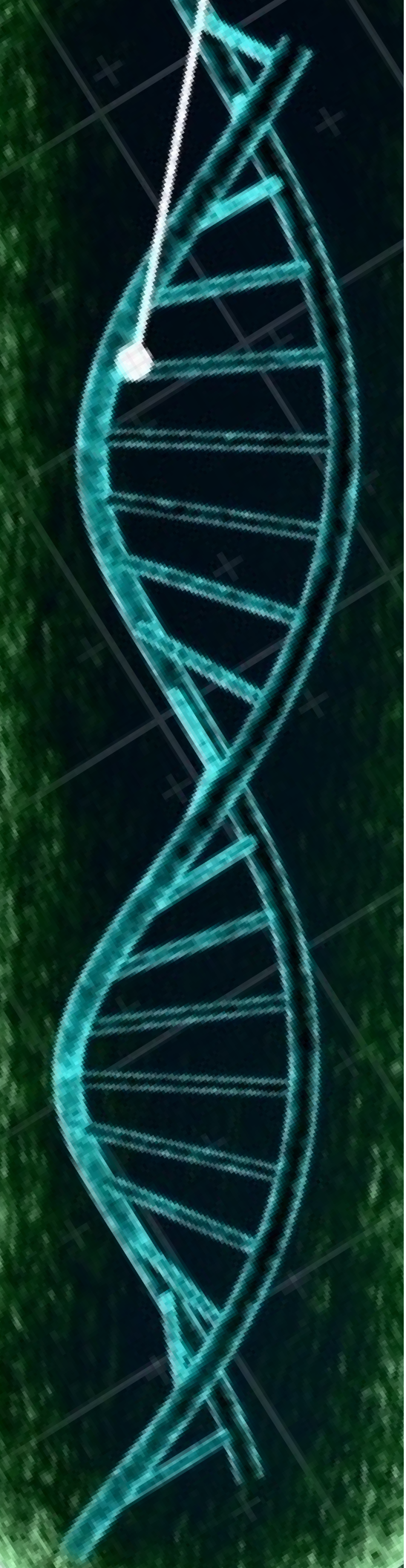

NALCO Water
An Ecolab Company

MAP

Unprecedented detection and precision control of microbial growth in papermaking systems.

INTRODUCING MAP MONITORING TECHNOLOGY

MAP (Metagenomic Analysis Protocol) is a patented approach that allows for detection, quantification, and identification of microbial DNA present in the papermaking process, sheet defects, deposits, and other materials. MAP allows an unprecedented level of detection and precision in troubleshooting microbial based problems. MAP provides advantages over traditional monitoring approaches by detecting all microorganisms and breaking them down into problem-causing categories.

In keeping with Nalco Water's tradition of delivering actionable data, MAP monitoring technology allows for quick and simplified analysis by grouping organisms into problem-based groups. This includes freshwater microorganisms, which are known to contribute to deposits, spoilage, and corrosion but difficult or impossible to enumerate with traditional techniques. Primary and Adaptive biofilm-formers can generate tremendous amounts of slime, making the process system more susceptible to fouling. Furthermore, bacteria protected in a biofilm are notoriously difficult to control.

The MAP approach is used to confirm the contribution of microbial growth to sheet defects or holes, confirm the infested process location, and target treatment to minimize the impact of microbial growth on process efficiency.

THE BENEFITS OF MAP

The benefits ofMAP are summarized in one word: PRECISION. Detection andquantification of problematic microbes that may be overlooked using traditional monitoring approaches. Identification of contamination 'Hot Zones'. Targeted treatment to eliminate problematic microbes and the problems they cause. This approach effectively controls microbial growth and surface deposition that leads to reduced process efficiency and product quality.

THEORY

The polymerase chain reaction (PCR) is a method for targeting sequences of nucleic acid (DNA or RNA) and increasing the copy number of the target sequence to obtain useful quantities of nucleic acid for analysis (Figure 1). The replication of the target DNA fragment is exponential; each new double stranded DNA becomes the template for two new strands of DNA and the process is repeated.

