# Advanced Diagnostic Strategies for Optimal Biocontrol

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### INTRODUCTION

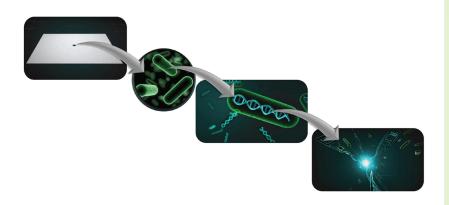
Papermaking systems utilise several raw materials that introduce microorganisms into the machine system. This includes virgin wood fibre, recycled fibre, freshwater, starch, dyes, and other chemical additives. Microorganisms proliferate in the warm, nutrient-rich environment of the papermaking system and diverse microbial communities result. Inadequate control of microbial growth allows for the formation of surface deposits that slough, leading to filter or nozzle plugging and defects (e.g. spots or holes) or breaks in the sheet. Microorganisms can also proliferate in the felts and machine fabrics, negatively impacting water removal and machine or operational efficiency.

Traditional key performance indicators, including conventional plating techniques and oxidant residuals, often indicate adequate dosing and control. However, deposition, defects and breaks may still be prevalent. There is a need for monitoring tools that provide more accurate information regarding microbial growth and biofilm formation in industrial water systems and allow for more rapid determination of the contribution of microorganisms to sheet defects. The presence of microorganisms in sheet defects and

ability to determine the location of problematic deposits in the process would allow for rapid implementation of the most appropriate microbial control strategy (Figure 1). Such information can be acquired through the use of quantitative polymerase chain reaction (qPCR) techniques. Optimised treatment strategies reduce the volume of treatment chemical required to control problematic deposits.

# PROBLEMS WITH TRADITIONAL MONITORING TOOLS

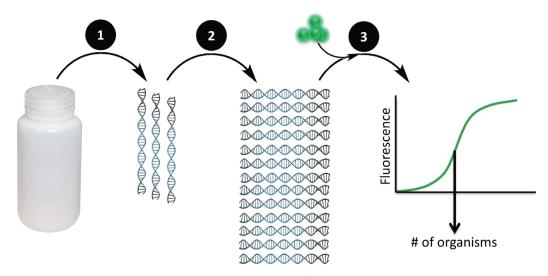
Many traditional monitoring approaches do not provide enough information for the papermaker to respond in a correct or timely manner to an upset situation. Techniques such as plate counts and adenosine triphophsate (ATP) measurements fail to represent and discriminate between the different microorganisms present in a sample. Analysis of microbial population using conventional plating techniques is labour intensive, time consuming, and relies on the ability of an organism to grow on a defined or selective medium. Moreover, lengthy incubation periods negate any possibility for pro-active control or preventative measures related to microbial growth in the process. While plating allows for a basic level of identification of organisms, it requires that the organism is viable. The need for viable bacteria and lengthy



**Figure 1.** MAP is used to determine the contribution of bacteria to sheet defects and holes and target treatment based on the type and quantity of DNA detected

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**Figure 2.** Overview of MAP. 1. Extraction: DNA is extracted from a sample. 2. Amplification: Extracted DNA is amplified using polymerase chain reaction (PCR). 3. Quantification: A fluorescent molecule is used to quantify the amount of DNA corresponding to a problematic organism.

incubation times limit the value of this approach as a problem solving tool, especially when it comes to analysis of sheet defects and felts.

A more rapid method for detecting microbial growth in process waters is by measuring the amount of ATP. While it is possible to quantify microbial activity in a sample with the use of the ATP assay, the reaction is unable to discriminate between ATP that is produced by one type of microorganism compared to another and it does not detect organisms that are viable but inhibited. Another disadvantage is that this method cannot be used to determine microbial contribution to sheet defects because most organisms are not viable following exposure to the heat of the dryer section.

In contrast to plating and ATP measurements, ninhydrin staining and Fourier transform infrared (FTIR) spectroscopy can be used to assess microbial contribution to sheet defects. These methods are not quantitative, indicating the potential presence or absence of microorganisms. They are

also non-specific, preventing the ability to identify the source of contamination or process location where growth is not adequately controlled. Furthermore, these methods are prone to false positive or false negative results caused by chemical additives that interfere with the test method and detection limits. In addition, if bacteria are detected in the defect, it is not possible to tell which organism is responsible for the defect and what part of the process it originated in.

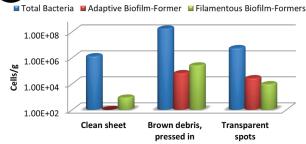
### METAGENOMIC ANALYSIS PROTOCOL (MAP)

MAP is a Nalco patented technology that fills technological gaps left by traditional monitoring approaches. This technology provides the ability to quantify and identify microbial DNA present in the papermaking process (Figure 2). All living things contain DNA, which is a robust molecule capable of surviving conditions found in papermaking systems. Once detected, microbial DNA is broken down into groups of bacteria that are known to cause problems in a papermaking environment:

- 1. Total bacterial load of the sample
  - The general bacterial population

- present in a sample
- Primary biofilm-formers Bacteria capable of colonising clean machine surfaces
- Adaptive biofilm-formers –
   Bacteria that exhibit tolerance to some biocontrol programs
- Fresh water bacteria Bacteria, including filamentous bacteria, that enter the papermaking process with raw water
- 5. Fungi
- Sulfate-reducing bacteria –
   Bacteria that reduce sulfate to form hydrogen sulfide gas
- Spore-formers Bacteria that have the propensity to form spores when experiencing an external stress (e.g. nutrient depletion or biocides).

