentine, Judd and the compilations of evidence and speculation by Bradford; and included in *Reduced Concentrations of Total Trihalomethanes but Increased Production of Brominated Forms* a synopsis of the performance of the Lake Murray Water Treatment Plant in reducing DBPs in distribution using mixed-oxidant solution – Text length 19.4 pages, 98 references; total length 27.4 pages.

8 February 2011 – conducted minor editorial revisions and added to the *Other Laboratory Evidence for the Presence of Oxidants Other than Chlorine* section a discussion of historic literature on the reactions between FAC and H_2O_2 which suggest that the conventional wisdom that H_2O_2 reacts rapidly with FAC may not be correct; rather at the pH of the mixed-oxidant solution (pH~9.5) H_2O_2 and FAC may coexist for several 10s of hours. Thus, the lingering presence of H_2O_2 in the mixed-oxidant solution cannot be discounted – Text length 21.5 pages, 101 references, total length 28.8 pages.

---

[1] Reiff, F.M., 1988, "Special Report: Drinking-Water Improvement in the Americas with Mixed Oxidant Gases Generated On-Site for Disinfection (MOGGOD)", PAHO Bull., 22(4):394-414.

[2] American Public Health Association, American Water Works Association, and Water Environment Federation, 2005, *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, APHA, Washington, DC.

[3] Trumm, J., 1994, "SCOLA Characterization: An Analysis of the Products of Brine Electrolysis", Master's Thesis, Department of Civil Engineering, University of New Mexico, Albuquerque, December, 1994.

[4] Dowd, M.T., 1994, "Assessment of THM Formation with MIOX", Master's Thesis, University of North Carolina, Department of Environmental Sciences and Engineering, School of Public Health, Chapel Hill, NC.

[5] Bradford, W.L., F.A. Baker, and R.I. Cisneros, 1997, "Results of Tests Comparing the Disinfection Effectiveness of Mixed-Oxidant Solution and Sodium Hypochlorite on Simulated Cooling Water", LATA/MX-97/0027, Los Alamos Technical Associates, Los Alamos, NM, and MIOX Corporation, Albuquerque, NM, January, 1997.

[6] Schroeder, G., Cedar Knox Rural Water Project, Nebraska, "On-Site Disinfection System Provides DBP Reduction, Residual Maintenance", Waterworld, October 2005, pp. 15-16.

[7] Aieta, E.M., P.V. Roberts, and M. Hernandez, 1984, "Determination of Chlorine Dioxide, Chlorine, Chlorite, and Chlorate in Water", J. Am. Wat. Wks. Assoc., 76(1):64-74.

[8] Venczel, L.V., 1997, "Evaluation and Application of a Mixed Oxidant Disinfection System for Waterborne Disease Prevention", Ph.D. Dissertation, Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.

[9] Kouame, Y. and C.N. Haas, 1991, "Inactivation of *E. coli* by Combined Action of Free Chlorine and Monochloramine", Wat. Res., 25(9):1027-1032.

[10] Rennecker, J.L., A.M. Driedger, S.A. Rubin, and B.J. Marinas, 2000, "Synergy in Sequential Inactivation of Cryptosporidium parvum with Ozone/Free Chlorine and Ozone/Monochloramine", Wat. Res., 34(17):4121-4130.

[11] Gordon, G.L., 1998, "Electrochemical Mixed Oxidant Treatment: Chemical Detail of Electrolyzed Salt Brine Technology", prepared for the U.S. Environmental Protection Agency, National Risk Management Laboratory, Cincinnati, OH, May 1998.

[12] Bradford, W.L. and F.A. Baker, 1995, "Design, Fabrication, and Testing of a Laboratory Test Electrolytic Water Disinfection Unit (EWDU): Addendum Number 3 Results of Studies Addressing Health Hazards Assessment Report Issues with the Electrolytic Water Disinfection Unit", prepared for the U.S. Army Tank-Automotive and Armaments Command, Mobility Technology Center -- Belvoir, Los Alamos Technical Associates, Los Alamos, NM, Report Number LATA/MX-95/0022.

[13] Vanden Plas, B., 1997, "Industrial Hygiene Survey Report for Gas Generation by MIOX Water Treatment Equipment", Los Alamos Technical Associates, Inc., Los Alamos, NM, January, 1997.