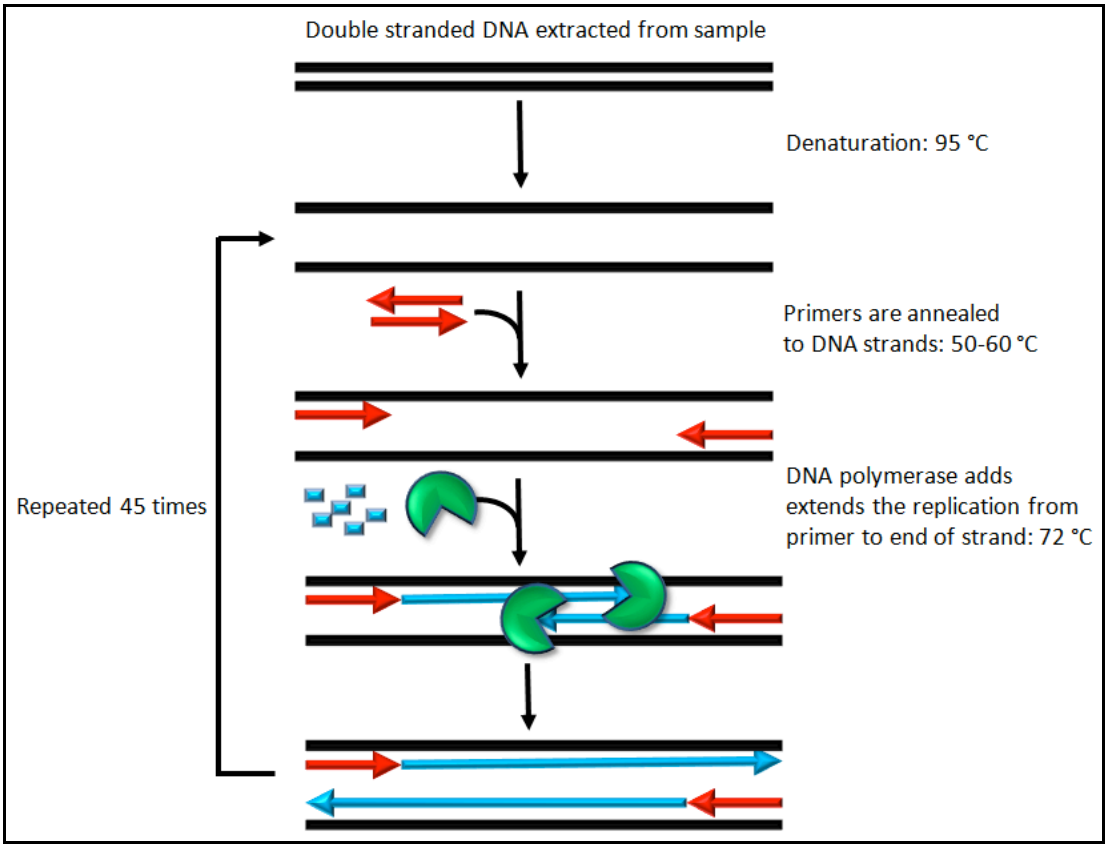

Figure 1 – *Overview of the polymerase chain reaction (PCR). Template DNA strands are shown in black. Primers are shown as red arrows. Nucleotides and complementary DNA are shown in blue. DNA polymerase is shown in green.*

Once DNA is extracted from the sample it can be analyzed in real-time using the quantitative PCR (qPCR) approach. Quantitative PCR employs methodology similar to PCR, but qPCR includes a real-time component allowing for DNA quantification as each replication cycle proceeds. In qPCR, primers target a DNA sequence of interest based on the identity of the target organism. Fluorescence is used to detect the resulting DNA and increased fluorescence is directly proportional to an increase in the quantity of target DNA. The number of cycles required to reach a pre-determined fluorescence threshold is compared to a standard that corresponds to the specific DNA target. The number of copies of target DNA present in the sample is calculated using the standard curve. The copy number per sample is then used to determine the number of cells per sample (Figure 2).

