Efficient Brewery Sanitation

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Date: April 22, 2015
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A MAJOR QUALIFYING PROJECT REPORT

SUBMITTED TO THE FACULTY OF THE

WORCESTER POLYTECHNIC INSTITUTE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF BACHELOR OF SCIENCE

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ABSTRACT

The Wachusett Brewing Company is a microbrewery that is looking to improve the sustainability of their quality assurance programs. Through process validation and optimization, we identified their current cleaning procedure of their bright tanks and subsequently developed a revised procedure based on a benchmarking matrix. Through analytical testing methods, both procedures were within the limit of our defined quality metrics. The use of this revised procedure would improve sustainability by saving time, money, labor, energy, and water.

EXECUTIVE SUMMARY

INTRODUCTION

The Wachusett Brewing Company has been producing beer since 1994, and they are continually striving to maintain the highest quality control practices. Currently, they are looking to optimize the cleaning procedure of their bright tanks so that it is more sustainable. It should be both energy efficient and effective. This report specifically focuses on the cleaning and sanitation processes performed on Wachusett Brewery's bright tanks.

OBJECTIVES

1. Define Current Operations and Procedures Used at Wachusett Brewery
2. Compare Standards to Brewing Industry
3. Develop a Revised Cleaning Procedure
4. Validate Current and Revised Procedures
5. Provide Recommendations for Sustainability

METHODOLOGY

In order to complete our objectives, we first investigated their current procedures by performing various analytical tests to create a standard baseline in terms of quality metrics. From there, we optimized the cleaning procedures of the bright tanks by developing a benchmarking matrix, and performed the same analytical tests to determine if the results were comparable. The analytical tests performed were ATP Bioluminescence testing, PCR and Gel Electrophoresis, Gas Chromatography, and pH testing.

RESULTS & FINDINGS

After reviewing the data collected from both the modified and original procedure, it was determined that both were able to meet Wachusett's quality threshold. Additionally, the modified procedure meets these thresholds with less water, chemicals, and time required.

CONCLUSIONS & RECOMMENDATIONS

The overall results indicate that the revised procedure is as equally effective as the original procedure in terms of meeting the quality thresholds set by Wachusett Brewery. The use of the revised procedure would improve sustainability by saving time, money, labor, energy, and water. By performing a cost analysis on the water and chemical usage expenses, there is a potential to save an average of $9.11 per wash. This would ultimately save the Wachusett Brewing Company nearly $3,600 per year. In addition to the direct monetary savings, there is a utility benefit by implementing these recommendations. By incorporating Hygiena's ATP Bioluminescence testing program, they are also able to obtain faster confirmation that they are meeting their quality standards.

AUTHORSHIP

All MQP members contributed equally with the writing, editing, laboratory testing, and execution of the overall work.

ACKNOWLEDGMENTS

Our MQP group would first like to thank our beloved advisor, Professor Kmiotek, PhD, for his continuous support and guidance throughout the project. We would also like to thank our co-advisor for his assistance and experience, Professor Wobbe, PhD. In addition, we want to extend our gratitude to the Wachusett Brewing Company who sponsored this project; specifically, Cullen Dwyer and Seth Wright. We also would like to recognize Olga Kashpur, whose expertise assisted with the biological components of this project. Lastly, we would like to thank the Chemical Engineering Department at Worcester Polytechnic Institute for the numerous opportunities for professional development provided to us throughout the years.

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1. INTRODUCTION

The Wachusett Brewing Company has been producing beer since 1994, and they are continually striving to maintain the highest quality control practices. By using proper cleaning techniques and microbiological checks, contamination of the beer can be avoided. With recent growth in size, a more cost effective and efficient cleaning procedure would be beneficial to maintaining their high quality beer. The cleaning procedure should be both energy efficient and effective, which are the main points of interest for this report.

