Tackling complex sheet production efficiency and effluent quality issues using advanced bioanalytical strategies.

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ABSTRACT

Incoming water is the single largest component in the production of paper and related products. Contamination problems, due to ineffective water treatment, may not only manifest themselves on the machine, but also at the wastewater treatment plant. Freshwater microorganisms form biofilms that can lead to reduced efficiency and product quality on the process side of the mill operation and filter plugging, poor clarification, and effluent turbidity issues in the wastewater treatment plant. These organisms often go undetected using standard monitoring techniques, making it impossible to treat consistently. Therefore, the use of sophisticated monitoring strategies is required.

DNA analysis of complex communities is valuable in assuring the correct prevention and treatment strategies are implemented. The use of Next Generation Sequencing and quantitative polymerase chain reaction (qPCR) approaches to analyze DNA from complex microbial communities makes it possible to trace organisms to their point of origin, where appropriate chemistry can be applied to eliminate them. Confocal Laser Scanning Microscopy (CLSM) is used to obtain information about the distribution of organisms in a biofilm and their susceptibility to biocides. Advanced biomonitoring technologies provide information necessary to execute a comprehensive water management strategy and bring chemical and operational cost-savings, process efficiency, and confidence in treatment strategies to the mill.

INTRODUCTION

Industries that are heavy users of water are dependent upon optimal preparation of this raw material to maintain their production efficiency and consistently meet product quality specifications. By reducing variability in the water, its utility is enhanced for the end user. One of the major variables in fresh water is microorganisms. When not treated properly, fresh water bacteria wreak havoc by forming slime on machine surfaces which reduces the efficiency of heat exchangers, plugs shower nozzles, and sloughs into the final product leading to losses in production time and revenue. It is estimated that 90% of microbiologically-related problems in the production of paper arise from inadequate fresh water treatment (5).

CASE STUDIES

Optimized Fresh Water: Improved machine performance

A graphics paper mill suspected that production could be improved by reducing variability in freshwater treatment caused by their chlorine gas program. The mill had been struggling with inconsistent fresh water treatment due to difficulties controlling the chlorine gas program. While on chlorine gas, the mill was struggling to maintain a stable chlorine residual and experienced sheet breaks due to defects. After switching to a monochloramine-based program, the mill was able to maintain stable chlorine residuals and reduce the impact of variable fresh water quality on the process.

The target chlorine residual in the raw water was achieved only 60% of the time. When out of spec, the chlorine residual swung between 0 and 0.9 ppm (Left side of Figure 1). Untreated incoming water, even for a short period of time, can carry microorganisms onto the machine where they rapidly multiply and contribute to

problems in the process. Conversely, a dose above 0.5 ppm free chlorine can degrade dyes, felts and contribute to corrosion. High variability in treatment of the incoming water resulted in large fluctuations of microbial activity on the machine, and difficulty maintaining effective chlorine residuals (Left side of Figure 2).

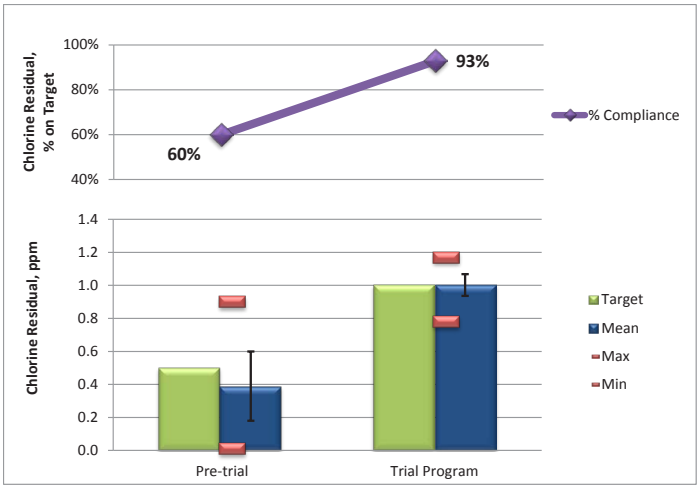

Figure 1: Freshwater treatment overview. Monochloramine improved treatment reliability. Residual chlorine variability was reduced and on target 93% of the time.