[14] Bradford, W.L., 1995, "Theoretical Oxidation-Reduction Potentials for the System HOCl/OCl⁻/Cl⁻ at pH 10.0 to 13.0 and Comparison with Measurements in Catholyte from MIOX Systems", LATA/MX-95/0017, Los Alamos Technical Associates, Los Alamos, NM, May 24, 1995.

[15] Venkitanarayanan, K.S., G.O.I. Ezeike, Y-C. Hung, and M.P. Doyle, 1999, "Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Plastic Kitchen Cutting Boards by Electrolyzed Oxidizing Water", J. Food Prot., 62(8):857-860.

[16] Venkitanarayanan, K.S., G.O.I. Ezeike, Y-C. Hung, and M.P. Doyle, 1999, "Efficacy of Electrolyzed Oxidizing Water for Inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*", Appl. Environ. Microbiol., 65(9):4276-4279.

[17] Kim, C., Y-C. Hung, and R.E. Brackett, 2000, "Roles of Oxidation-Reduction Potential in Electrolyzed Oxidizing and Chemically Modified Water for the Inactivation of Food-Related Pathogens", J. Food Prot., 63(2):.

[18] Deev, A.M. and M. Okumura, 2003, "Mass Spectrometric Analysis of MIOX Headspace: January 2003 Progress Report", prepared for Dr. Wes Bradford, Los Alamos Technical Associates, California Institute of Technology, Pasadena, CA, January 2003.

[19] Jeong, J., J.Y. Kim, and J. Yoon, 2006. "The Role of Reactive Oxygen Species in the Electrochemical Inactivation of Microorganisms", Environ. Sci. Technol., 40(19):6117-6122.

[20] Jung, J.J., K.W. Baek, S.Y. Park, B.S. Oh, A. Kang, and J-W. Kang, 2006, "Water Disinfection and Control Strategy of Disinfection By-Products in Electrochemical Process", *Proceedings* of the AWWA Water Quality Technology Conference, 5-9 November 2006, Denver, CO.

[21] Weinberg, H., S. Rodriguez-Mozaz, and A. Sykes, 2008, "Characterization of the Chemical Constituents of Mixed Oxidant Disinfection", Final Project Report, presented to MIOX Corporation by the University of North Carolina, Department of Environmental Sciences and Engineering, Chapel Hill, NC, 23 July 2008.

[22] Rodriguez-Mozaz, S., H.S. Weinberg, A.M. Saenz de Jubera, and A.C. Sykes, 2008, "Novel Strategies for the Characterization of Mixed-Oxidant Solutions for Drinking Water Disinfection", poster with paper, AWWA Conference, June, 2008, Atlanta, GA.

[23] Gordon, G., W.J. Cooper, R.G. Rice, and G.E. Pacey, 1992, *Disinfection Residual Measurement Methods*, 2nd Edition, AWWA Research Foundation and American Water Works Association, Denver, CO, 889 pp.

[24] Connick, R.E., 1947, "The Interaction of Hydrogen Peroxide and Hypochlorous Acid in Acidic Solutions Containing Chloride Ion", J. Am. Chem. Soc., 69(6):1509-1514.

[25] Held, A.M., D.J. Halko, and J.K. Hurst, 1978, "Mechanisms of Chlorine Oxidation of Hydrogen Peroxide", J. Am. Chem. Soc., 100(18):5732-5740.

[26] Hurst, J.K., P.A.G. Carr, F.E. Hovis, and R.J. Richardson, 1981, "Hydrogen Peroxide Oxidation by Chlorine Compounds. Reaction Dynamics and Singlet Oxygen Formation", Inorg. Chem., 20(8):2435-2438.

[27] Espenson, J.H., 1986, "Homogeneous Inorganic Reactions", Chapter VII in *Techniques of Chemistry*, Volume 6, *Investigation of Rates and Mechanisms of Reaction*, Part 1, General Considerations and Reactions at Conventional Rates, C.F. Bernasconi (editor), John Wiley and Sons, Wiley Interscience Publications, New York, NY.

[28] Snyder, S.A., B.D. Stanford, A.N. Pisarenko, G. Gordon, and M. Asami, 2009, "Hypochlorite – A Assessment of Factors That Influence the Formation of Perchlorate and Other Contaminants", American Water Works Association, Denver, CO, 31 July 32009.

