A Special Case Study of Bacteria Issues in Molded Fiber Process using DMAIC Methodology

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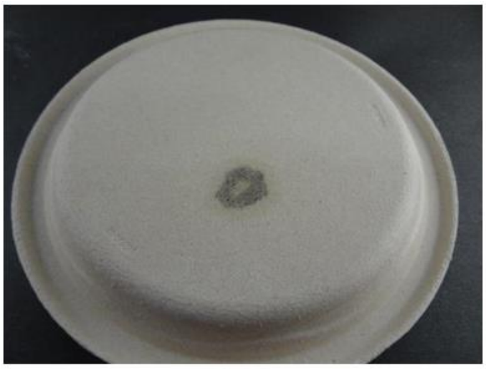
Abstract: The future market for food packaging calls for the development of sustainable and environmentally friendly products that meet the criteria of the FDA for safe consumer usage. Molded fiber processing techniques can be applied to achieve this need. However, bacteria control in a wet pulp mill environment can be challenging. This case study effectively analyzes bacteria sources and creates a solution to bacteria control, applying DMAIC methodology, along with noting the effects on the process and finished product.

Keywords: molded fiber, food packaging, pulp process, bacteria, biofilm, DMAIC

1. Introduction

Paper and pulp mills are prone to bacteria growth due to the nature of the wet environment with a naturally decomposing composition of the pulp slurry. The byproducts created from the metabolism of the bacteria put the mill at risk of explosion, fire, poor working conditions for employees, and defects in the product produced. Molded fiber production is an emerging market for food packaging. There are few published study documents on the subject.

The emerging molded fiber products, such as dinnerware. Formers, dies, tanks, piping, etc. are not difficult to produce. However, due to the process uniqueness, such molded fiber products can have many special defects that were of unknown causation. One of the most concerning defects is a black spot often surrounded by a ring of staining, refer to Figure 1. The black spots were seen on multiple formers and cavities and were intermittent in nature and size. Products with such a defect are unacceptable to customers.



a) Contamination on a plate

b) Zoom-in view of contamination

Figure 1 Fiber Plat Contamination

Addressing this quality issue, this case study demonstrates the necessity of hygiene programs and strives to understand the microbiology within the pulping process and its possible effects on the equipment and products produced. The study applies the proven DMAIC methodology and focuses on the measurement and analysis methods for quantifying bacteria issues. Based on the findings, this case study develops a good understanding and preventive controls in the production processes.

2. Problem Solving and Discussion

2.1 Define Phase

2.1.1 Introduction to Pulping Process

Paper and pulp mills have large tanks and pipe systems of wet slurry and are often enclosed, which perpetuates an environment for the bacteria to thrive, refer to Figure 2. Water temperatures rise during the mixing process, simply from the mass agitation of the slurry alone. Inside the tanks, the environment provides a breeding ground for bacteria with its humid and moist conditions, often devoid of light.

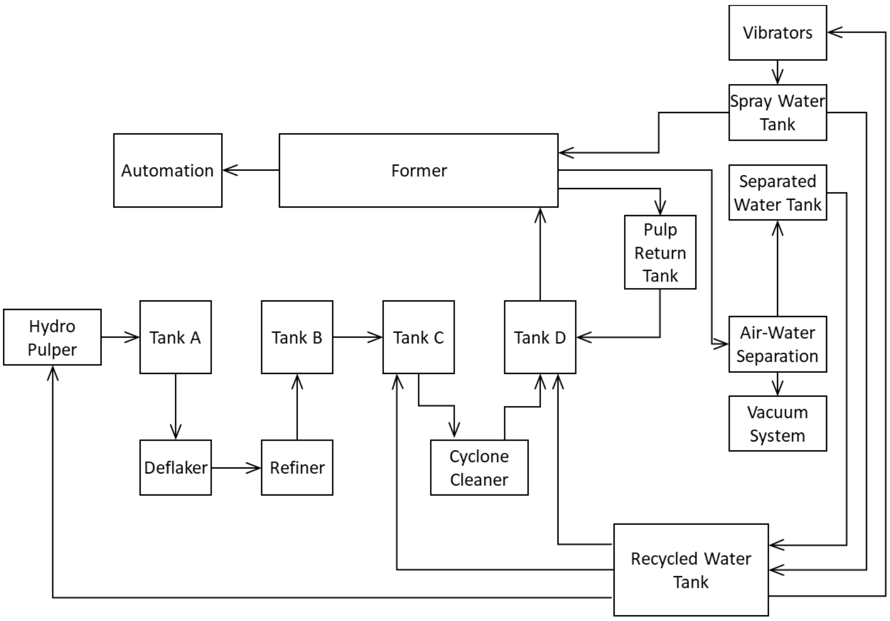


Figure 2 An overview of the piping system for the case study

2.1.2 Problem Description

To better understand and define the problem, the process was also scheduled a shutdown to inspect and deep clean the process lines, as the processing equipment has been used for approximately two years. During the scheduled shutdown, every pipe, valve, dead leg, tank, etc. and bleach and sanitize them were opened. The inside of pipes was inspected with borescopes, and the observations were documented.