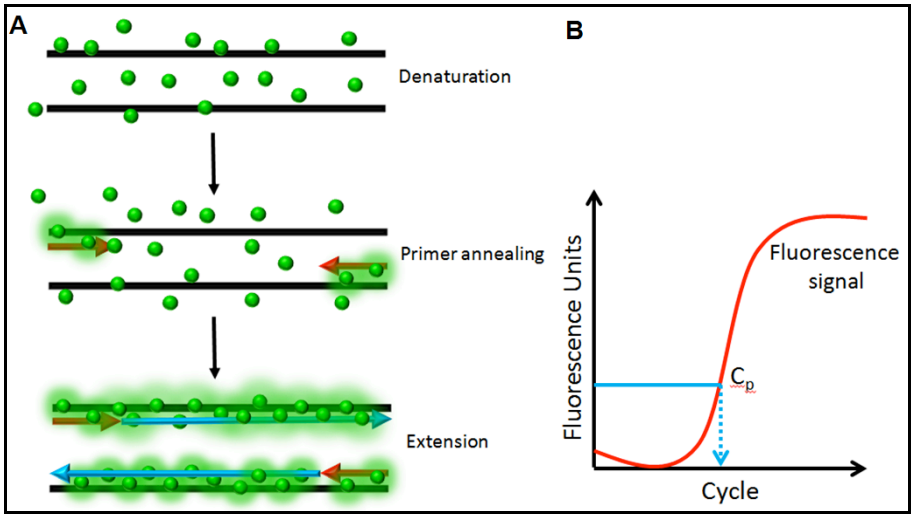

Figure 2 – *SYBR Green Assay for DNA quantification. **A**) SYBR Green reaction schematic is shown. Template DNA strands are in black, complement DNA is in blue, primers are in red. The green spheres represent SYBR green molecules. **B)** Real-time signal accumulation during a qPCR reaction. Threshold fluorescence level is shown with a solid blue line. Cp represents the cycle number at which the fluorescence signal crossed the threshold.*

The ability to quickly and accurately identify microorganisms in papermaking systems provides a wealth of information that can be used to optimize the efficacy and performance of a biocontrol program. This technology can be used to identify bacterial contamination in samples where traditional monitoring techniques fail. These samples include sheet defects, machine felts, fresh water and machine deposits. Traditional monitoring techniques are dependent on microbial viability and are not suitable due to low moisture content of the samples and exposure to the high temperature of the machine dryers in the case of sheet defects. Typically microorganisms cannot be recovered from such samples using traditional plating techniques. Therefore, the contribution of microorganisms remains unknown.

Having the ability to accurately determine the amount and the type of organism causing a problem and then being able to link it back to a specific part of the papermaking process or source of inoculation is a huge opportunity to differentiate Nalco Water from competitive suppliers. This method of solving problems can lead to decreased chemical usage, improved machine operation and product quality, which in turn will translate into cost savings and decreased environmental impact for Nalco Water's customers (Figure 3).