This report specifically focuses on the cleaning and sanitation processes performed on Wachusett Brewery's bright tanks. This was done by first investigating their current procedures by performing various analytical tests to create a standard baseline in terms of quality metrics. From there, we optimized the cleaning procedures of the bright tanks, and performed the same analytical tests to determine if the results were comparable. This paper elaborates, in depth, on our optimization process, as well as the testing methods used to obtain our data.

2. BACKGROUND

Microbreweries are breweries that produce 10,000 hectoliters or less of beer annually. Considering the smaller volume of product made and the area of sales as a result, these microbreweries must be extremely cautious of the opinion that the general market has of their product. The packaging, advertising, and most importantly, the quality of the beer define this branding. This is why these microbreweries pay close attention to the cleanliness of their equipment, as contamination of the beer can lead to a spoiled batch. *Lactobacillus* and *Pediococcus* are two of the primary concerns that brewers have, as they can quickly cause an off flavor of the beer.

2.1 *LACTOBACILLUS* AND *PEDIOCOCCUS*

Lactobacillus Brevis grows primarily in an environment that is around 30°C with a pH of 4-5. This is a common temperature and pH range for brewing conditions, which is why contamination by this bacteria is likely. This type of bacteria is capable of fermenting starches, but the result of contamination can range from the development of haze and sediment to acidification. There are also other substrains of *Lactobacillus*, but *L. Brevis* is the strain most commonly found. This bacterium is harmful to beer as it can change the flavor, clarity, and viscosity. Because many would prefer not to have a drink with the consistency of honey, equipment must be properly cleaned to avoid growth of this bacteria.

Pediococcus, similar to *Lactobacillus*, can also alter the taste of beer. It can produce a buttery or cheesy flavor. It can also produce sediment, which would accumulate on the bottom of the bottle or tank. These cells will not be visible until they have formed colonies or flocculated, as the cells have a diameter of 0.7-1.0 micrometer. They develop well when CO_2 is present, which can be worrisome to the majority of breweries, as many pump CO_2 into the beer throughout the brewing process. *Pediococcus* prefers an environment similar to *Lactobacillus*, as they both grow best in a temperature around 20-30°C. [1]

2.2 SANITATION AND CLEANING METHODS USED IN BREWING INDUSTRIES

Stainless steel is a commonly used material for brewing equipment. This is because it is easy to clean, highly resistant to corrosion, and it is relatively ductile. Due to the varying sizes and shapes of the equipment, it is necessary to ensure a proper cleaning technique. This is because the welding used to build the equipment can cause crevices for bacteria to build up in. There are four different methods of washing that can be performed; they include sterilization, disinfection, sanitization, and cleaning. Sterilization is intended to destroy any form of life, while disinfection is only meant to kill the microorganisms that are of concern. Sanitization is defined as lowering the level of contaminants to what is deemed acceptable, and cleaning is simply removing the visible and larger scale dirt.[2] Brewers use a combination of these four techniques to ensure a safe and healthy environment for their product.

The brewing process does inhibit the majority of possible bacterial growth. This is because it incorporates many different hurdles that bacteria must overcome in order to develop. When brewing, extremely hot temperatures are used to boil the wort in the kettle. Then hops are incorporated, which produce acids that prevent bacterial growth. There is also a low pH, along with low oxygen and nutrient levels. There is a high concentration of ethanol as well, which prevents cell membrane functions, causing bacteria to have difficulty colonizing.[1] This is why there are not many forms of bacteria that are able to survive during brewing. Brewers are often not necessarily concerned with types of bacteria that are dangerous to people because of the unlikelihood that they would form. Instead, they are more focused on the bacteria that could damage their beer.

