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BIOFILM REMOVAL

Biofilm is a substance that forms readily in water distribution lines, water storage tanks, and any other aqueous environment. A biofilm forms when bacteria begin to excrete a slimy, sticky substance that allows them to adhere to surfaces. The biofilm mass usually consists of many species of bacteria, and can also include fungi, algae, and protozoa. Biofilm is resistant to chlorine and is difficult to remove once initial adhesion occurs.[1] The biofilm slime shelters disease-causing microorganisms, protecting them from chlorine disinfection. In addition, biofilm exerts an oxidant demand, consuming chlorine residuals in the distribution line and requiring higher doses at the treatment station for residual maintenance at the end of the line.

A safe, user-friendly and viable method for controlling biofilms would have a significant impact on any industry that utilizes treated purified water, including municipal drinking water, cooling towers, and swimming pools. A mixed-oxidant solution generated on-site through the use of salt, water, and an electrolytic cell has clearly exhibited the ability to remove biofilm, unlike traditional chlorination technologies. The evidence includes third party research, visual documentation from municipal and industrial operators, and a number of improvements in water quality that are understood to result from removal of biofilms. MIOX Corporation, an equipment supplier producing on-site mixed-oxidant generators, has proposed to work with the Montana State University's Center for Biofilm Engineering to conduct additional laboratory studies in the future.

Independent Research

Initial observations of biofilm removal were reported by Montana State University during a field study conducted at a KOA Kampground in Great Falls, Montana.[2] The campsite has a small potable water supply, as well as showers and a swimming pool. The site previously used powdered sodium hypochlorite and experienced frequent positive coliform hits, even with free chlorine slug dosage levels as high as 1000 ppm. The positive hits in the presence of free chlorine were indicative of biofilm contamination, also evidenced by a black biofilm slime in the showers. The distribution system contained accumulated biofouling that had to be flushed from the system whenever there was a power outage. The existing cartridge filters also had to be cleaned every 2-3 days due to the heavy accumulation of biofilm.

After conversion to mixed oxidants, the KOA Kampground has not experienced a single non-compliance coliform event. The black slime in the showers disappeared soon after the conversion. Although biofilm initially sloughed visibly from the pipelines, the water eventually ran clear, and currently the filters only have to be cleaned every 3-4 weeks. Whenever power outages occur now, no flushing is required and there is no discoloration of the water, indicating that the biofilm does not start to regrow, even when disinfection is temporarily interrupted.

The swimming pool was opened at the same time that the MIOX system was installed, so there is no comparative data between operations with sodium hypochlorite versus mixed oxidants.

However, there has been no growth of algae on pool surfaces, a common problem for outdoor pools, and no algaecide is necessary, not even on the unpainted concrete areas. The pool pipes, connections, and filters remain visibly clean, and no slimy biofilm is apparent when touching the surfaces. (In contrast, the potable water distribution system components rapidly coated with a slimy substance with use of sodium hypochlorite.)

The mixed-oxidant system was responsible for elimination of biofouling at the Great Falls KOA Kampground, ultimately resulting in safer drinking water with no bacterial contamination, ease of maintenance with no flushing required, and greatly extended filter runs.

Hot Springs and Swimming Pool Facilities

Hot springs are very popular in Japan, with over 30,000 facilities across the country. The warm aqueous environment provides an ideal breeding ground for bacteria, and a number of the sites suffer from positive coliform hits and biofouling. Inono Hot Springs in Akita, Japan, was no exception.[3] Although dosing at 1.5 mg/L of free available chlorine (FAC) with sodium hypochlorite, the site was often unable to maintain even a 0.2 mg/L residual, and experienced positive coliform and *Legionella* hits. The decision was made to evaluate an on-site mixed-oxidant generator to replace their existing sodium hypochlorite disinfection. Before removing the sodium hypochlorite system, the MIOX distributor in Japan filmed the interior of the feed water pipe and the filter recirculation pipe and recorded a heavy, filamentous mat of biogrowth in both locations. The interior of the pipes was subsequently videotaped at six and 22 days after treatment with mixed oxidants began.