The inspections reveal some areas with mold-like appearance and the expected slime that is normal in a wet environment.

Accordingly, the problem statement was developed: “There are unknown amounts and species of mold/bacteria growth in the process, leading to quality and regulatory concerns.”

Note that the mold/bacteria in the pipes and on equipment could not be linked to the black spots on the products. These were considered two separate issues until proven otherwise.

2.1.3 DMAIC Project Preparation

This problem-solving project is unique and new in the field, significant efforts were made on the project preparation, including:

- Gaining understanding of food industry requirements vs. actual conditions

- Preparing management and resources for the project
- Having a kickoff meeting to review the problem, necessity, action items, etc.
- Searching for vendor with the accreditation to analyze bacteria and conducting quoting process
- Starting baseline investigations, such as about 150 swabs taken throughout the process to set baseline bacteria counts, and additional swabbing for product contact surfaces and compressed air testing
- Consulting experts in the field of chemical interactions in slurry systems, papermaking experts, water analysis
- Reviewing risks associated with design, process steps, and bacteria speciation
- Reviewing the current cleanout practices and limits for bacteria counts
- Gaining understanding of food industry requirements vs. actual conditions

2.2 Measurement Phase

2.2.1 Technical Information related to Bacteria

Bacteria formation and its effects, such as biofilm growth is a byproduct of bacteria, are the unique foundation of this project's solutions and new treatment plans. Within the biofilm, there are microbial cells that produce a self-secreted substance that allows the cells to attach to themselves, or a surface such as the side of a slurry tank or pipe wall That is referred to as an extracellular polymeric substance (EPS).

The EPS secretion consists of polysaccharides, proteins, lipids, and nucleic acids (Verderosa, 2019, p. 3). It provides protection against harsh environments by providing structure and allowing the bacteria to capture nutrients needed for survival. Due to the EPS consisting of several types of bacteria, a mature biofilm has increased survival rates against antimicrobial agents. It often takes 10-100 times stronger concentration to kill the biofilm than it would to kill each bacterium in its most basic state (Torres, 2012, p. 340).

The stages of biofilm production include:

- Individual bacteria find a surface to attach to
- The EPS is released which strongly affixes the bacteria to the surface, causing clusters of the individual bacteria
- Layers are formed, with water channels and larger groupings of bacteria
- The biofilm reaches its fullest potential of cellular density
- Small sections of bacteria are released, finding new surfaces to expand their growth

Oxygen reduction within the slurry occurs as a byproduct of this metabolism and the production of volatile fatty acids and hydrogen sulfide. These conditions, accompanied by the moist, warm environment previously mentioned, accelerate the corrosion of some metals within the process, such as that in tanks and pipes. Additionally, certain types of bacteria, known as sulfate-reducing bacteria (SRB), can take the hydrogen that is normally produced from the metabolism of bacteria and use it to produce hydrogen sulfide or methane. Iron that is normally present in piping systems can be corroded from SRBs, especially if the sulfur and thiosulfate are metabolized into sulfuric acid (Chaudhari, 2022, p. 2).

2.2.2 Bacteria Found

In this study, the biofilm of the production process was considered a level 1 when reviewed by the experts, with only a thin layer of slippery slime attached to the tank walls. In some areas, the formation of biofilm exceeded level 1 growth, or a thicker film, in which the EPS secretion and bacteria colony formation would be more difficult to remove.

In addition to the thickness of the biofilm, a critical part of the measurement phase was to understand the speciation of the bacteria within the system. Different types of bacteria can play distinct roles in biofilm and slurry, often leading to different types of destruction or danger. For example, the production of hydrogen, hydrogen sulfide, and volatile fatty acids results from anaerobic bacteria and the normal process of metabolism. This can be extremely dangerous, with the design and monitoring of the industrial process critical to overall performance of the slurry, as it is the resultant toxic gases from these bacteria that puts paper mills at risk of explosion and deadly odor. It was discovered that the process had several types of bacteria, notably three of which are SRBs. The speciation of bacteria found in the system includes: acinetobacter modestus, agrobacterium radiobacter, elizabethkingia miricola, microbacterium oxydans, stenotrophomonas maltophilia, pseudomonas mosselii, fictibacillus halophilus, sphingomonas parapaucimobilis, staphylococcus species, bacillus cereus, and salmonella.