First and foremost, companies are aware of the different forms and causes of contamination. By first preventing this, it becomes easier to maintain the quality of the beer. Raw materials, equipment, bottling lines, kegs, and even employees can track-in bacteria. This is why operators must treat the facility as a sanitary environment, and must wear proper gear. Pumping CO_2 into the conditioning tanks can also inhibit the development of bacteria. Many companies also pay close attention to the yeast they are using. If the pitching yeast used has bacterial components, it can quickly become a widespread problem as this is the yeast that is recycled across batches.[1]

Cleaning in Place (CIP) is a common practice for the industry, as it does not require the removal or changing of equipment when cleaning. Rather, CIP incorporates permanent pieces of equipment that can clean tanks, such as spray balls. COP, or Cleaning Out of Place, is sometimes used because it makes visual inspection easier, but it is very time consuming and the cleaning process has a lot of variability between operators. Ensuring consistent procedures is important for maintaining sanitary equipment that is reproducible over every shift. When cleaning, the following general steps are taken: wetting of the equipment, reaction between the soil and the water/chemical used, removal of both, and prevention of reoccurrence. Disinfection is important as it can help prevent regrowth. Some disinfectants used in industry include peracetic acid, hydrogen peroxide, and chlorine.[1] Both physical and chemical reactions must take place in order to clean the equipment. Depending on the material being cleaned and the extent of the situation, the temperature, chemical concentration, duration of washing, and flow rate of the solution can

be adjusted to appropriately match the material being scrubbed. The factors listed above should also be checked to ensure that they are environmentally and economically friendly. It is understandable that companies would like to wash and sanitize to the maximum extent, but more is not necessarily better. For example, according to the paper titled "Development of Guildelines for Microbiological Control in Microbrewery" by Elio Moretti, a common CIP procedure is listed below.

1. Pre-rinse
2. Detergent circulation
3. Intermediate Rinse
4. Drain

There are also other possible detergent circulations and rinses that can be performed if desired. This may include the use of a disinfectant or an acid. Depending on the volume of beer being produced, as well as the type and size of the equipment, the flows and temperatures can be adjusted in order to create a more efficient process.[1]

2.3 METHODS USED BY OTHER BREWERIES

Many microbreweries use similar cleaning procedures, as they are effective and easily reproducible between companies. Highland Brewing Company and Magic Hat Brewing Company were interviewed to further understand what other facilities use to clean and validate their processes, in order to build upon the current methods used by Wachusett Brewery.

2.3.1 HIGHLAND BREWING COMPANY

Highland Brewery, located in Asheville, North Carolina, uses similar cleaning processes as Wachusett Brewery. Before washing, Highland purges the tank of any CO_2 as it can react with the sodium hydroxide that is used later on. From there, they perform an initial rinse on the fermenters and bright tanks to remove any remaining beer before starting a caustic cycle. Any of the water that is used in the brewery is run through a carbon filter, as they already have soft water. The caustic cycle is run at 130°F and is performed in half hour bursts. After the caustic cycle, a water rinse is used, followed by an acid cycle. Wachusett Brewery only performs the acid cycle on an as needed basis, but Highland uses it during every wash cycle. This is done because acid can breakdown the protein and organic buildup that may develop over time. Another water rinse is used to clean out the remaining acid before incorporating the sanitizer, which is peracetic acid. The sanitizer is used to wash the tank for 10 minutes before it is ready for another batch of beer.

To ensure a sanitary environment, Highland tests multiple locations for bacteria. This includes the manway, due to of ease of access, before and after sanitation. They also test the last rinse water because it is considered to be a homogenized sample. Currently, they use both surface and rinse water swabs for ATP bioluminescence testing to verify cleanliness. If their ATP results are beyond the fail limit, they will redo their cleaning in place process and will then retest the tank. They also said that breweries should rely more on experience rather than data results alone.[3]

2.3.2 Magic Hat Brewing Company

Magic Hat Brewery in Vermont also uses surface ATP testing to monitor their bright tanks. They swab more locations for microorganisms than Highland Brewery and Wachusett Brewery. They test inside the bottling line, specifically in the filler tubes, capper, and rinsers. Magic Hat also tests the filling spear of the keg and all of the tanks that have been cleaned, by swabbing the surface, rather than analyzing at the rinse water.[4]

2.4 ATP BIOLUMINESCENCE TESTING

Wachusett Brewery is interested in incorporating a testing protocol that could give them more rapid results. They wanted to receive validation as close to real-time as possible. Currently, the only quality tests performed at the brewery consist of plating cell culture samples of beer to check for bacterial contamination. However, cell culture tests are limited with respect to the fact that it could take four to seven days for bacteria to grow. An additional testing method that is proven to be proactive for quality control would benefit the brewery immensely.