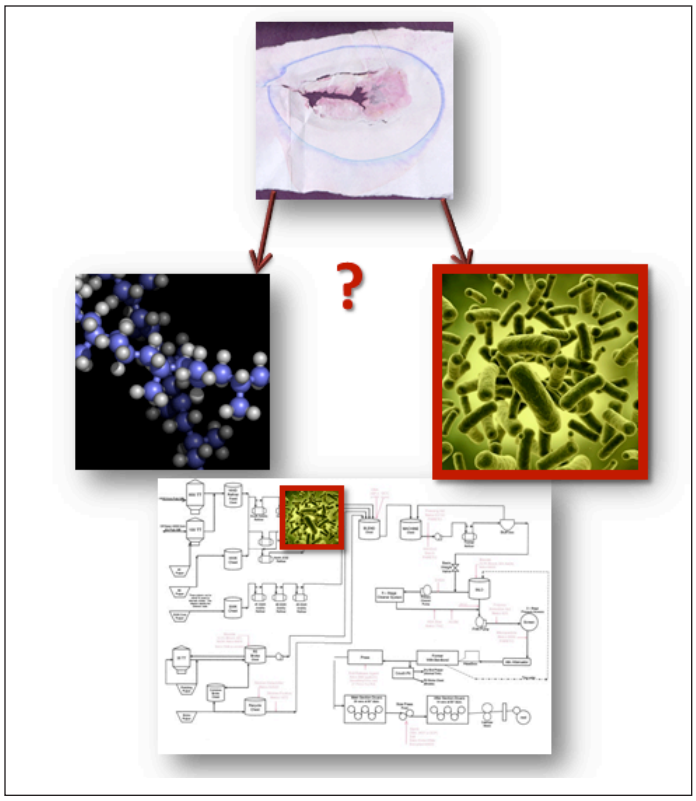

Figure 3 – *geoMAP can be used to determine the contribution of microorganisms to sheet defects and identify contamination 'Hot Zones' for targeted, more efficient chemical control of microbial growth.*

CASE STUDIES

Reduced Process Efficiency caused by Freshwater Microorganisms

A coated free sheet mill was experiencing trouble with an ongoing deposition problem throughout the machine. An increase in the amount of defects in the final product was also on the rise. In the summer months the mill experienced good microbial control. There was less deposition observed on the machine, and few defects were detected in the final product.

In the month of September the mill's fresh water treatment system experienced unscheduled downtown time. Two of four sand filters experiencing major corrosion and were taken off line for repairs for a period of four weeks. During this period approximately two thirds of the mill's fresh water was bypassing the two remaining sand filters. Chemical treatment of the water was not adjusted to account for increased turbidity and BOD of the incoming unfiltered water. Increased deposition on the machine was observed

during this time period (Figure 4). In addition, the number of defects in the final sheet was beginning to increase. In order to compensate for the lax in fresh water treatment, the paper machine was boiled out in late October.

In order to determine the root cause of the deposition and product quality issues, samples of process water, on-machine deposits and sheet defects were analyzed by quantitative polymerase chain reaction (qPCR). This technology allows for accurate and fast detection of specific groups of organisms as well as the total microbial contamination. The samples were analyzed for the presence of total microbial contamination as well as select fresh water organisms known to originate from the environment and enter the paper mill through incoming fresh water.

Total microbial contamination was observed to increase between August and October (Figure 6). When the level of fresh water contamination was compared to that of three other mills, it was determined that the amount of microorganisms present in the fresh water at this mill was significantly higher than those mills with good fresh water control (Figure 7). In addition, a fresh water organism that is tolerant to some oxidant-based biocontrol programs and forms biofilms was detected in samples from October and November (Figure 8). This organism was not detected in water samples from the month of August. The relative abundance of this bacterium in the broke increased significantly following the boilout in October (Figure 9).

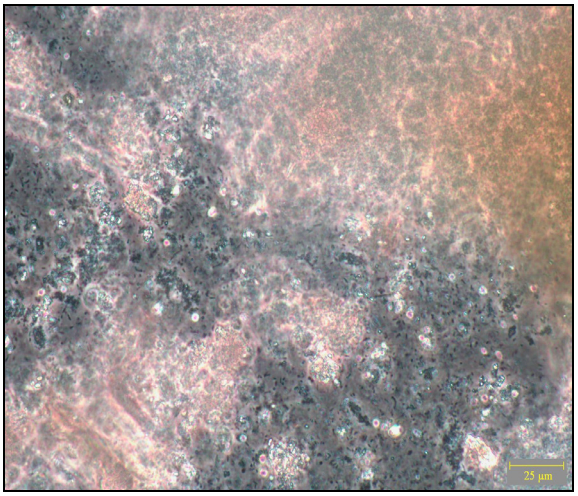

Figure 4 – *Foil Box Deposit (October). Macroscopic: Red-orange liquid deposit with darker, orange-red flakes. Phase-Contrast (400X): masses of fiber and amber-colored debris; masses of single-cell bacteria; a few thin bacterial filaments*

