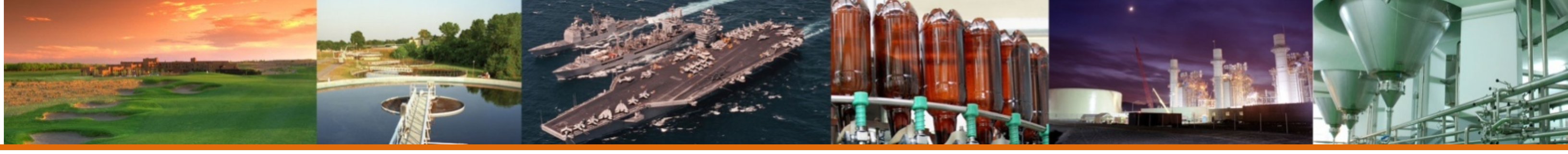

Biofilm Control Strategies in Beverage and Dairy Processing

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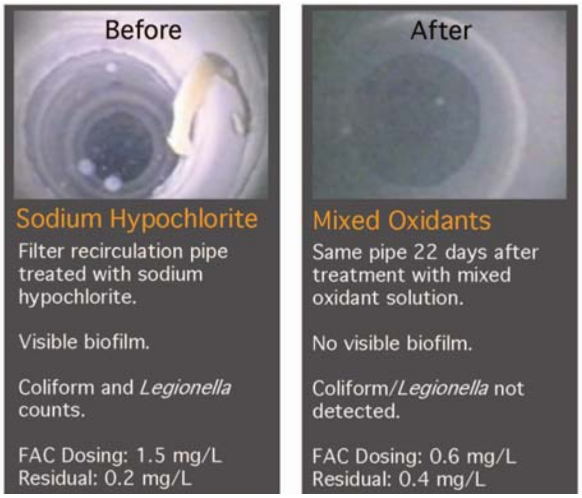
August 17, 2010

Summary:

Bacterial biofilms cause a number of serious problems for industrial fluid processing operations. Mechanical blockages, impedance of heat transfer processes and biofilm induced corrosion result in billions of dollars of losses each year. Product spoilage and possible risk to public health are also consequences of biofilm-mediated contamination. This brief article will review aspects of biofilm control strategies for Clean-in-Place (CIP) in the beverage and dairy industries and introduce a promising disinfectant via case studies.



Overview of Biofilms

Biofilms are aggregates of predominately bacterial cells attached to and growing on a surface.[i] These biofilms are found in aqueous environments and are often resistant to disinfection. A biofilm forms when bacteria begin to excrete a slimy, sticky substance that allows them to adhere to surfaces. An additional structural feature called the extracellular polymeric substance (EPS) is what is thought to provide the biofilm with increased resistance to antimicrobial agents and biocides. The biofilm mass often varies with location within a given system, and is typically composed of many species of microorganisms, including bacteria fungi, algae, and protozoa. Biofilm is difficult to remove once initial adhesion occurs.[ii] Even small numbers of surviving organisms can regrow, damaging beverage and dairy products or putting a company's reputation at risk in the event of a product recall due to negative health outcomes. Biofilms can also shelter disease-causing microorganisms, such as *Legionella*, *Listeria*, and temperature resistant bacterial spores, which are normally inactivated readily in their planktonic, or single cell form.

A safe, user-friendly and viable method for controlling biofilms would have a significant impact on any industry that utilizes CIP or must control bacterial populations such as in water distribution systems, cooling towers, and swimming pools. Improved biofilm control technologies used in CIP applications should also minimize both the number of steps required for CIP as well as the use of high temperature steps, both of which provide the added benefit of increased production time.

CIP Strategies

There are many CIP strategies and chemical regimens for controlling biofilms. These may be divided into flooded CIP systems and the air-injected CIP systems often used in the dairy industry. Flooded CIP systems involve completely filling all the pipes exposed to product with water, chlorine, biocide, caustic or other chemical for a prescribed amount of time according to plant protocol. Air-injected CIP systems are commonly used in milking and cheese operations to assist with physically removing organic soil and biofilm. When designed properly, the turbulence and type of biocide can assist with both cleaning and disinfection. Even a small amount of biofilm left after the CIP process can propagate and spoil the milk or cheese.[iii]

MIOX – A Novel Biofilm Control Solution

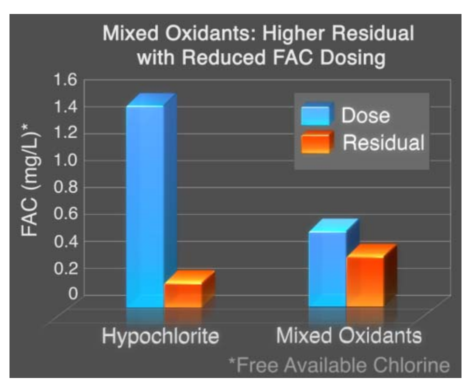
Many biocide treatment regimes exist, including a multitude of combinations of cleaning (hot caustic, such as sodium hydroxide) and disinfection (quats, chlorine, proprietary biocides) chemicals. An alternative to these variable regimes is MIOX's Mixed Oxidant Solution (MOS), a cost-effective, simple cleaning and disinfection solution for beverage applications that has the potential to also provide enhancements to biofilm control strategies for CIP applications. MOS, a proprietary blend of hypochlorite and other oxidants, has been generated on-site by MIOX Corporation since 1994 through the use of salt, water, and an electrolytic cell. The chlorine-based product of electrolysis has clearly exhibited the ability to remove biofilm, unlike traditional chlorination technologies. Evidence for the biofilm control attributes of MOS 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.