A feasible testing method to include into the brewery's quality assurance program could be ATP Testing, which is based on the ATP bioluminescence reaction. ATP is an organic molecule that is used by living cells as their main source of energy.[5] Animal, plant, bacterial, yeast, and mold cells produce and break down ATP to aid in several biological processes.

One limitation of measuring the ATP on a surface or in rinse water is that the assay cannot tell which ATP molecules originate from yeast, product residue, bacteria, or other possible sources. Even so, the presence of ATP on a surface or in rinse water, regardless of the source, indicates that it has not been adequately cleaned and can possibly support further bacterial growth. Therefore, ATP is an ideal indicator of surface cleanliness and it can be both quickly and easily used for validation purposes.

The bioluminescence reaction is a two-step reaction, and occurs as follows:

(1) Luciferin+Luciferase+ATP → Luciferin-Luciferase-AMP + PPi
(2) Luciferin-Luciferase-AMP →Oxyluciferin+Luciferase+AMP+CO_2 + photon

The light emitted is proportional to the amount of ATP in the sample when all other reactants are in excess.[6]

Because there are a large number of measurement systems and testing protocols that exist, this project focuses on an evaluation of the EnSure System by Hygiena. When the Luciferin/Luciferase enzymes are exposed to ATP, light is produced. The light is detected and measured by the Hygiena EnSure luminometer that uses a photodiode sensor. The amount of light detected (in units of RLU, or Relative Light Units) is linearly correlated to the quantity of ATP collected. Concerning technical specifications for the EnSure System, the correlation is one femtomole ATP equal to five RLU when using either the AquaSnap or UltraSnap swabs. In a general sense, a higher reading indicates a higher level of contamination in the sample.

Hygiena produces several swab types. The first is UltraSnap, which is a swab that tests hard surfaces. The surface swabs come with a buffer moistened swab bud that aids in the removal of any biological material (ATP) on either a wet or dry surface. Once the sample is collected and exposed to the buffer solution in the Hygiena device, the ATP contained inside the collected cells will be released. ATP released from inside these cells, along with any free ATP picked up from the surface by the swab, is now available to react with the chemistry in the bulb of the device.

The other types of swabs are the AquaSnap Free and Total swabs, which collect ATP from water samples. The AquaSnap Free swab collects ATP that is free in solution, or unbound inside of cells. The AquaSnap Total swab contains a detergent to release ATP that is bound to microbial or organic matter, or is inside microbes, and thus it measures both free ATP in solution and ATP from microbial sources. The honey dipper shaped tip of the AquaSnap swabs allow for consistent collection of 100 μl of sample each time. Measuring both the total and free ATP, and taking the difference in RLU, will result in a good estimate for how much ATP is coming from bacterial sources. The SuperSnap and AquaSnap swabs both have a shelf life of 15 months at refrigerated temperatures, and one month at room temperature.

When researching acceptable RLU results that we should set for Wachusett Brewery, Hygiena was able to provide RLU value guidelines that are typical of those found in the food and beverage industries for stainless steel surfaces. As seen in *Figure 1*, these guidelines represent those typically used in the sectors listed. These should only be used for guidance in establishing *Pass*, *Caution*, and *Fail* thresholds for similar surfaces and products**.** The EnSure System comes with standard pass/fail guidelines that match high-risk general food processors, which is highlighted in the figure below. The guidelines are Pass: <10 RLU, Caution: 10-30 RLU, and Fail: >30 RLU.