Upon conversion to mixed oxidants, sloughing was immediately apparent as particles of biofilm began to appear on the pool surface. Operators manually removed the biofilm and within one week of operation, the pool water was clear.

INONO HOT SPRINGS

Biofilm removal started within hours after MIOX system start-up

After 6 days, the pool was clean

Even more impressive was the videotape showing biofilm removal in the 4-inch pipelines. In the feed water pipe, substantial removal was evident after 6 days, and during the last videotaping at

22 days, removal of biofilms was total. The same phenomenon occurred in the filter recirculation pipe with the entire filamentous mass completely eliminated.

FEED WATER PIPE

Feed water pipe with sodium hypochlorite

After 6 days with mixed oxidants

After 22 days with mixed oxidants

FILTER RECIRCULATION PIPE

With sodium hypochlorite

With mixed oxidants

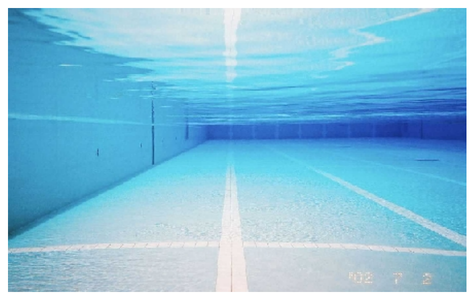
After conversion to mixed oxidants and removal of biofilm, the chlorine dosage was reduced by 60% to only 0.6 mg/L, while the residual more than doubled to 0.4 mg/L. Positive bacterial hits were eliminated, indicating a healthier recreational environment for the swimmer.

Other hot springs in Japan using MIOX equipment have experienced similar improvements. For example, the Hyuga Sun Park Hot Springs in Miyazaki, Japan, which utilized sodium hypochlorite for disinfection, had such a severe *Legionella* outbreak during their first month of operation that 9 customers became infected and lost their lives. The facility was shut down for over a year to research alternatives after 1.5 million CFU/cm^2 of *Legionella* was detected in the pipelines. In October of 2003, the hot springs opened again with newly installed MIOX equipment. As part of the authorization to re-open the facility, daily *Legionella* counts are required. Since opening in October of 2003, there have been no positive counts for coliforms or *Legionella*. The absence of biofilm allows no protective covering for dangerous bacteria.

Installations at Shibasaki Gymnasium in Tachikawa City and Miwa Town in the Aichi Prefecture of Japan, both initially using sodium hypochlorite disinfection, reported drastically improved clarity of the water after only 4-5 days of operation with MIOX mixed oxidants. Improved water clarity indicates a removal of biofilm contamination and/or algae growth responsible for the cloudiness in the water.

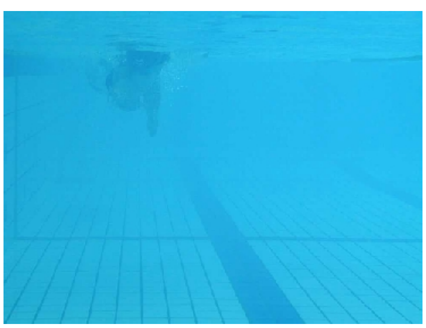
MIWA TOWN

With hypochlorite

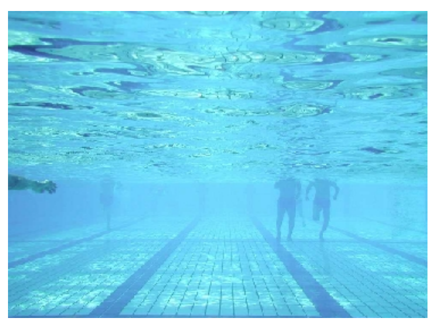


With mixed oxidants

TACHIKAWA CITY



With hypochlorite



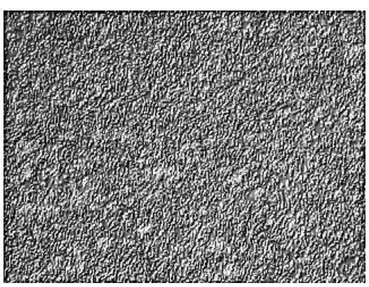
With mixed oxidants

Another interesting incident validated the effectiveness of mixed oxidants at a hotel in Hawaii. Mixed-oxidant generators at the Embassy Suites swimming pool in Honolulu were converted in 2002 to generation of sodium hypochlorite. Although the FAC residual in the treated water did not change, black and brown algae deposits formed on the sides and bottom of the pool after only a few days. The operator converted one of his sodium hypochlorite generators back to its original mixed-oxidant configuration, and the algae disappeared within a few days.