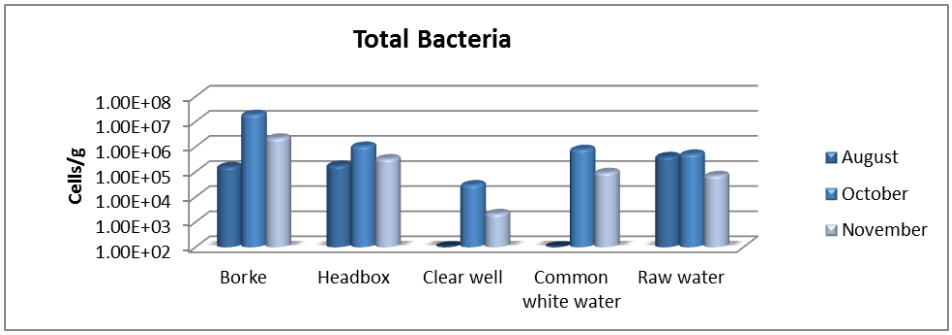

Figure 6 – *Total bacterial contamination of water samples from August to November as detected by MAP.*

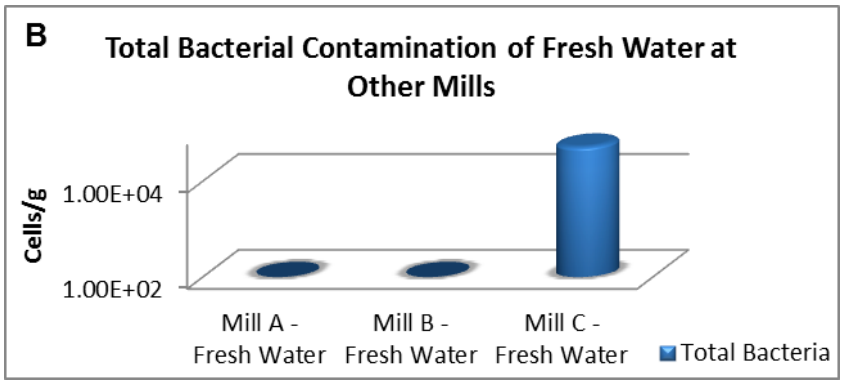

Figure 7 – *Total bacterial contamination of fresh water from three mills. Mills A and B were practicing good fresh water treatment, while Mill C was not.*

The same tolerant biofilm former, in addition to another group of fresh water organisms, was detected in sheet defects and machine deposits collected in the month of October (Figure 10). The presence of fresh water organisms in machine deposits and sheet defects is an irrefutable sign of insufficient fresh water treatment.

Despite the boilout, which took place in October, the water samples from November suggest that the organisms introduced into the system through poor fresh water treatment have proliferated in the paper machine. One such place where the fresh water organisms have accumulated is the broke (Figure 6). This is evident from the detection of high levels of an adaptive biofilm former in the broke in October. Following the boilout, the broke was retested in November and <1 log decrease in the amount of this organism was observed post boilout. Additionally,the relative abundance of this organism increased (Figure 7). In addition, the adaptive biofilm former was detected in the headbox and the common white water. The standard boilout procedure used to clean up the machine did not take into the account the full month-long inoculation event that occurred due to lack of inadequate fresh water treatment.