High Risk Product Surfaces/CIP (Clean-In-Place) systems

PRODUCTION ENVIRONMENTS	PASS	CAUTION	FAIL
Dairy (milk, cream products)	< 15	16 – 29	> 30
Juice products	< 15	16 – 29	> 30
Water bottling	< 15	16 – 29	> 30
Brewing plant and equipment	< 15	16 – 29	> 30
CIP (Clean-In-Place) Systems	< 5	6 – 9	> 10

Typical Product Surfaces

PRODUCTION ENVIRONMENTS	PASS	CAUTION	FAIL
Raw meat *Abattoirs* *Butchery*	< 100 < 50	101 – 199 51 – 99	> 200 > 100
Cooked meat products (Meats & all product w/meat) *Low risk* *High risk*	< 50 < 25	51 - 99 26 - 49	> 100 > 50
Fish products	< 30	31 - 59	> 60
Shellfish	< 100	101 - 199	> 200
Cheese processing	< 25	26 - 49	> 50
General food processors *Low risk* *High risk*	< 30 < 10	31 - 49 11 - 29	> 50 > 30
Vegetable processing *Low risk* *High risk*	< 50 < 10	51 - 74 11 - 29	> 75 > 30
Baked products *Low risk* *High risk*	< 30 < 10	31 - 49 11 - 29	> 50 > 30

FIGURE 1: ATP THRESHOLD GUIDELINES

2.5 PCR AND GEL ELECTROPHORESIS

Polymerase Chain Reaction, or PCR, is best known for identifying DNA to solve crime investigations. We decided to use it in conjunction with ATP bioluminescence testing at Wachusett Brewery. It is a tool that works by selectively amplifying certain DNA fragments. This process can also be applied to bacteria, so it can be used to determine whether the Wachusett Brewery cleaning procedure is effective. Considering the small volume of bacteria that was expected to be present, PCR was used rather than plating. PCR works by denaturing the DNA strand, or splitting the strand by breaking the hydrogen bonds. DNA primers then attach onto each strand during the annealing stage, and the nucleotides attach onto the rest of each individual strand during the elongation phase. Following PCR, gel electrophoresis can then be

used to determine growth from PCR. Below is a list of the necessary materials in order to run this test:[7]

- Sample – Whatever the experimental material is. It is helpful to place the sample in a small container with glass beads and to then vortex it. This breaks open the nuclear membrane, making the DNA more accessible.
- Template- The template helps to target the desired portion of DNA that will be amplified.
- Primers-They are small sections of DNA that compliments the desired region of the original DNA that will be replicated. There should be two in order to create a region to replicate (one for each end).
- Deoxynucleoside Triphosphates – DNTPs provide the basis for the DNA to bind and polymerize.
- Magnesium-The enzymes used to lyse and polymerize are magnesium dependent. An appropriate concentration can help the enzyme work more efficiently.
- Buffer for pH – The enzymes are also pH sensitive.
- Polymerase – DNA Polymerase builds a new strand of DNA complimentary to the single strand of DNA that is present.
- Water- This is used to obtain the correct concentration of primers and templates.
- *E. Coli* – The standard that can be used to determine a growth curve. This can be used to create a ratio to find the initial volume of experimental bacteria.
- GoTaq- The master mix contains the template, magnesium, DNTPs, and buffer.

After combining the materials above, the samples can be placed in the PCR. Once the run is complete, the samples can be stored in the 4°C refrigerator. From there, the gel electrophoresis can be used to separate DNA. The agarose should first be mixed and solidified, then the samples can be placed into the gel, and the equipment turned on. Be sure the positive and negative electrodes are aligned correctly before running. Below are the materials needed to run gel electrophoresis.