2.2.3 Link between bacteria and black spots

The hydrogen sulfide created as the byproduct from some anaerobic bacteria is known at times to react with iron, chemically creating iron sulfide. This iron source is readily available from some tank or piping materials, groundwater inputs, and is also found in many types of wood in which the slurry is composed. Often, this condition is visible on the products in the form of black specs (Schwingel, 1997, p. 140).

The first notation of this type of black speck that could be found was in reference to the term "sulphur stinker". This term was first documented in 1927 in a study by Werkman and Weaver. This phrase describes the spoilage sometimes found when canning food products, when the food can show blackening, the brine is blackened, or when there are spangles present on the inside of the can (Speck, 1981, p. 150). Military Standard MIL-STD-900 was created in response to these spoilages and provides insight to a cannery on acceptable levels of bacteria. In this study, hydrogen sulfide was from cystine, commonly found in the ground. Therefore, there is risk to a pulp mill if they are importing starches that have not been given a sterilizing treatment.

The chemistry package that is being used for oil and grease resistance contains paraffin wax. When the finished goods product was tested with Fourier transform infrared spectroscopy, the areas with black spots were found to contain elevated levels of this paraffin wax chemistry. This was confirmed by three separate testing labs.

The process uses stainless steel 304 wherever possible due to the wet environments. This grade contains roughly 66% iron, 18% chromium, and 8% nickel, with other alloying metals making up the rest of the composition.

Ball valves, bolts, bracing within the former's structural framework, and the dies are composed of the best available choices, designed for the same wet environment. Considering this, sometimes the black spots contained little flecks of metal that were barely visible but confirmed in testing from both the internal analytical laboratory and an outside vendor.

2.3 Analyze Phase

2.3.1 Lab Analysis

To compare, fiber plates were cut into portions with a notable contamination spot and portions without. Both portions were digested with concentrated nitric and hydrochloric acid (1:3 ratio). The samples were analyzed using inductively coupled plasma - optical emission spectrometry (ICP-OES) and the results were then normalized, and major and minor analytes are reported. The total metals recovered by ICP-OES from these portions were 0.12% and 0.04%, respectively (Table 1).

Table 1 Analysis of Fiber Plate Contamination

Sample Name	**Contaminated**	**Uncontaminated**
Inferred Components	Iron, Calcium, Chromium, Nickel	Calcium
% Metals Recovered	0.12%	0.04%
Analyte	**% of Analyte in Acid Soluble Portion Recovered by ICP-OES**	
Iron (mg/L as Fe^{+2})	50.38%	0.00%
Calcium (mg/L as Ca^{+2})	34.02%	100.00%

Chromium (mg/L as Cr^{+3})	11.19%	0.00%
Nickel (mg/L as Ni^{+2})	4.42%	0.00%

A separate portion of the plates was soaked in dichloromethane to extract oil soluble organic molecules out of the plates. The solvent was then evaporated, and there remained a wax-like substance extracted from both the contaminated and uncontaminated portion. There appeared to be significantly less wax in the uncontaminated portion compared to the contaminated portion. An FTIR was performed on a wax-like substance, and it was found to match closely with the spectra for paraffin (Figure 3).

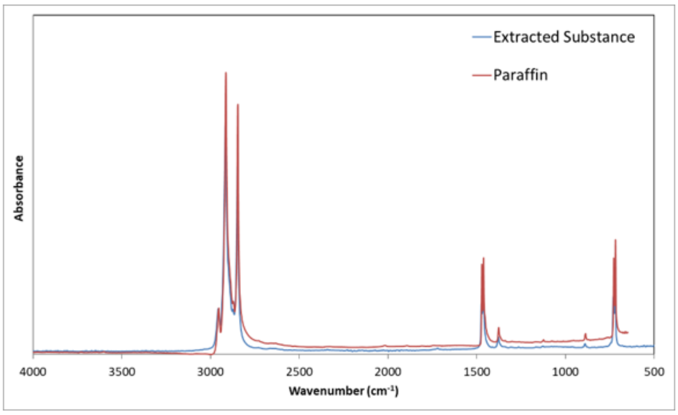


Figure 3 Contaminated Fiber Plate Extraction FTIR

2.3.2 Lab Findings

The lab analysis results indicated the spots to be a combination of paraffin, iron, calcium, chromium, and nickel, and that the metal was visibly embedded into the fiber. The only consistent analyte between the spotted and unspotted is calcium, thus leaving paraffin, iron, chromium, and nickel. Potential sources of contamination include:

- Stainless steel corrosion leaching iron, chromium, nickel. Metal reacts with additives during the forming process.
- Paraffin is deposited on stainless components within the system. Microbiologically induced corrosion (MIC) occurs under deposit, eventually sloughing off into the process with time.
- Paraffin is deposited as the result of biofilm growth/corrosion cell formation on stainless steel surfaces.

The black spots contained pieces of metal. Tiny silver specks of metals exist in the stereo microscope image (Figure 4). Soaked contaminated spots in acid, iron, calcium, nickel, and chromium are present. The uncontaminated piece only had calcium in it – no other metals. The contaminated black spot also contained more wax than the uncontaminated piece. The wax matched closely with paraffin when looking at it on the FTIR.

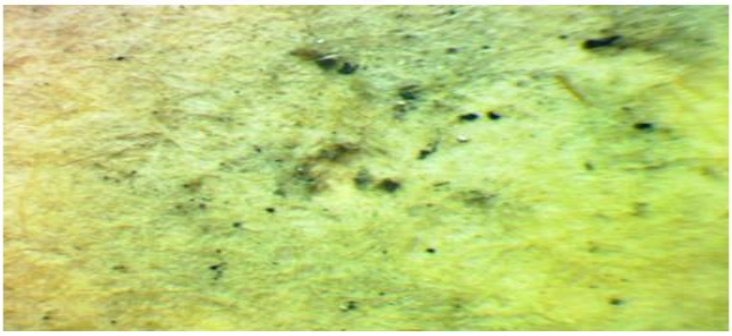


Figure 4 Fiber Plate Stereoscope Image

2.4 Improve Phase

2.4.1 Clearning Process

The development of cleaning regimens was a lengthy process, as they started with bleaching processes. The bleaching is merely sanitizing the surface with but not actually penetrating any biofilm growth, nor does the bleach act as an agitator to the surface. Therefore, bleaching does not remove any substantial film from the tanks or pipes due to bacteria. However, the routine cleaning was proving to be effective, as the defects were all but eliminated after the initial implementation in the first part of September (Figure 5).