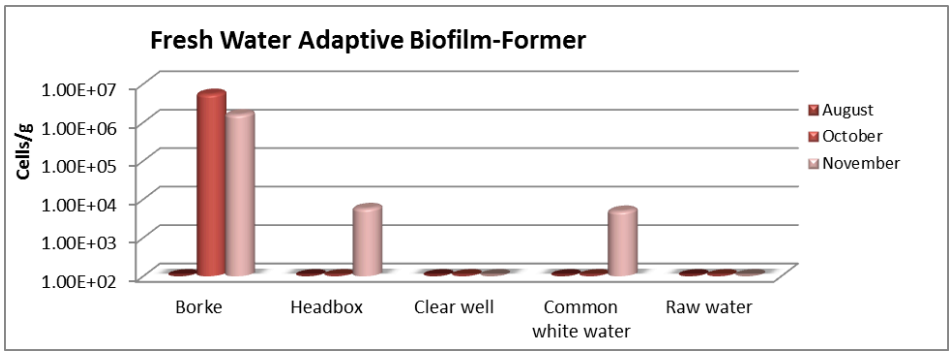

Figure 8 – *Contamination in water samples by an adaptive biofilm-former from August to November as detected by qPCR.*

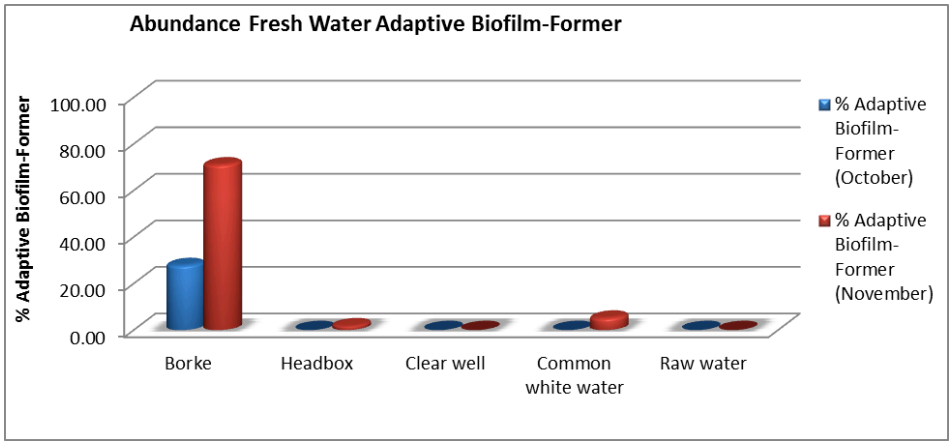

Figure 9 –*Relative abundance of the fresh water adaptive biofilm-former expressed as percentage of the total bacterial population.*