Case Study: KOA Kampground Facilities

Biofilm removal by MOS was initially observed in New Mexico in 1995, and later reported by Montana State University during a field study conducted at a KOA Kampground in Great Falls, Montana.[iv] 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 1,000 ppm. The positive counts 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 required flushing from the system whenever a power outage occurred. The existing cartridge filters also required cleaning every 2-3 days due to the heavy accumulation of biofilm.

As a potential solution to this problem, MIOX installed a MOS generator at this site to replace the hypochlorite that had been used previously. Since conversion to MOS, the KOA Kampground has not experienced a non-compliance coliform event. The black slime in the showers disappeared within a few weeks after the conversion. Initially, biofilm visibly sloughed from the pipelines. The water eventually ran clear, indicating that the system had reached a stabilization point. Camp operators reported that the filters are cleaned every 3-4 weeks, rather than every few days. Whenever power outages occur now, no discoloration of the water occurs, indicating that the biofilm does not regrow, even when disinfection is temporarily interrupted. The MOS 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.

Case Study: Hot Springs and Swimming Pool Facilities



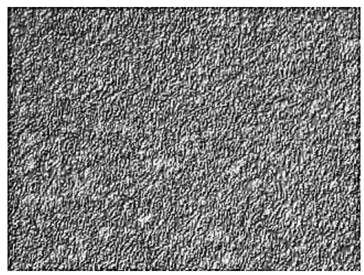
Hot springs are very popular in several cultures. However, the warm aqueous environment provides an ideal breeding ground for bacteria, and a number of the sites suffer from positive coliform counts and biofouling. At one such facility in 2002, dosing at 1.5 mg/L of free available chlorine (FAC) with sodium hypochlorite barely maintained a 0.2 mg/L residual. Coliforms and *Legionella* were frequently detected. After establishing a baseline borescope camera image (see before picture), the interior of the pipes was subsequently videotaped at 6 and 22 days after treatment with MOS began. Upon conversion, sloughing was immediately apparent. In the feed water pipe, substantial removal was evident after 6 days (photo not shown), and after 22 days (see after image), biofilm removal appears to be complete. Bacterial monitoring and residual chlorine measurement provides quantitative data to complement the compelling boroscope images. After conversion to MOS 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. Coliforms and *Legionella* were not detected.[v]

Case Study: Hospital CIP Method

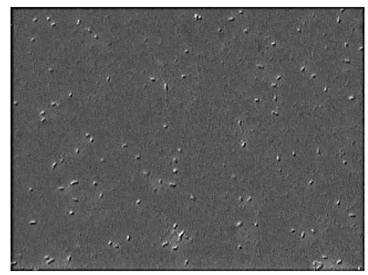
In 2006, a new hospital commissioning was delayed due to non-compliance with the water system. *Legionella* and *Pseudomonas* (a biofilm-former) were consistently detected despite use of chlorine dioxide (0.5 ppm dose) to clean the system. A premise plumbing case study was designed with the goal of cleaning the system in a short period of time using MOS. Disinfectant residual and bacterial sampling was conducted at 1400 sampling points. Within 8 days, MOS dosed at 50 ppm showed non-detectable *Pseudomonas* and *Legionella* counts, an indication that biofilm had been controlled.

Day	*Pseudomonas* (CFU/mL)	*Legionella* (CFU/L)
Day 0	0-95	Detected
Day 5	60 (hot) 0 (cold)	ND/1 L
Day 8	0 (all points)	ND/1 L

One hypothesis for the consistently better biofilm control exhibited by MOS as compared to hypochlorite is that MOS removes more of the polysaccharide attachment matrix constituting the framework that protects biofilms and allows them to thrive. Research supporting this hypothesis was conducted at the Orange County Water District in California. Researchers performed several studies comparing hypochlorite and MOS efficacy at controlling *Psuedomonas putida* biofilms on cellulose acetate RO membranes.[vi] Based upon several lines of analysis including microbe staining and microcopy techniques that indicate areas where DNA is present, researchers reported that MOS appeared to remove the polysaccharide biofilm substrate, while chlorine had less effect at the same dose and exposure times.



Biofilm Removal at 35 min



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

Black and white pictures created using differential interference contrast optics demonstrating MOS effect on a *Pseudomonas putida* biofilm. The first view shows unchecked biofilm growth at 35 minutes. At 5 hours and 40 minutes, the biofilm cells were nearly undetectable. DNA fluorescence data confirmed the biofilm removal.

Conclusions

MIOX Corporation has amassed a large body of laboratory and field data indicating that MOS can provide both cleaning and disinfecting properties, excellent features for an ideal biofilm control strategy in CIP processes. Now, more research is needed to understand how best to optimize process cleaning, especially in dairy applications where soils and ideal biofilm growth conditions exist.

Recognizing the need for application data, MIOX is building a laboratory-scale CIP flow-reactor system capable of testing several chemical and biological parameters. These types of bench-top flow systems have been previously described in the academic literature.[vii] MIOX's system is designed to test a variety of applications and conditions, including a coupon system and a method to add and remove pipe sections so that different pipe materials can be tested for biofilm growth and control. This system can also be used to further examine the issue of chemical corrosion, complementing nearly two years of research already conducted in our laboratory on this issue. The CIP system will be upgraded as needed and will provide an excellent test bed for comparative studies. Studies will be customized for each application to best understand how to apply MIOX technologies to optimize biofilm control during CIP.

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About MIOX Corporation

MIOX Corporation (www.miox.com) is focused on solving one of the world's most pressing issues: the need for affordable, safe, and healthy water. MIOX's patented on-site water disinfection technology safely and economically generates either hypochlorite or advanced mixed oxidant using just salt, water and power, replacing the need to purchase, transport and store dangerous chemicals. MIOX is safely used in over 30 countries for public drinking water systems, water reuse projects, and non-municipal applications including the food and beverage, power, and aquatics and leisure industries.