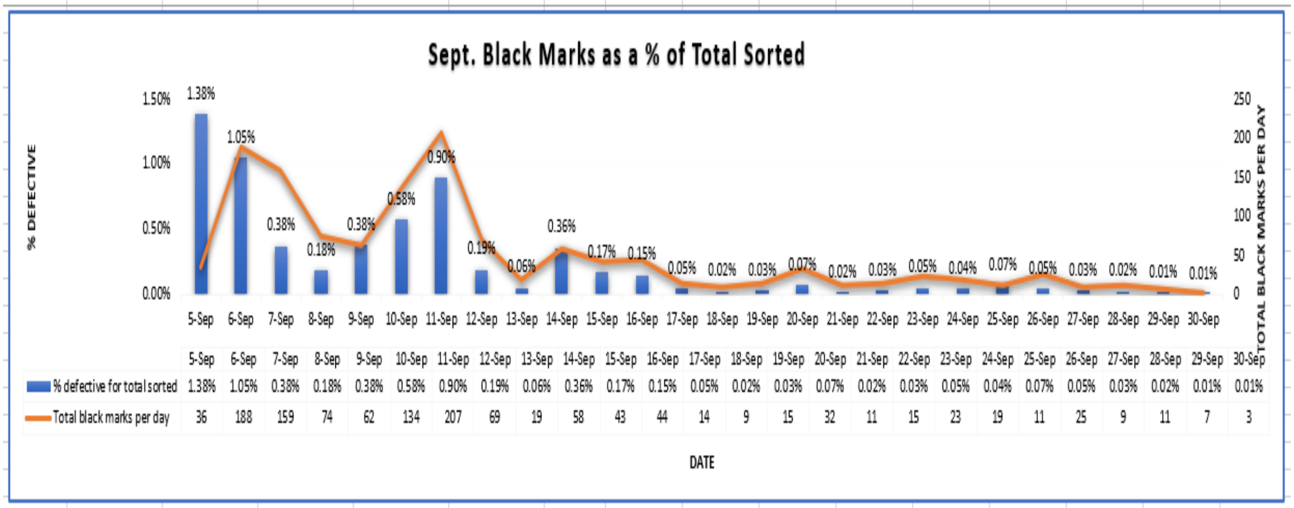


Figure 5 Chart of Sort Results for September

A filtration system was implemented to "catch" the black spots (wax and bacteria deposits), with immediate positive results (Figure 6). Since there is a recirculation system for the slurry, this helped to prevent the deposits from remaining in the slurry until they were formed into finished goods. A vision system for long-term detection of the black spots on finished products is to be used, and sorting of the finished goods as a short-term solution is implemented. Noting that these are both necessary reactive controls, due to the design of the process.

Figure 6 Filtration Bags with Evidence of Black Contamination

Rather by accident, the process quality discovered that the black spots seemed to flare up after a period of cleaning, or any notable amount of downtime, once the equipment was restarted. The cleaning process itself, including spraying off tanks, actually increased the part defects, as immediately after the cleaning it could be expected that the following 24 hours would show increased defects in the product (Figure 7).

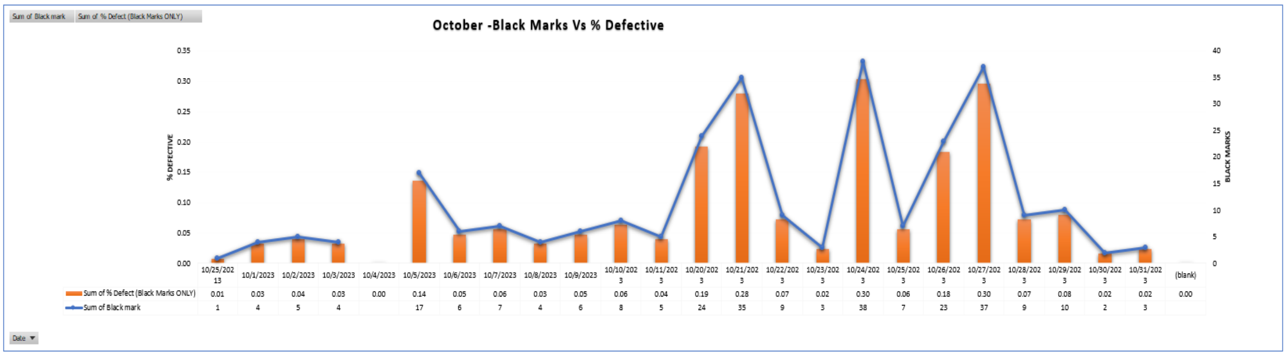


Figure 7 Chart of Sorting Results for October

The process further reduced the amount of chemistry (OGR) in the system, to further reduce the amount of paraffin wax in the slurry (Figure 8). This took place while monitoring the performance of the finished goods product.