- Buffer – A solution that works as a medium for the DNA to move through the gel. 1x Tris-acetate-EDTA (TAE) was used in this case.
- Agarose gel, 1% in TAE
    - 0.05 g agarose
    - 50 ml TAE
- Ethidium Bromide – Binds to DNA and stains it. Should be mixed with the sample before running the gel.
- Marker – Used as another control to compare with samples. Its marks can be used to determine the mass of the DNA.

The TAE and agarose should be mixed and microwaved. Once boiling, they can be poured into the stand, with the dividers in place. After the gel is solidified, the dividers can be removed, leaving wells for the samples to be placed in. The gel should then be covered with TAE. The samples can be put into the gel, and the electrodes placed into the lid of the gel container. It should then be run until the marker reaches the end of the gel. The gel can be removed and imaged.

2.6 GAS CHROMATOGRAPHY

Brewing beer leads to a variety of side products and waste. This waste can be on the macro scale, such as dirt, plant material, and other leftover ingredients. These soils can be easily washed away by rinsing the tank. On the micro scale, the soils are more varied and complex. These types of soils can be grouped into two basic categories: biological and chemical. Typical biological contaminates are the bacteria and yeast from fermentation. Chemical contaminates come from many sources such as side reactions of fermentation, organics, inorganics, dissolved minerals in the water, and even the chemical solutions used to clean the tank.

Using analytical chemistry, two pertinent aspects of the process verification can be determined. It is not only important to know the composition of the effluent wastewater, but also to model the changes in composition throughout the cleaning process. To validate the current cleaning process, we will use gas chromatography (GC) to define the composition of organic material in the water and to see how it changes over time.

Gas chromatography is a chemical analytical technique that can be used to determine organic compounds in the wastewater. A sample is injected into a heated system. The liquid is then vaporized, separated, and eluted though a column by an inert gaseous mobile phase. The sample is then passed through a detector where the sample is analyzed. GC is especially useful because it can analyze compounds that are highly dilute and can vaporize the material without decomposition.

Wachusett Brewery cleans its tanks using a cleaning solution consisting of water and an industrial caustic mix. Because the cleaning process uses a caustic chemical solution to clean the tanks, it is also a contaminant that needs to be removed. Although the solution is mostly sodium hydroxide (an inorganic compound) it does contain a significant amount of sodium gluconate, a chelating acid that is organic, and thus can be detected.

Typically, GC is used in laboratory settings for non-food grade applications for the separation and identification of organic compounds. However, the use of this type of spectral analysis is becoming more prevalent for quality control in breweries. GC is now being used for food science applications to identify problems or changes in the brewing process that can affect the taste or quality of the final product.

Flavor Characterization

Instead of using GC for sanitation purposes, breweries like Boulevard Brewing Company use GC to study their beer for taste purposes. They measure the concentration of esters, sulfur compounds, diacetyl, and most importantly, hop oils. From this information, brewers can adjust brewing conditions and hopping rates to more effectively produce desired flavors. Characterizing the beer by developing flavor profiles gives Master Brewers the ability to define beer flavors without relying solely on subjective taste testing.[8]

Alcohol Proof

GC can be used as an alternative method to determine the alcohol proof (ethanol content) of the beer instead of using a typical hydrometer. Based on the strength of the signal and the area under the curves, concentration of alcohol can be calculated more accurately than when using hydrometer estimations. The GC can also be used to monitor other types of alcohols and aldehydes. Combined with a mass spectrometer (MS), GC can identify the different alcohols present in the beer which can be indicative of undesired side reactions during fermentation. Monitoring these compounds can allow brewers to refine their fermentation techniques to produce the desired chemicals and have an accurate alcohol proof for the more complex craft beers. [9]

Chemical Characterization

GC can also be used in other quality assurance applications. Besides flavor compounds and alcohol modeling, GC can be used to monitor unwanted chemicals in beer. The typical groups of contaminates found in beer are vicinal diketones, acetaldehyde, trihalomethanes, and sulfur compounds. These chemicals are important to monitor because they can contribute to off-flavors, and they are indicative of undesired side reactions, bacterial spoilage, and contamination. [10]

2.7 ULTRAVIOLET RIBOFLAVIN TESTING

Another important part of process validation is ensuring that the cleaning chemicals and other residuals have been removed from the process equipment and associated piping. Efficient cleaning of the tanks in the process system requires that the surfaces be completely covered by the spray pattern introduced through the spray devices. This is usually examined by performing a "Riboflavin Validation" test.