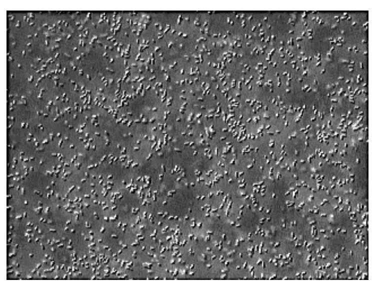
Another pool operator at the YMCA in Valdosta, Georgia reported similar results. Mixed oxidants eliminated a black algae deposit underneath a water slide that was not removed with hypochlorite disinfection.

<u>Membrane Filtration</u>
At the Orange County Water District in California, Dr. Don Phipps, Chemist and Microbiologist, studied the effects of mixed oxidants and of sodium hypochlorite on the removal of *Psuedomonas putida* biofilms on cellulose acetate RO membranes.[4] He reported a higher initial oxidant demand of 17 mg/L with mixed oxidants versus only 9 mg/L with chlorine; however, mixed oxidants destroyed the polysaccharide biofilm substrate, while chlorine had no effect. Many biofilms deposit an extra-cellular substrate upon attachment to a surface, which expedites further attachments and protects the organisms from disinfectants. After removal of the oxidant-demanding substrate, FAC requirements with mixed oxidants would likely decrease, and the surface would remain clean as long as mixed oxidants were periodically applied.

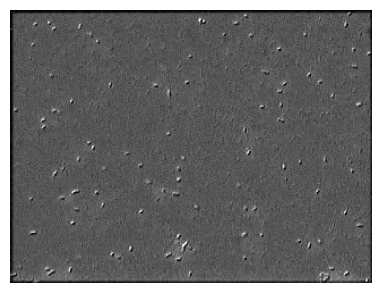
DAPI staining showed no intact DNA remaining on the mixed-oxidant samples. Phipps was impressed with the thoroughness of kill that occurred with mixed oxidants, the removal of the substrate itself, and the speed with which these events occurred once the proper dosage was reached. Black and white pictures of the microscopic activity from his preliminary studies are reproduced below. The first view shows unchecked biofilm growth at 35 minutes. Removal of biofilms began suddenly at 4 hours and 40 minutes and was well underway at the 5-hour mark. At 5 hours and 40 minutes, 1 hour after removal began, the biofilm was basically eliminated.



Biofilm Removal at 35 min



Biofilm removal at 5 hrs



Biofilm removal at 5 $^{2}/_{3}$ hrs

<u>Cooling Towers</u>
Rapid removal of biofilms and maintenance of a clean system has been observed in industrial applications as well. Paul Petersen and Dr. Wes Bradford[5] conducted a study of mixed oxidants versus the organic biocide hydantoin on Gen-Probe cooling towers in California. Within a few days of replacing the hydantoin biocide with MIOX disinfection, biofilm scales on the cooling surfaces were eliminated. The scales did not reappear with continued use of mixed oxidants. In addition, the occurrence of detectable planktonic aerobic bacteria in the cooling water basin was

below detection (< 1000/mL). Elimination of biofilms and aerobic bacteria to this extent in cooling towers is considered unusual.

In an unrelated study, Charles Smith of Quality Water Systems installed a small MIOX system on a bakery cooling tower for a pilot test.[6] Upon start of the test, the clear pipelines were completely obscured by a "leathery" black slime. Previous disinfection with hypochlorite had minimal impact on the biofilm contamination, and the pipes required cleaning within 10 days after disinfection was stopped. In contrast, mixed oxidants thoroughly broke down the biofilm. When the MIOX pilot system was removed, the bakery owners reported that it took 3 weeks for the biogrowth to reform, and even at that point, the "leathery" texture was not apparent, so cleaning was unnecessary. Mixed oxidants removed even the tough substrate that allows more rapid regrowth of biofilm.