[29] Shelton, S.P. and L.L. Barton, 1992, "A Review of the Effectiveness of SCOLA [MIOX mixed-oxidant solution] in the Disinfection of Water Contaminated with Microorganisms", a report for Los Alamos Technical Associates, Inc., University of New Mexico, Albuquerque, Department of Biology, July, 1992.

[30] Barton, L.L., 1993, "Evaluation of the MIOX System for Killing Coliform Bacteria in Sewage Water -- Bacteriological Study", University of New Mexico, Albuquerque, Department of Biology, July 25, 1993.

[31] Barton, L.L., 1994, "Evaluation of the MIOX System for Killing Coliform Bacteria and T-2 Virus -- Bacteriological Study", University of New Mexico, Albuquerque, Department of Biology, March 25, 1994.

[32] Barton, L.L., 1996, "Disinfection of Simulated Cooling Tower Water", submitted to Dr. Wesley L. Bradford, MIOX Corporation. University of New Mexico, Albuquerque, Department of Biology, March 4, 1996.

[33] Bradford, W.L., 1993, "Design, Fabrication, and Testing of a Laboratory Test Electrolytic Water Disinfection Unit (LTEWDU)", Prepared for US Army Belvoir RD&E Center, Los Alamos Technical Associates, Inc., Los Alamos, NM, LATA/MX-93/0003.

[34] Bradford, W.L. and F.A. Baker, 1994, "Design, Fabrication, and Testing of a Laboratory Test Electrolytic Water Disinfection Unit (EWDU): Addendum No. 1 Results of Tests Conducted at Joint Task Force - Bravo (JTF-B) and for Integration with the Reverse Osmosis Water Purification Unit (ROWPU)", Prepared for US Army Belvoir RD&E Center, Los Alamos Technical Associates, Inc., Los Alamos, NM, LATA/MX-94/0008

[35] Bradford, W.L. and F.A. Baker, 1994, "Design, Fabrication, and Testing of a Laboratory Test Electrolytic Water Disinfection Unit (EWDU): Addendum No. 2 Results of Reinfection Studies", Prepared for US Army Belvoir RD&E Center, Los Alamos Technical Associates, Inc., Los Alamos, NM, LATA/MX-94/0009

[36] Waldroup, Amy, 1997, "Results of Initial Laboratory Studies Comparing MIOX Mixed-Oxidant Solution with Hypochlorite in Disinfection of a Poultry Processing Line", presented at Poultry Industry Conference, University of Arkansas, Fayetteville, January 27, 1997.

[37] Nolen, G.M., 1999, "Use of MIOX Onsite Disinfect (sic) Generators in Food Processing Industries", a presentation summarizing results of numerous studies, received November 4, 1999.

[38] Russell, S.M., 2001, "A Comparison of MIOX versus Bleach for Eliminating Pathogenic Bacteria Associated with Poultry Products", Department of Poultry Science, University of Georgia, Athens, June 1, 2001.

[39] Son, H., M. Cho, H. Chung, S. Choi, and J. Yoon, 2004, "Bactericidal Activity of Mixed Oxidants: Comparison with Free Chlorine", J. Ind. Eng. Chem., 14(5):705-709.

[40] Son, H., M. Cho, J. Kim, H. Chung, B. Oh, and J. Yoon, 2005, "Enhanced Disinfection Efficiency of Mechanically Mixed Oxidants with Free Chlorine", Wat. Res., 39(4):721-727.

[41] Venczel, L.V., C.A. Likirdopulos, C.E. Robinson, and M.D. Sobsey, 2004, "Inactivation of Enteric Microbes in Water by Electro-Chemical Oxidant from Brine (NaCl) and Free Chlorine", Wat. Sci. Technol., 50(1):141-146.

[42] Dekonenko, A. and G. Bajszar, 2009, "Disinfection of Feline Calicivirus (a Human Norovirus Surrogate) on Surfaces with Chlorine-Based Mixed Oxidant Solution", manuscript in preparation 1 September 2009.

[43] U.S. Environmental Protection Agency, 1998, "Environmental Protection Agency; Announcement of Small System Compliance Technology Lists for Existing National Primary Drinking Water Regulations and Findings Concerning Variance Technologies", Federal Register, 63(151):42032-42048, Thursday, August 6, 1998.