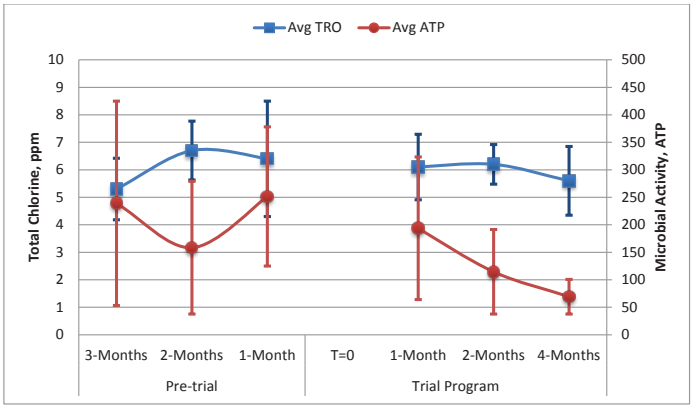

Figure 2: Paper machine key performance indicators: total residual chlorine (TRO) and ATP. Key process indicators with the chlorine gas program show high variability in microbial activity. After switching to monochloramine microbial activity decreased and becomes less variable while oxidant dose is reduced.

The incoming water was analyzed for problematic fresh water organisms, such as filamentous and nitrifying bacteria, using quantitative polymerase chain reaction (qPCR). qPCR is a DNA-based technique that detects and quantifies DNA from specific bacterial targets within a sample (3). Filamentous and nitrifying bacteria contribute to slime on machine surfaces and increase oxidant demand in process waters (5). These organisms cannot be detected by standard plating techniques and require incubation times of up to three weeks. qPCR analysis demonstrated variability in bacterial diversity of incoming water; filamentous bacteria were detected in every sample taken, with the occasional presence of nitrifying bacteria (Figure 3A).

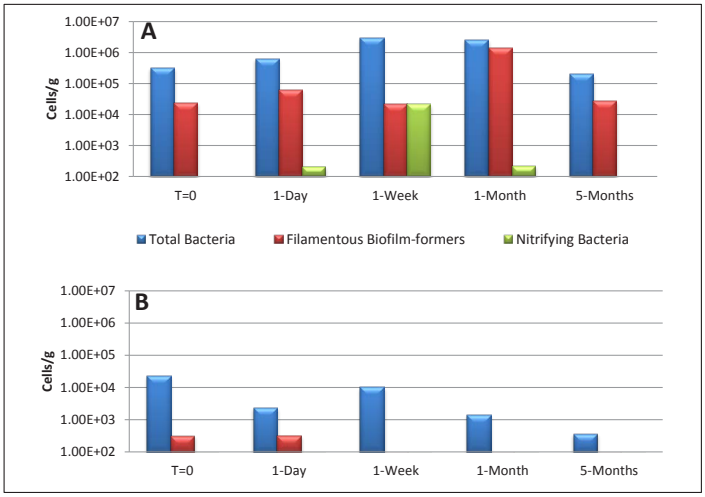

Figure 3: A) qPCR analysis results of untreated incoming water. Variable bacterial populations, and high levels of bacteria, including filamentous biofilm-formers and nitrifiers, were detected. T=0 indicates the start of the trial program. B) qPCR analysis of treated incoming water. Reduced bacterial variability and eventual elimination of problematic bacteria were observed. T=0 indicates the start of the trial program. The limit of detection of qPCR assays was 100 cells/g.

In order to reduce variability in treatment and microbial activity in the process, a monochloramine-based chemical program was implemented on the incoming raw water as a chlorine gas replacement. The process was treated with a combination of oxidizing biocides on the machine and in the stock chests. The treatment change improved program reliability. After the program change, the target chlorine residual was satisfied 93% of the time (Right side of Figure 1). Furthermore, DNA-analysis of treated incoming water showed a consistent reduction of microorganisms and problematic bacteria entering the process (Figure 3B). The use of a reliable combined chlorine treatment also improved the persistence of chlorine in the water going to the machine. Only 17% of the chlorine gas residual remained by the time it reached the paper machine (0 – 0.15 ppm). The new program reached the machine with 96 – 100% of its original dose (Figure 4).