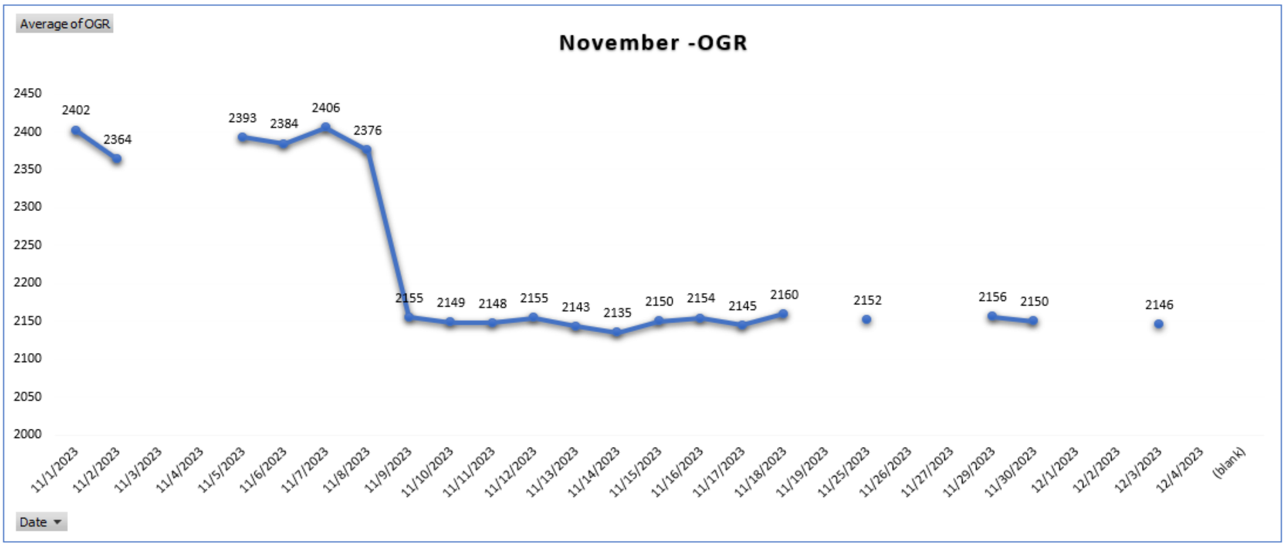


Figure 8 Chart of Oil and Grease Resistance Chemical Added to the Process

2.4.2 Biofilm Formation

The link between the bacteria and the black spots indicated that methods to manage the formation of biofilm needed to be set in place. The project team worked on many action items, with some of the most prominent noted here:

- Set in place cleanout methods that are standardized, and at set frequencies
- Each location for bacteria swabbing evaluated for risk to finished goods, and set at a frequency for monitoring that is adequate for the risk
- Re-swab continued at set interval, with data tracking and analyzation
- Establish the minimum amount of chemistry needed to obtain oil and grease resistance on the finished goods product (less chemistry = less paraffin wax in the slurry)

The process initially reduced the amount of chemistry (OGR) in the system to reduce the amount of paraffin wax in the slurry. This took place while monitoring the performance of the finished goods product (Figure 9).

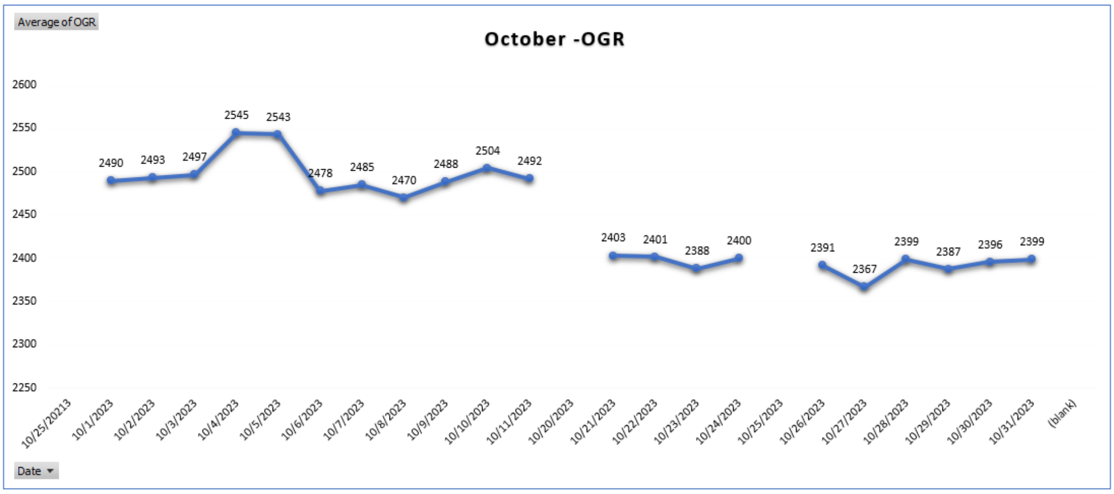


Figure 9 Chart of Oil and Grease Resistance Chemical Added to the Process

2.4.3 Discussion

Realizing the costs of third-party testing for bacteria, speciation, and the overall water quality would be long term, the management began investigating the implementing of a hygiene program for monitoring bacteria in the wet pulp system. While it was tempting to send all bacteria samples from throughout the process to an accredited third party for colony count analysis, this is not a requirement for the food packaging industry within the United States. In fact, product contact surfaces and finished product sampling are of higher risk and while sending these to an accredited laboratory makes sense, the internal slurry monitoring in the wet state does not. It was expected that within this wet system there would always be bacteria present, but the process needed to gain a state of control over the biofilm formation. The cost and resources of the process were considered, along with the accuracy of the measurement method being chosen.

Methods and tools needed to count bacteria are widely available and easily obtained from online vendors. Although plate-count methods can be time and labor intensive, they also require a sterile environment, making them difficult to implement industrially (Mueller, 2009, p. 401). There are several methods for bacteria counts that are field appropriate. Dipslides are one of the easiest methods to deploy as they are simply a plastic handheld covered in a nutrient agar coating that is pressed onto a surface to be tested for bacteria count, and then placed in an aqueous solution and incubated. The manufacturer of the dipslide prepares a density chart that is then used to interpret the results of the bacteria count. Due to this ease of use, and the minimal risk of the slurry in its wet state while inside the tanks, valves, dies, etc. the dipslide method was chosen to be deployed.