[44] Yozwiak, M.L., W.L. Bradford, F.A. Baker, S.D. Olfers, M.M. Marshall and C.R. Sterling, 1994, "Effect of a Mixed-Oxidant Solution on *Cryptosporidium parvum* (oocyst) viability", Poster Session, *Proceedings* of the 47th Annual Meeting of the Society of Protozoologists, June 24-29, 1994, Cleveland, OH.

[45] Venczel, L.V., M. Arrowood, M. Hurd, and M.D. Sobsey, 1997, "Inactivation of *Cryptosporidium parvum* Oocysts and *Clostridium perfringens* Spores by a Mixed-Oxidant Disinfectant and by Free Chlorine", Appl. Environ. Microbiol., 63(4):1598-1601.

[46] Sobsey, M.D., M.J. Casteel, H. Chung, G. Lovelace, O.D. Simmons III, and J.S. Meschke, 1998, "Innovative Technologies for Waste Water Disinfection and Pathogen Detection", pages 483-493 in *Proceedings of Disinfection '98, The Latest Trends in Wastewater Disinfection: Chlorination versus UV Disinfection, Water Environment Federation Technical Exhibit and Conference, Baltimore, MD, April 19-22, 1998*, WEF, Alexandria, VA.

[47] Casteel, M.J. M.D. Sobsey, and M.J. Arrowood, 1999, "Inactivation of *Cryptosporidium parvum* Oocysts in Water and Wastewater by Electrochemically Generated Mixed Oxidants", presented at the International Conference on Minimizing the Risk from *Cryptosporidium* and Other Waterbourne Particles, Paris, France, April 19-23, 1999, International Association on Water Quality, International Water Services Association, and the International Ozone Association. Published at Wat. Sci. Technol., 41(7):127-134, 2000.

[48] Sobsey, M.D., M.J. Casteel, J. Kase, and M. Andersen, 1999, "Inactivation of *Cryptosporidium parvum* and Other Test Microbes in Water by MIOX Mixed Oxidants Technologies", Progress Report Number 1 under the DARPA Contract, prepared for MIOX Corporation, Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, July, 1999.

[49] Sobsey, M.D., J.A. Kase, M.E. Anderson, M.J. Casteel, C. Likirdopulos, and E. Sickbert-Bennett, 2000, "Inactivation of *Cryptosporidium parvum* Oocysts and Other Waterborne Microbes by Oxidants Generated Electrochemically from Sodium Chloride from Portable Pen and Bench Scale Systems", in *Proceedings of the 2000 American Water Works Association Water Quality Technology Conference*, November 6-9, 2000, Salt Lake City, UT.

[50] Bajszar, G., Burbidge, T., and M. Bajszar, 2001, "Mixed Oxidant Pen Characterization via *Cryptosporidium* Inactivation Utilizing Quantitative PCR Techniques -- Report #1: Pen Cell Geometry, Voltage Tests and *Cryptsporidium* oocyst Inactivation Assays", Report to MIOX Corporation, Albuquerque, NM, M3C Systems, Colorado Springs, CO, January 2, 2001.

[51] Chauret, C.P., C.Z. Radziminski, M. Lepuil, R. Creason, and R.C. Andrews, 2001, "Chlorine Dioxide Inactivation of *Cryptosporidium parvum* Oocysts and Bacterial Spore Indicators", Appl. Environ. Microbiol., 67(7):2993-3001.

[52] Sasahara, T., M. Aoki, T. Sekiguchi, A. Takahashi, Y. Satoh, H. Kitasato, and M. Inoue, 2003, "Effect of the Mixed-Oxidant Solution on Infectivity of *Cryptosporidium parvum* Oocysts in a Neonatal Mouse Model", J. Japanese Assoc. Infectious Diseases, 77(2):82-.

[53] Bajszar, G. and A. Dekonenko, 2010, "Stress-Induced Hsp70 Gene Expression and Inactivation of *Cryptosporidium parvum* Oocysts by Chlorine-Based Oxidants", Appl. Environ. Microbiol. 76(6):1732-1739.