After changing to a more persistent oxidant, freshwater consistently entered the paper process without contributing to microbial demand. Improved incoming freshwater quality also allowed for an optimized biocontrol strategy over the whole production process. The impact on key process indicators was a reduction in microbial activity achieved by a lower oxidant dose to the headbox (Right side of Figure 2). Consistent control of microbial activity has been linked to reductions in holes and sheet breaks resulting in improved runnability (1). A decrease in sheet breaks due to holes was also observed

(Figure 5). An effective raw water treatment strategy decreased the impact of microbial growth on process variability and allowed treatment of the process to be more effective.

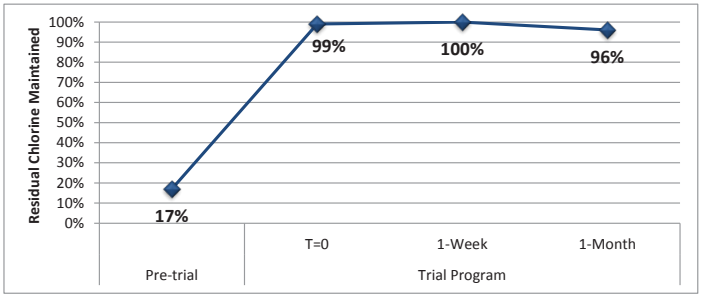

Figure 4: Oxidant persistence from water treatment plant to machine. Oxidant persistence increase after switching to a monochloramine-based incoming water treatment program. T=0 indicates the start of the trial program.

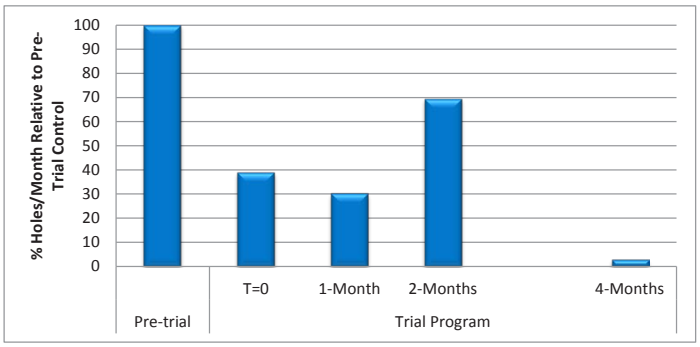

Figure 5: Percent reduction in holes observed during the trial. T=0 indicates the start of the trial program.

Ineffective Fresh Water Treatment: Surprising Impact on Paper mill Wastewater

A paper mill was experiencing excessive biofilm buildup in the wastewater effluent pipe. The high volume and density of slime caused it to slough and be expelled from the pipe into the waterway. The mill spent a lot of resources trying to resolve this issue. A physical barrier inside the pipe was installed to capture solids as they exited to the river. However, due to the high levels of sloughing biofilm, the barrier required frequent cleaning, which put an additional strain on resources. In order to develop a cost effective treatment plan to eradicate the slime, it was critical to determine its root cause.

Identification of major bacterial components of the biofilm would allow them to be traced through the process to determine their points of origin. Based on the clear quality of the effluent water, it was hypothesized that the biofilm was initiated by a group of organisms known as early colonizers. These bacteria have an ability to attach to clean surfaces and create a hospitable environment for attachment of other organisms and debris. An experiment was designed to study biofilm succession at the effluent pipe.

Twenty stainless steel coupons were suspended into the effluent stream. Two coupons were recovered from the effluent stream every hour. The coupons were submitted for speciation analysis using Next Generation Sequencing (NGS). NGS is a platform for performing sequencing of millions of small fragments of the 16S rRNA in parallel. Bioinformatics analyses are then used to compare these fragments against all known bacterial DNA sequences in the Ribosome Database Project (2, 4). Additionally, NGS also provides information about the relative abundance of microorganisms present in samples. qPCR was used to quantify the total bacterial loading on the coupons.