MAP utilises quantitative polymerase chain reaction (qPCR) to provide accurate, timely, and representative information about the population of microorganisms in any given sample. By focusing on the potential problem developed by uncontrolled growth of these problematic organisms, more





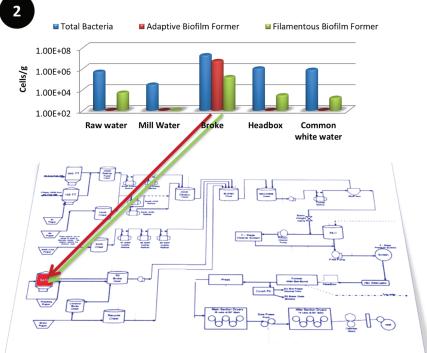
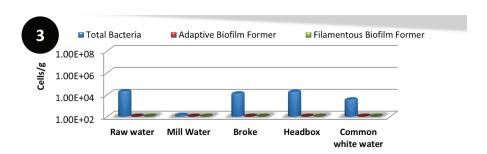


Figure 3. Utilising MAP to resolve a product quality issue

- **3.1.** Identification that the root cause of sheet defects was microbiological
- **3.2**. Identification of the "hot zone" of problematic bacteria in the process.
- **3.3.** Confirmation that the targeted treatment was successful



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precision can be applied to the design and deployment of microbial control programs.

# CONNECTION BETWEEN MACHINE DEPOSITS AND SHEET DEFECTS

The root cause of sheet defects is notoriously difficult to determine. High temperatures in the dryer section desiccate all microbial contaminants making them impossible to analyse using traditional plate counts or ATP techniques. The burden of identifying whether the defect is microbial or chemical in nature then falls on mill personnel's experience, FTIR, or ninhydrin spray. The experience of mill personnel can be subjective, and FTIR and ninhydrin spray detect chemical groups that are not unique to microorganisms. All of these approaches can result in inaccurate information that may lead to an improperly treated process.

DNA is a robust molecule that can withstand extreme conditions of the dryer section. MAP makes it possible to detect bacterial DNA in sheet defects and identify problematic species present therein, thus making it possible to determine, with certainty, whether the root cause of

the problem is chemical or bacterial in nature (Figure 1). Based on the type and quantity of microorganisms present, their origin can be traced back to a specific source in the process.

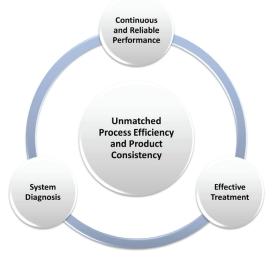
# IDENTIFICATION OF HOT-ZONES OF PROBLEMATIC BACTERIA IN THE PROCESS

Once the root cause of a sheet defect has been recognised as microbiological and the implicit bacterial species identified, it is important to eliminate the source of these bacteria from the process. Using MAP to perform a thorough process survey allows for the type and quantity of target bacteria to be determined to track their origin back to a specific location or raw material source in the process or locations that serve as proliferation zones for these bacteria.

# TARGETING THE MOST EFFECTIVE CHEMICAL TO THE MOST RESPONSIVE ADDITION POINT

When the identities and location of problematic organisms are known, it is possible to accurately target the right chemicals to correct the problem. Knowing which organisms are present makes it easier to select

Figure 4. MAP Technology allows for proactive diagnosis of microbial problems leading to targeted treatment strategies that ensure process efficiency and quality



Patented,
DNA-based
monitoring
strategies
are available
to provide
more
accurate
information
regarding
microbial
growth
and biofilm

an appropriate chemical for their elimination.

### **CUSTOMER EXAMPLE**

A coated freesheet mill was experiencing product quality issues due to an outbreak of sheet defects. Onsite biomonitoring, using traditional plating and ATP tools, did not indicate a microbiological problem. Analysis of sheet defects with MAP showed that the root cause of the problem were problematic bacterial species, including adaptive and filamentous biofilm-formers (Figure 3-1). Following a process survey, the location of these bacteria in the process was identified to determine the most responsive addition point for the biocide (Figure 3-2). Subsequent MAP testing confirmed eradication of problematic bacteria from the process (Figure 3-3).

### CONCLUSION

Conventional plating techniques and oxidant residuals may indicate adequate biocide dosing and control of microbial growth, while deposition, defects and breaks remain prevalent. Patented, DNA-based monitoring strategies are available to provide more accurate information regarding microbial growth and biofilm formation in industrial water systems. These strategies allow for rapid analysis of the contribution of microorganisms to deposit formation, and can be used to rapidly determine whether or not deposits containing microorganisms are contributing to defects. Quantitative PCR techniques allow for rapid analysis of sheet defects to determine the contribution of microorganisms to quality issues. This new approach has been demonstrated to allow for a more proactive diagnosis of problems leading to improved machine efficiency and product quality (Figure 4).

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