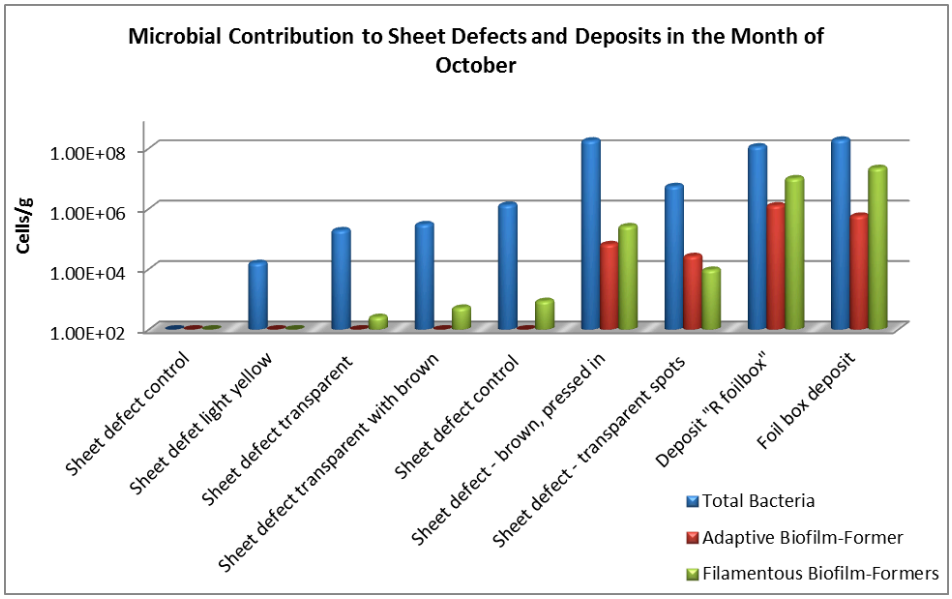

Figure 10 – *Microbial communities present in machine deposits and sheet defects collected at time of poor fresh water treatment.*

FELT PERMEABILITY AND FELT LIFE

An uncoated freesheet mill was suffering from a felt permeability problem on one of its paper machines. The permeability of the felt fabrics on one of the machines at the mill decreased at a significantly greater rate than on the other two machines. All chemical means to resolve this problem were exhausted and the mill began to look toward microbial contamination as a possible problem. However, the mill's biocontrol program was perceived to be running well due to low bacterial counts, low ATP measurements, and lack of slime deposition on machine surfaces.

In order to solve this problem, felt samples from the unaffected machine were compared to those from the struggling machine using qPCR (Figure 11). While both felts contained the same amount of total bacteria (~107 cells/g), it was immediately obvious that the problematic felt had a bacterial population that was dominated by a single group of organisms: adaptive biofilm formers. These bacteria were present in small amount on the other felt.

Following the discovery of a dominant bacterial population growing in the felt, the felt cleaning chemical was changed. The fabric was reanalyzed during the subsequent shutdown period. It was observed that the adaptive biofilm former population was reduced to 25% of the total population, with the overall bacterial load remaining unchanged (Figure 12).

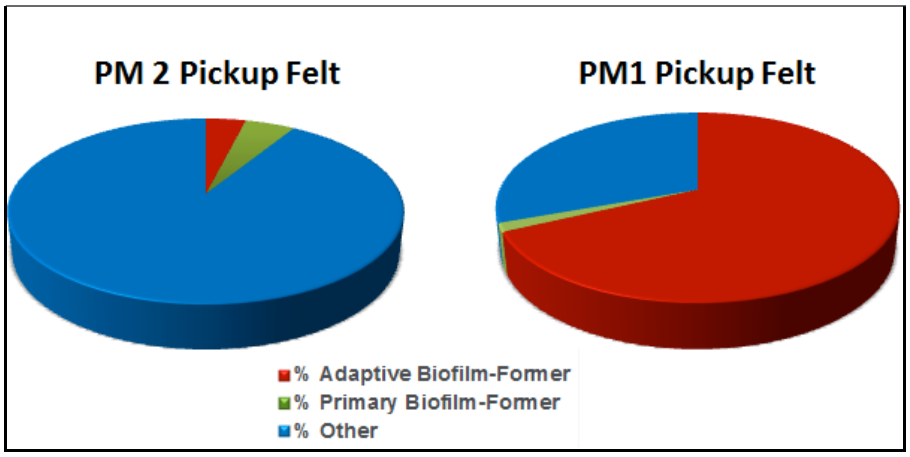

Figure 11 – *qPCR analysis of felts from a paper machine with no felt permeability problems (PM2 pickup felt) and felt samples from the machine with felt permeability problems (PM1 pickup felt).*