The use of the petrifilm aerobic plate count method requires a bit more skill and handling care as it is a nutrient rich film that has a gelling agent and dye that can be rehydrated. This method places diluted samples onto the film, spread out evenly (Mueller, 2009, p. 401). Error can occur due to uneven or insufficient spreading of the sample, or if too much force is used. Error can also occur in the dilution method preparation. It can be difficult to view colonies on the petrifilm without magnification. Due to these limitations, petrifilm will only be used in the process for compressed air testing going forward.

2.5 Control Phase

2.5.1 Prevention Recommendations

The management contacted expert vendors who separately but consistently recommended both a biocide and a surfactant with an agitating agent such as hydrogen peroxide. Biocide was recommended for daily use, to be included in the product recipe. The surfactant plus hydrogen peroxide was determined to be used for sixty-day deep cleanings, and twice monthly "quick" treatments on high-risk areas. Note that the speciation of bacteria type should be the driver for biocide choice.

For the development of the cleaning regimens, several factors were considered such as risk related to finished product, time to dismantle equipment, need for specialty staff such as maintenance, confined space areas, downtime of the system, etc.

2.5.2 Assessing the Risk to Finished Goods

One of the prevention measures that was at the forefront of the risk-based thinking was the desire to use predictive pathogen modeling to predict the process settings that are required to inactivate the bacteria as a known liability and risk to the end consumer. For example, the process had tooling temperatures that were set around 355°F, with a three-tool process each taking 34 seconds of cycle time. Knowing the pressures, time and temperatures should have allowed for a predictive model in which scientific claims could be made that the bacteria were inactivated. However, the only pathogen models that could be found were from the USDA regarding cooking food, and the model did not allow for inputs outside of the accepted cooking parameters.

There are certain risks on the finished goods, via testing the product at its end state, each contact surface for the product, and each step within the process back to the beginning of the raw materials. This finished product testing was reported by third party labs on many occasions to be bacteria free. Therefore, the cleaning regimen rated each location is shown in Table 2.

Table 2 Risk Analysis Matrix

	Likelihood of Exposure to Finished Goods					
Location		1	2	3	4	5
	5	Medium (6)	High (7)	High (8)	Extreme (9)	Extreme (10)
	4	Medium (5)	Medium (6)	High (7)	High (8)	Extreme (9)
	3	Medium (4)	Medium (5)	Medium (6)	High (7)	High (8)
	2	Low (3)	Medium (4)	Medium (5)	Medium (6)	High (7)
	1	Low (2)	Low (3)	Medium (4)	Medium (5)	Medium (6)
Rating	Location			Likelihood of Exposure		
1	Sample location is far from exposed contact surfaces/ production forming equipment/ finished goods/packaging.			Extremely unlikely to occur.		
2	Sample area is exposed to biocide and surfactant regularly.			Unlikely to occur.		
3	Sample area is on the production forming equipment or tools used on the production forming equipment.			Likely to occur.		
4	Sample area is a product contact surface.			Good chance it could occur.		
5	Sample area is a finished good or packaging material.			Almost certainly will occur.		

2.5.3 Dispersants and Surfactants

Surfactants are first generally used and introduced into the pulping process with hydroxides and silicates when deinking of recycled fibers is required (Torres, 2012, p. 327). Surfactants can include soaps, detergents, foaming agents and more. They work under the premise of reducing surface tension and the dissolution of grease and other buildup.

Foaming agents are especially effective for the pulp industry, as the large tanks of slurry are constantly being mixed, and they are susceptible to bacteria growth on the highest levels where residual materials cling to the tank sides. Rinsing these sidewalls down regularly and removing this buildup helps to prevent the biofilm growth. Since most anaerobic bacteria cannot survive in an oxygen rich environment, foaming agents are particularly effective. Some surfactants are mixed with hydrogen peroxide and are recommended for pulp mills. This is one of the chemical types to be used.

Further study on surfactants for the management could include the emerging market of nanomaterials that have shown to be effective. These nanomaterials are being used within the food packaging industry and have grown widely over the past few years. One nanomaterial family can be called ROS, or reactive oxygen species. Some of the staphylococcus bacteria have shown inhibitory reactions when subjected to nanomaterials that use zinc compounds when released in water solutions. With this application, the staphylococcus species can be found to be 95% effective (Shen, 2022, p. 1).