[54] Bradford, W.L., 2004, "SUMMARY – *Cryptosporidium parvum* oocyst Inactivation Studies Using the MSR MIOX Purifier (aka Pen)", performed by BioVir Laboratories, Inc., Benicia, CA directed by Dr. Rick Danielson, reported 18 April 2003, Summary Prepared by Wesley L. Bradford, PhD, LATA/MIOX, Los Alamos, NM, 1 April 2004

[55] Bajszar, G., 2006b, "Summary Report: Analysis of Disinfection Efficacy of Mixed Oxidant Solutions Produced in MIOX Pen Units with Different Anode Coatings", M3C Systems, Albuquerque, NM, 30 August 2006.

[56] Bajszar, G., 2006d, "Report: Analysis of Disinfection Efficacy of Mixed Oxidant Solutions Produced in MIOX Cap Units with Different Electronic Control Boards for DC Output", M3C Systems, Albuquerque, NM, 2 November 2006.

[57] Keegan, A.R., 2003, "Cell Culture-Taqman PCR Assay for Evaluation of *Cryptosporidium parvum* Disinfection", Appl. Environ. Microbiol., 69(5):2505-2511.

[58] Bradford, W.L., 1996, "Notes on the Chemistry, Microbiology, and Removal of Iron and Manganese in Water Supplies and Enhanced Coagulation by Mixed-Oxidant Solution", LATA/MX-96/0025, Los Alamos Technical Associates, Los Alamos, NM, September 25, 1996, revised and reissued May 17, 1997 and August 29, 1997.

[59] Herrington, R.E. and W.L. Bradford, 1997, "Pilot Study Report Mixed-Oxidant Disinfection System at Greenfield, Iowa", poster session, Water Technology Conference, American Water Works Association, Denver, Co., November 9-13, 1997.

[60] Faust, S.D. and O.M. Aly, 1998, *Chemistry of Water Treatment*, 2nd Edition, Ann Arbor Press, Chelsea, MI, 581 pp.

[61] Becker, W.C. and C.R. O'Melia, 1996, "Optimizing Ozonation for Turbidity and Organics (TOC) Removal by Coagulation and Filtration", American Water Works Research Foundation, Denver, CO, 209 pp.

[62] Hodges, W.E. Jr., H.A. Mora, G.F. Stolarik, C.H. Tate, and R.R. Trussell, 1979, "Effect of Ozonation and Chlorination Pretreatment", Presented at the ASCE National Conference on Environmental Engineering, San Francisco, CA, July 9-11, 1979.

[63] Jekel, M.R., 1983, "The Benefits of Ozone Treatment Prior to Flocculation Processes", Ozone Science and Engineering, 5(1):21-35

[64] Jekel, M.R. and U.F. Reicherter, 1987, "Ergebnisse des Essener Verfahrens zur Ruhrwasseraufbereitung", Vom Wasser, 69:165-175.

[65] Richard, Y., 1993, "Use of Ozone and Flotation for the Treatment of a Reservoir Water: The Dinan Case History", Ozone Science and Engineering, 15(?):465-480.

[66] Richard, Y., L. Brener, P. Thebault, and A. LePrince, 1983, "Influence of Preozonation on Clarification by Flotation for Drinking Water Treatment", Ozone Science and Engineering, 5(1):3-20.

[67] Dufresne-Henry, 1999, "Report on Winter Session Water Treatment Pilot Study at North Dike Pumping Station, Wachusett Reservoir", Clinton Department of Public Works, Clinton, Massachusetts, July, 1999.

[68] Herrington, R.E., W.L. Bradford, and M. Hamman, 1999, "Performance of a Conventional Surface Water Plant Using Mixed Oxidants for Microflocculation and Final Disinfection", presented at the American Water Works Association (AWWA) 1999 Annual Conference, Chicago, IL, June 20-24, 1999.

[69] Lawrence A. Lipe and Associates, 2001, "Rend Lake Intercity Water System Claricone and MIOX Pilot Study Results, Fall 2000", Benton, IL, 8 June 2001.

[70] Ghadimkhani, A.A., A. Torabian, and A.R. Mehrabadi, 2006, "Preozonation and Prechlorination Effect on TOC Removal in Surface Water Treatment", Pakistan J. Biol. Sci., 9(4):708-712.