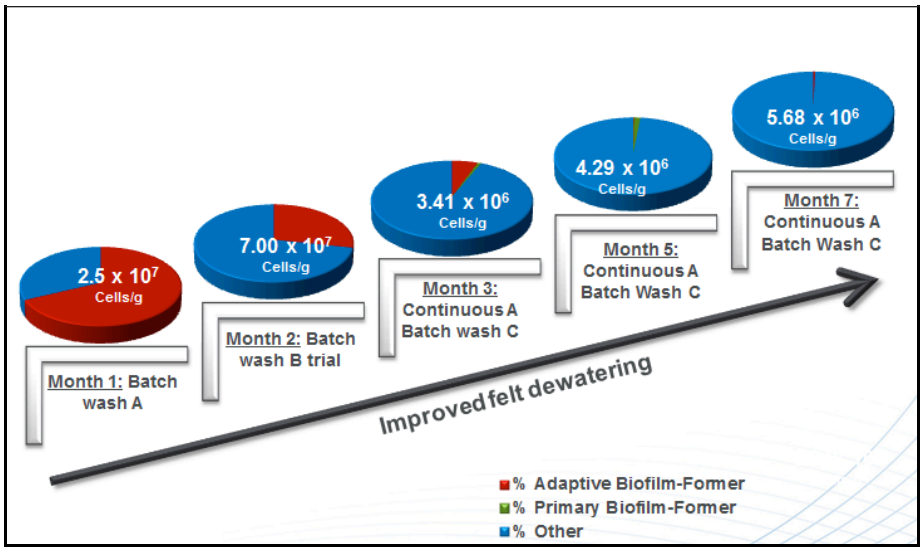

Figure 12 – *Relative abundance of bacteria in felt fabrics over time as determined by qPCR. White numbers over the pie graphs represent total bacterial loading of the sample.*

Based on these data, a second chemical, shown to have good efficacy against the adaptive biofilm formers, was added to the felt washing protocol. After this second chemistry adjustment, the felts were reanalyzed during a shutdown period. DNA results showed that the total bacterial load stayed unchanged, while the adaptive biofilm formers were reduced to roughly 10% of the total population (Figure 12). The new chemistry combination was allowed to run on the machine for the next four months. Over the course of these months, the adaptive biofilm former population was eliminated from the felt fabrics on this machine. The elimination of this group of bacteria resulted in improvement in dewatering efficiency of the felts, and the extension of the life of the fabrics by 4 days (Figure 13).

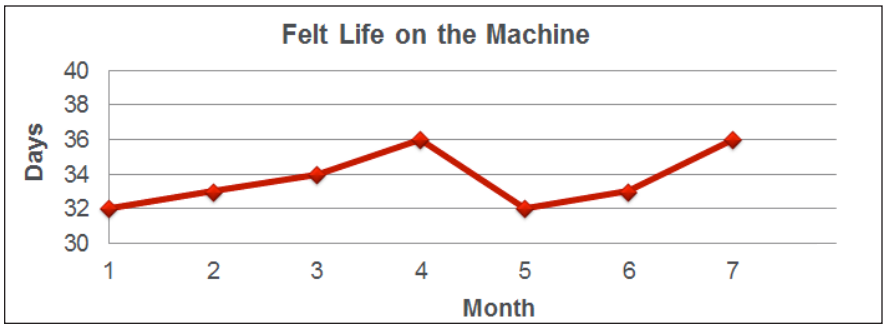

Figure 13 – *Felt fabric lifetime (in days) on the machine before (Month 1) and during (Months 2-7) program optimization.*

MAP MONITORING TECHNOLOGY VALUE PROPOSITION

The unparalleled detection capability provided by MAP Monitoring Technology creates new strategies for delivery of the most effective chemical control agents to the most responsive addition point. Microbial control programs that have incorporated MAP into their operations have achieved:

- Continuous and reliable performance
- Unmatched product consistency and process efficiency
- Significant improvements in biocide efficacy.
- Reduced chemical consumption.

GUIDELINES FOR MAP MONITORING

Contact the appropriate Paper Services Division Grade Team or the Expertise Center for Deposit Control to determine if OxiPRO™ Deposit Control Technology is suitable for your application. Approval and scheduling is required prior to submission of samples for analysis.

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