Black biofilm coating before application of mixed oxidants

Breakdown of biofilm begins with mixed oxidants

Tests conducted at Cadbury Trebor Bassett in the United Kingdom[7] showed significant activity against biofilm bacteria with the start of MIOX equipment operations and removal of biofilms that were prevalent with earlier use of biocides. Controlling microbiological growth, including biofilms on cooling surfaces, is one of the major challenges in cooling tower maintenance. Of particular concern is *Legionella*, which tends to be present in biofilm masses, and can be difficult to eradicate by conventional means. In contrast to disinfection with biocides, no *Legionellae* species were present on any coupon test throughout the 1-year mixed-oxidant trial, representing a 99% reduction from previously observed biofilm formation. Mixed oxidants not only eliminated all existing biofilm but also prevented the re-growth of new biofilm. Removal of biofilms not only is useful for control of *Legionella*, but also enables more efficient cooling tower operations and eliminates under deposit corrosion by iron- and sulphate-reducing bacteria.

<u>Municipal Installations</u>

Diana Water Supply in Texas has a number of well sites and has been gradually converting from chlorine gas disinfection to MIOX mixed-oxidant generation, beginning in 1996. Several years ago, two line breaks occurred around the same time, one on a line treated with chlorine gas and the other on a line treated with mixed oxidants. Both pipelines had been in use for more than ten years, and both were originally on chlorine gas. The conversion to mixed oxidants on the second line had taken place about one year prior to the line break. The chlorine treated line was only 200 feet from the disinfection station, yet it was coated with a brown slimy substance. In contrast, the mixed-oxidant treated line was half a mile from the disinfection station, but the line was completely clean.

Biofilm contamination on chlorine-treated line (left) versus mixed-oxidant line (right) at Diana Water Supply

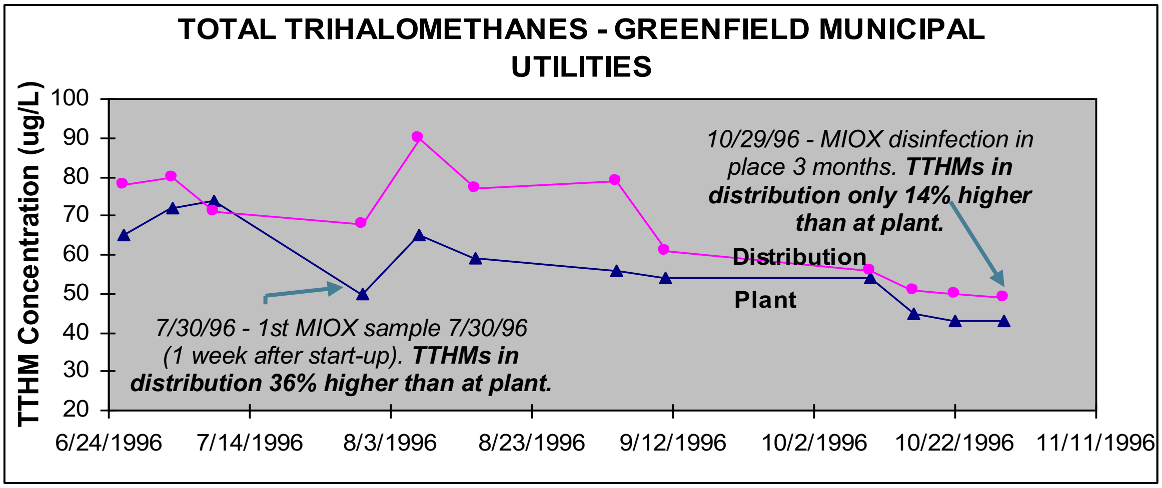
Visual removal of biomaterial has been evident at a number of other municipal sites. A pilot study of a CBI Claricone™ unit and a MIOX SAL-80 system was conducted at a midwestern water system. The MIOX solution was applied in concentrations sufficient to meet the chlorine demand of the raw water. Resulting TTHMs were only 3 ppb and algae growth in the clarifier was visibly impacted. Sloughing occurred on tube settlers with 8 hours, and within a week, solids on the tube settlers turned brown and dropped off.[8]