[71] Crayton, C., B. Warwood, and A. Camper, 1997, "Final Report on the Validation of Mixed-Oxidants for the Disinfection and Removal of Biofilms from Distribution Systems", Montana Water Resources Center, MSU, Bozeman, MT, October, 1997.

[72] Crayton, C., A. Camper, and B. Warwood, 1997, "Evaluation of Mixed-Oxidants for the Disinfection and Removal of Biofilms from Distribution Systems", presented at the Water Quality Technology Conference, American Water Works Association Meeting, November 9-12, 1997, Denver, CO.

[73] Petersen, P. and W.L. Bradford, 2000, "Cooling Water: Potential Benefits from Mixed-Oxidant Application in Cooling Tower Maintenance", UltraPure Water, 17(5):53-61, May/June, 2000.

[74] Rossman, L.A., R.A. Brown, P.C. Singer, and J.R. Nuckols, 2001, "DBP Formation Kinetics in a Simulated Distribution System", Wat. Res., 35(14):3483-3489.

[75] Phipps, D., Jr. and G. Rodriguez, 2001, "Comparison of the Efficiency of Bacterial Removal and Reduction of Bacterial Viability by Mixed Oxidant Solution (MOS) and Chlorine (as NaOCl) on a Reverse Osmosis Membrane", Water Resources and Technology Department, The Orange County Water district, Fountain Valley, CA, June 2001.

[76] Donlan, R.M. and J.W. Costerton, 2002, "Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms", Clinical Microbiology Reviews, 15(2):167-193.

[77] Sanmiya, H., 2005, ""Improvement of Water Quality of Tachikawa Citizen Swimming Pool — Surprising Effectiveness and Performance of Mixed Oxidant Generator Made in USA", Heating Piping and Air Conditioning (Japanese), November, 2005.

[78] Walker, D., 2008, "Final Report of Phase II Biofilm Removal Experiments", MSU/CBE, Bozeman, MT, 13 June 2008.

[79] Bradford, W.L., 2008, "FINAL REPORT – On-Site Generation", Department of Homeland Security, Small Business Innovative Research, Phase II, Contract Number: NBCHC060059 – Period of Performance 4/27/06 – 7/23/08, MIOX Corporation, Albuquerque, NM, 6 August 2008.

[80] Bradford, W.L., 2009, "IMPORTANT PROJECTS IN MIOX APPLICATIONS – West Columbia, SC: Synopses of Issues, Significance to MIOX, and Status — With Updates", Update #5 – 5 June 2009, MIOX Corporation, Albuquerque, NM.

[81] Li, J. and E.R. Blatchley, III, 2007, "Volatile Disinfection Byproduct Formation Resulting from Chlorination of Organic–Nitrogen Precursors in Swimming Pools", Environ. Sci. Technol., 41(19):6732-6739.

[82] Bradford, W.L. and R.I. Cisneros, 1995, "Oxidation of Ammonia in Aqueous Solutions Using the MIOX Mixed-Oxidant Ammonia Solutions Using the MIOX Mixed-Oxidant Solution at Chlorine Concentrations Near Stoichiometric Equivalency with Ammonia Nitrogen", Los Alamos Technical Associates, Inc., Los Alamos, NM, LATA/MX-95/0015

[83] Daniel, E., 1995, "Pilot Study/Engineering Report for 'MIOX On-Sight [sic] Mixed Oxidant Generator for Cash Water Supply Corporation'", Cash Water Supply Corporation, Greenville, TX, July, 1995.

[84] Bradford, W.L., 1996, "A Primer on Ammonia Reactions with Chlorine in Solution; With Appendices of Notes on Evidence for Ammonia Oxidation by Mixed-Oxidant Solution, Terminal Analyses of Test Solutions, and Quantifiable Sources of Oxidant Demand in Waters", Los Alamos Technical Associates, Los Alamos, NM, August 20, 1996.

[85] Kuehl, N.R., 2000, "Mixed Oxidant Ammonia Removal Project", presented at the 25th Annual Conference of the Iowa Rural Water Association, February, 2000; Kuehl & Payer, Ltd., Storm Lake, IA.

[86] Bradford, W.L., 2000, "Some Benefits of the Use of Mixed Oxidants in Swimming Pools: Speculation on Chemical Mechanisms for Lack of Swimmer's Complaints Using Mixed-Oxidant Solution", Los Alamos Technical Associates, Inc., Los Alamos, NM, 23 May 2000; updated 2 January 2004.