Speciation analysis identified many groups of bacteria present in the biofilm on the coupons. However, two groups of bacteria were identified as being the most prevalent members in the biofilm over time: *Sphaerotilus* and *Flectobacillus* genus. These organisms form long, tangled filaments and thrive in low oxygen and high nutrient conditions. *Sphaerotilus* can attach to surfaces by one end of the filament while extending the rest of the filament into the water column to trap debris, nutrients, and other bacteria (7). This organism is notorious for growing massive biofilms in wastewater treatment plants. *Flectobacillus* is a filamentous organism that cannot digest complex sugars. This bacterium forms symbiotic relationships with the organisms in the biofilm and uses the biofilm's byproducts as its source of carbon (6). Other biofilm formers detected using NGS included genera: *Sphingomonas, Bosea,* and *Deinococcus*, among many others.

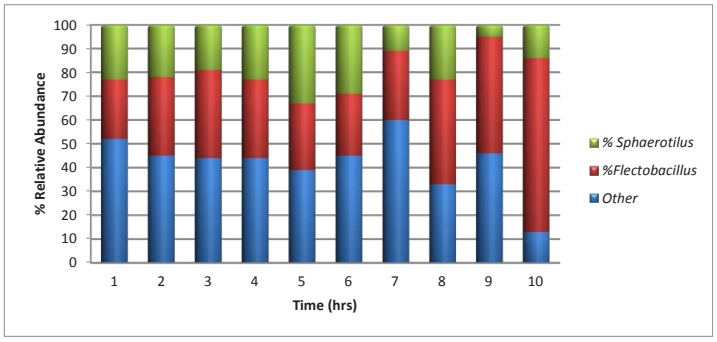

Figure 6: Relative abundance of Sphaerotilus and Flectobacillus genera in the effluent biofilm over time.

Based on the increase in relative abundance of *Flectobacillus* and *Sphaerotilus* bacteria in the biofilm over time, it was possible to extrapolate succession of biofilm formation (Figure 6). It is hypothesized that there is normally-occurring biofilm present on the surface of the pipe. This initial biofilm likely contains *Sphingomonas, Flavobacterium, Clostridium*, among other biofilm-forming species. Next, *Sphaerotilus* attaches to this biofilm, or

clean pipe surface, and forms brush-like structures that trap debris and other bacteria, like *Flectobacillus*. As the biofilm grows larger, *Flectobacillus* utilizes the influx of nutrients to grow and out compete the rest of the biofilm population in a matter of hours. Eventually, the biofilm becomes so dense and heavy that it sloughs and inoculates a new spot down the pipe.

To determine the source of *Flectobacillus* and *Sphaerotilus* samples were collected throughout the process, starting at the incoming raw water. NGS was used to analyze microbial communities in the fresh water treatment plant, paper machines, raw materials, and the wastewater treatment plant (Figure 7). The data showed that both types of bacteria were absent on the paper machines, but were detected in the raw water and again in the wastewater treatment plant. It was determined that these organisms were being trapped by the anthracite filters, as the incoming river water passed through them, and backwashed directly to the wastewater treatment plant. Low dissolved oxygen and high nutrient levels presented ideal conditions for rapid growth.