The Village of Hazlet in Saskatchewan converted from sodium hypochlorite bleach to mixed oxidants at their well site. Within one month of operation, the black film on the cistern ladder and walls was virtually gone.[9]

Thibodaux, Louisiana converted from chlorine gas to mixed oxidants. With the use of chlorine gas, they had to manually scrub their filter walls to remove a black deposit from iron and manganese.[10] Even with vigorous effort, the deposits were very difficult to remove. After conversion to mixed oxidants, the black film would rinse off with a hose with no scrubbing required. It is likely that the black deposits were held in place by a biofilm substrate that was subsequently destroyed with mixed oxidants, expediting the removal of the iron and manganese deposition.

The Village of Swain's Lake, New Hampshire utilized mixed oxidants for a pilot study during the peak of a severe algae bloom.[11] With use of chlorine gas, they had to backwash their filters every 6 hours on average. With mixed oxidants, the filter backwash interval was extended to 37.6, showing dramatic control of algae contamination.

Greenfield Municipal Utilities in Iowa monitored total trihalomethanes (TTHMs), by-products of chlorine disinfection applied to water containing organic material.[12] TTHMs in the distribution system are typically higher than at the plant, due to organic material (biofilm) in the lines that continues to react with chlorine in the water stream. While Greenfield's TTHM levels both at the plant and in the distribution system were lowered with mixed-oxidant treatment, the results were more drastic in the distribution system. At the beginning of the monitoring period in late July, TTHMs were 36% higher in distribution than at the plant. At the end of the 3-month study, TTHMs were only 14% higher in distribution than at the plant, indicating removal of organic material from the pipelines.



Conclusion

One of the common concerns of municipal customers removing biogrowth is the possibility of excessive flushing that may cause customer complaints or difficulties. Although this is a possibility, it is important to note that very few customers experience this dilemma. Should excessive sloughing occur, the recommended remedy is to flush the water lines for the first several weeks of operation. The distribution system typically stabilizes after a few weeks, and after the biofilm is removed, water quality will improve, disinfection by-products will be lowered, and required dose rates will be reduced.

Although few peer-reviewed publications have been produced on removal of biofilms using MIOX mixed oxidants, the anecdotal evidence is compelling. Biofilm experts in the industry, including Montana State University, continue to investigate the MIOX technology. Future studies are planned with these organizations as the importance of removing biofilm becomes an increasing priority in municipal, commercial, and industrial applications.

[1] Montana State University, The Center for Biofilm Engineering web page. “What is biofilm?” http://www.erc.montana.edu/CBEssentials-SW/bf-basics-99/bbasics-01.htm

[2] Crayton, Cyndi, et al. Montana Water Resources Center, Montana State University. *Final Report on the Validation of Mixed Oxidants for the Disinfection and Removal of Biofilms from Distribution Systems.* October 1997.

[3] NSP Co., Ltd. Okinawa, Japan. Videotaping, photography, and customer interviews.

[4] Phipps, Don Jr. and Grisel Rodriguez, Water Resources and Technology Department, Orange County Water District, California. *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.* June 2001.

[5] 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.

[6] Interview with Charles Smith, Quality Water Systems. April 2004.

[7] Lewis, J.A., et al. Waterman Environmental Services. *An Evaluation of the Efficacy of the Mixed-Oxidant Solutions Produced from “Activated Water” in Cooling Tower Biological Control.* December 24, 2001.

[8] Gould, Brad, Lawrence A. Lipe & Associates. *Midwestern Water System Claricone and MIOX Pilot Study Results*. Fall 2000.

[9] Interview with Larry Sletten, Operator for the Village of Hazlet, Saskatchewan, Canada. April 20, 2000.

[10] Conversation with Joe Van Marcke, former operator of the MIOX equipment. May 2002.

[11] Skip Wolfe, Kinetico Incorporated. *Pressure Filtration System Pilot Plant Study Final Report.* Swain’s Lake Village Water District, Barrington, NH. October 31, 1995.

[12] Duben, Mark. Howard R. Green Company Consulting Engineers. *Pilot Study Final Report: Mixed-Oxidant Disinfection System at Greenfield, IA.* December 5, 1996.