2.5.4 Biocides-using chemistry to control bacteria

Pulp and paper mills will benefit the most from prevention measures such as inhibiting the growth of the biofilm formation in the first place. There are two methods commonly used for this remediation, including biofilm-inhibition and biofilm-dispersal agents. Neither method fully resolves the biofilm growth issue, so it is in the best interests of profitability and safety that further remediation is sought (Verderosa, 2019, p. 1). In fact, once pathogenic biofilms are created, they often become as much as 1,000 times more resistant to antimicrobial treatments. This resistance is problematic and a byproduct of the insufficient methods to develop the treatment itself, as the standard development is for treatment against bacteria in the free-living mode, and not the mass of the protected layers of biofilm (Venderosa, 2019, p. 2).

A certain biofilm family called isothiazolone has been examined through gas chromatography, mass spectrometry, and trace analysis specifically for the use of desulphurization of an aqueous solution. Since the process had known sulfur issues from the previously mentioned metabolism of bacteria, this application was a good fit. One study even showed that this biocide family could target intracellular thiols of bacteria, and even some residuals left by proteins. These thiols play important roles in the enzymatic reactions of the bacteria. The study proved that the use of isothiazolone biocides can successfully prevent the malodor formation from hydrogen sulfide in the resulting finished goods that they are used in (Frerot, 2014, p. 80-81).

One disadvantage of antifouling biocides is that they can degrade in some circumstances, such as through a breakdown of chemicals due to a reaction to water, a separation of molecules when exposed to light, or even biodegradation of any biologic ingredients. These biocides can also cause issues with the marine ecosystems (Lee, 2020, p. 562).

Zinc pyrithione is a biocide used to prevent the division of bacterial and fungal cells and is known to work on the toxicity of iron-sulfur proteins in studies conducted on algae (Lee, 2020, p. 559, 560). Since anaerobic bacteria, including sulfate-reducing bacteria, exist in the pulp environment, this biocide is of particular interest for future investigation.

2.5.5 Other Environmentally friendly controls

Aeration techniques have been used in the industry, and the process investigated adding to the current aeration that is already in place in the recirculation process. These are commonly used in wastewater systems and have been investigated in large-scale reservoirs as methods of controlling bacterial colonies in water. Normally, these applications utilize a permeable layer and the influx of oxygen into the aqueous solution, at a minimum and have shown that this artificial mixing and aeration had a profound effect on the microbial communities (Zhou, 2019, p. 15). While aeration in a pulp mill can be as simple as adding an airline, certain stagnant areas such as chests or dead leg zones can be dangerous for installing aeration if they are not first treated with a biocide due to hydrogen sulfide burning readily in an excess of air (Robichaud, 1991, p. 152-153).

Even metal-organic frameworks such as zeolitic imidazolate framework-8 (ZIF-8) are being looked at for bacterial resistance in food packaging. ZIF-8 can release active zinc compounds in an aqueous solution like the slurry environment in a pulp mill. The zinc compounds then work to inactivate the bacteria when exposed to light due to the generation of reactive oxygen species (ROS). This method is similar to the use of hydrogen peroxide in that degradation of the cell wall and cytoplasmic membrane occur from the presence of ROS, which eventually can destroy the entirety of some bacteria. When this ZIF-8 is constructed into a film in the shape of a cross, it can kill E. coli, and is found to be safe for humans (Shen, 2022, p. 2, 11). ZIF-8 is also effective against S. Aureus, however, at a lower level.

3. Conclusive Remarks

This case study integrates DMAIC methodology and technical analysis, which is a usual situation. While the special subject requires in-depth analysis, understanding, and discussion.

In the processes of wet formed molded fiber food packaging products, bacteria control methods should be effective, environmentally friendly, and safe for food consumption use. This subject is relatively new and needs more study specific to this industrial environment.

Pulp mills engaging in the processing of wet molded fiber products should contain little to no bacteria. This case study is one of the pioneer explorations on this challenging subject, combining DMAIC and technical analysis. This case study established good understanding, found the complex technical root causes, improved the situations, and proposed control/prevention measures, which provide a useful reference for the DMAIC applications and the molded fiber pulp industry.

As a problem-solving DMAIC project, it achieves good understanding and results. With the mindset of continuous improvement, this project will continue for better understanding, more effective process quality control, and more sustainable solutions for the processes. For example, the possibility of using some plant-based essential oil to control bacterial growth, genetically specific customer plant products and their enzymes to counter the resistance of bacteria.

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