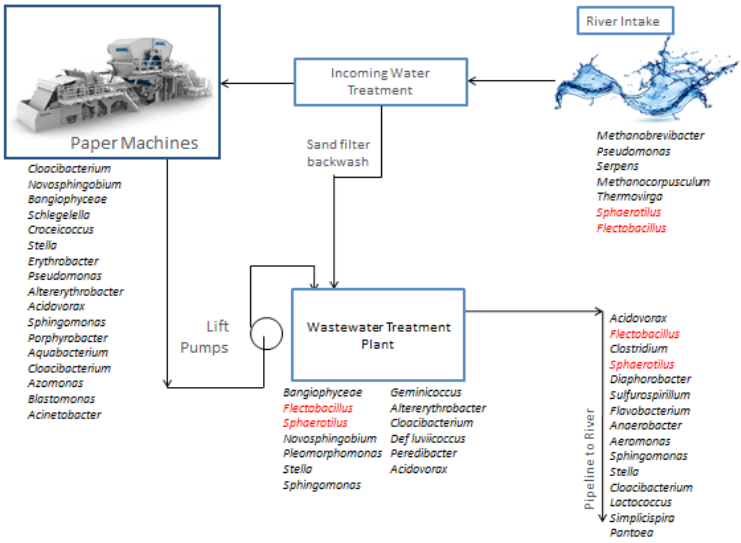

Figure 7: NGS analysis of process water samples. Bacteria at each sampling location are listed in the order of their abundance at the time of sampling.

To determine the most effective strategy to eradicate biofilm in the effluent pipe, inhibition and removal was evaluated using confocal laser scanning microscopy (CLSM). CLSM provides insight into biofilm morphology, and when coupled with a viability stain, is a powerful tool to evaluate biofilm penetration of biocides. Sterile stainless steel coupons were placed at the bottom of three biofilm reactors. Mill effluent water was subjected to varying biocide treatments while being recirculated through the reactors. Sodium hypochlorite, monochloramine, and chlorine dioxide were tested as a means to inhibit biofilm formation. The same treatments were also tested as a means to remove week-old biofilm formed by the organisms in the effluent water. Coupons were removed daily, stained with FilmTracer™ Live/Dead Biofilm Viability kit for confocal laser scanning microscopy analysis and observed with CLSM (Figure 8). The Viability kit provides a two-color fluorescence assay of bacterial viability. When used alone, the SYTO® 9 stain generally labels all bacteria in a population—those with intact membranes and those with damaged membranes. In contrast, PI penetrates only bacteria with damaged membranes, causing a reduction in the SYTO® 9 stain fluorescence when both dyes are present. Thus, with an appropriate mixture of the SYTO® 9 and PI stains, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red.

In the biofilm inhibition study, it was observed that sodium hypochlorite was ineffective against biofilm prevention. Conversely, both monochloramine and chlorine dioxide showed the highest levels of biofilm inhibition (Figure 8). This is also evidenced in the volume of biofilm that formed with each treatment. The control and sodium hypochlorite-treated samples did not inhibit biofilm formation (Figure 9). Monochloramine treatment was the most effective inhibitor of biofilm formation (Figure 9).

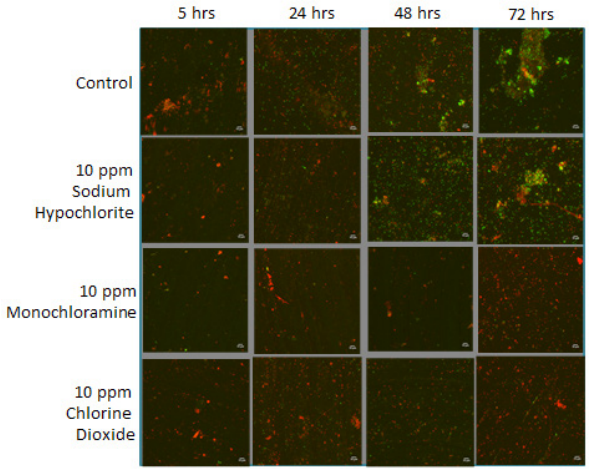

Figure 8: Corresponding Z-projected images for untreated, sodium hypochlorite treated, monochloramine treated, chlorine dioxide treated samples ranging from 5 hours to 72 hours. Green color is indicative of intact cellular membranes, while red is an indication of membrane damage.