[87] Hery, M. and G. Hecht, 1998, "Occupational Exposure to Chloramines in Swimming Pools and Vegetable Processing Plants", presented at the *2nd International Conference on Pool Water Quality and Treatment*, March 4, 1998, Cranfield University, Bedfordshire, UK.

[88] Shang, C., W-L. Gong, and E.R. Blatchley III, 2000, "Breakpoint Chemistry and Volatile Byproduct Formation Resulting from Chlorination of Model Organic-N Compounds", Environ. Sci. Technol., 34(9):1721-1728.

[89] Bernard, A., S. Carbonnelle, O. Michel, S. Higuet, S. C. de Burbure, J-P. Buchet, C. Hermans, X. Dumont, and I. Doyle, 2003, :Lung Hyperpermeability and Asthma Prevalence in Schoolchildren: Unexpected Associations with the Attendance at Indoor Chlorinated Swimming Pools", Occupational and Environmental Medicine, 60:385-394.

[90] Weaver, W.A., J. Li, Y. Wen, J. Johnston, M.R. Blatchley, and E.R. Blatchley, III, 2009, "Volatile Disinfection By-Product Analysis from Chlorinated Indoor Swimming Pools", Wat. Res., 43(13):3308-3318.

[91] Bradford, W.L. and R. Dempsey, 2005, "Observations on the Use of Mixed Oxidant in Swimming Pools: Mechanisms for Lack of Swimmer's Complaints in the Presence of a Persistent Combined Chlorine Measurement", Los Alamos Technical Associates, Inc., Los Alamos, NM, and Simply Water, LLC, Houston, TX, 17 June 2005.

[92] Judd, S.J. and G. Bullock, 2003, "The Fate of Chlorine and Organic Materials in Swimming Pools", Chemosphere, 51:869-879. [also presented at the Aquatic Health Conference, 2-4 October 2004, Atlanta, GA]

[93] Javfert, C.T. and R.L. Valentine, 1992, "Reaction Scheme for the Chlorination of Ammoniacal Water", Environ. Sci. Technol., 26(3):577-586

[94] Bradford, W.L. 2008, "IMPORTANT PROJECTS IN MIOX APPLICATIONS – Australian Pools: Synopses of Issues, Significance to MIOX, and Status — With Monthly Updates", MIOX Corporation, Albuquerque, NM, Initial Compendium 25 April 2008

[95] Hamm, B., 2002, "DBP Reduction Using Mixed Oxidants Generated On Site", Field Report, J. Am. Wat. Wks. Assoc., 94(11):49-53.

[96] Burns & McDonnell, 1998, "On-Site Electrolytic Generation of Disinfectants", a report to the Electric Power Research Institute (EPRI), Burns & McDonnell Project No. 971464-EPRICL2, Burns & McDonnell, St. Louis, MO, February 1998.

[97] Amy, G.L., M.S. Siddiqui, K. Ozekin, H.W. Zhu, and C. Wang, 1997, "Empirically Based Models for Predicting Chlorination and Ozonation By-Products: Haloacetic Acids, Chloral Hydrate, and Bromate", EPA Report CX 819579.

[98] White, G.C., 1992, *The Handbook of Chlorination and Alternative Disinfectants*, Third Edition, Van Nostrand Reinhold, New York, NY, 1308 pp.

[99] Bradford, W.L., R.I. Cisneros, and F.A. Baker, 1995, "Mixed-Oxidant Solution Reactions with Gas Phase Hydrogen Sulfide (H_2S): Interim Report", Los Alamos Technical Associates, Los Alamos, NM and MIOX Corporation, Albuquerque, NM, LATA/MX-95/0016

[100] Reyes, S., 1995, "Use of Mixed Oxidants to Control Sewage Odors and Concrete Pipe Corrosion in the Albuquerque Wastewater Collection System", LATA/MX-95/0023, MIOX Corporation, Albuquerque, NM, August 8, 1995.

[101] Barrett, W., 2001, "Town of Green Cove Springs, Florida Harbor Road WTP: MIOX Disinfection Pilot Project", letter report, Barrett Supply Inc., Jacksonville, FL, 14 March 2001.