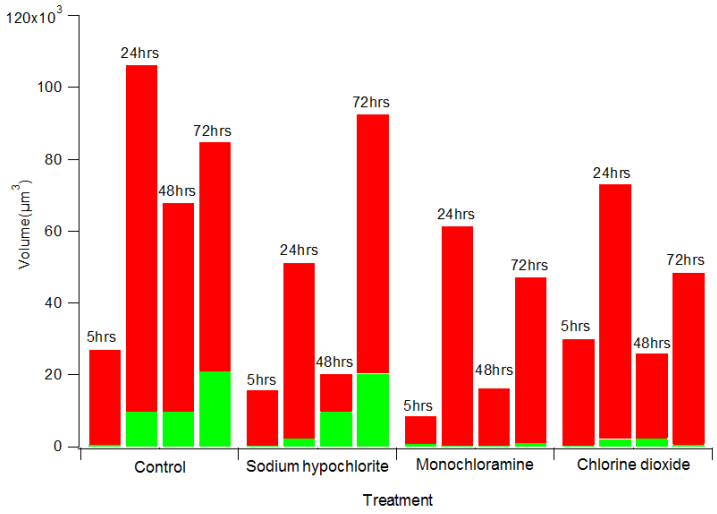

Figure 9: Biofilm measurements from CLSM during biofilm inhibition. Images in Figure 9 were split into red and green channels, and converted into binary images for 3D segmentation. Red represents volume with red fluorescence signal only. Green is volume with green fluorescence signal only. Areas where both green and red signal were present were excluded from the chart.

When the same oxidant-based chemistries were tested for their ability to remove week-old biofilm, sodium hypochlorite was not as effective as monochloramine or chlorine dioxide to remove cells from the biofilm (Figure 10). It is likely that the contact time for sodium hypochlorite was insufficient to penetrate the biofilm (8). Compared to chlorine dioxide, the monochloramine treated sample had the most total biofilm volume and blend biofilm volume for the imaged region. This suggests monochloramine cannot remove biofilm effectively although it can penetrate biofilm to kill most live cells.

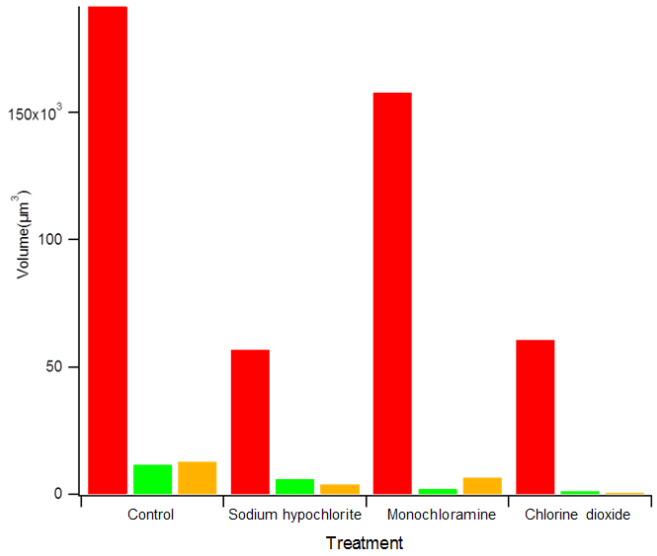

Figure 10: Biofilm volume measurements based CLSM images. Red represents volume with red fluorescence signal only, Green is volume with green fluorescence signal only and blend is volume with both red and green fluorescence signals, indicated in orange.

Based on the biofilm inhibition and removal data, chlorine dioxide was determined to be the most effective against removing existing biocide and keeping the pipe surface clean. A treatment strategy was developed to target this oxidant at the inoculation and exit points of water into the wastewater treatment plant.

CONCLUSIONS

Water is the largest raw material used in papermaking. This material is also highly variable in quality. Microbial loading can change based on weather, season, and environmental factors. By eliminating this variability with treatment programs that maintain their performance through the constant changes, many issues caused by microorganisms can be eliminated from the process. Effective and consistent fresh water treatment was shown to improve biocontrol effectiveness thereby reducing the bioburden of the system. Concurrently, machine performance exhibited an improvement due to a reduction of sheet breaks caused by holes. Inadequate fresh water treatment can lead to massive problems that not only manifest themselves in the production process, but also affect the wastewater treatment plant leading to issues with water reuse and discharge. Water touches all parts of the papermaking process, it is important to prepare this raw material so that its utility is enhanced to the maximum for the papermaker.

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