In industry, the riboflavin test is used for surface inspection of components, vessels, machines, and other process equipment that require maximum surface cleanliness.[11] The goal of this control procedure is to detect critical areas on the test surface, or weak points that are not being adequately covered. In addition, it can demonstrate complete wettability and capability of vessel washing. This is an analysis that is completely visual, and is based upon the response of the riboflavin compound to ultraviolet light.[12] If blind spots are identified during spray coverage testing, then corrective actions should be performed to ensure that the blind spot is cleaned.[13]

The general steps for the Ultraviolet Riboflavin Test are as follows:[14]

Step 1: Riboflavin solution is evenly sprayed, by means of a sprayer, over the entire vessel surface, most thoroughly around openings, connectors, and joints.
Step 2: Then it is checked with an ultraviolet lamp if the solution was thoroughly sprayed over the surface. The riboflavin solution fluoresces when illuminated by the ultraviolet light.
Step 3: Afterwards, the vessel is washed with clean water. The washing is done over the existing connectors on the vessel (cleaning head, cleaning nozzle, etc.). Make sure to follow the normally used flow rates and rinse times. Perform coverage testing utilizing a water rinse that is equivalent to the shortest phase of the cleaning cycle. Verify the equipment is drained after testing is

complete.
Step 4: After washing the vessel, the entire surface is again checked with the ultraviolet lamp for remains of any fluorescent substance.

Three detailed procedures for Riboflavin Testing were found. The first procedure was prepared by the Riboflavin Test: Working Party of the Sterile Process Engineering Group of VDMA(Verband Deutscher Maschinen- und Anlagenbau, German Engineering Association) [15], the second was published documentation of a procedure from AB Process Group[16], and the third was from the Journal of GXP Compliance[13]. Our own testing procedure was based closely on the previous procedures from these reputable sources.

2.8 PH

Digital pH meters can be used to determine the pH values of various samples at a brewery. This testing method can be utilized on the house water, as well as the water from each rinse cycle after the caustic rinse. Using this data, a baseline pH can be established from the house water. This baseline can then be compared to the water from the post-caustic rinse cycles. Knowing that caustic is a basic solution that contains sodium hydroxide, by testing the rinse water, we can then determine if all of the caustic has been removed from the bright tank. For example, if the last burst rinse has a pH closer to ten, and not a neutral seven, then it can be concluded that further rinses are necessary. It is important that all of the caustic cleaning solution is removed from the bright tanks to prevent the fermented beer from coming into contact with hazardous material. This can result in off flavors and possibly unsafe amounts of caustic in the beer. This pH test can also help determine the minimum number of post-caustic rinses that are necessary to return the water to the same pH as the house water.

2.9 HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP)

A sanitation quality control plan is a preventative program, as opposed to a troubleshooting program, that identifies sources of the problem after the fact.[17] It should be designed to measure and control all factors that could affect product quality, as well as maintain the criteria and methods of measurement, records and reports, and safety standards. The plan must be specific to the areas of application. This includes the associated surfaces and equipment, the methods used, and the monitoring methods and frequency. Standards for all testing values should be clearly defined. For example, some breweries have a pass/fail benchmark for the number of organisms remaining on beer contact surfaces or in CIP rinse water.

The principles of Hazard Analysis and Critical Control Points (HACCP) were incorporated into our process analysis and final recommendations within the scope of the MQP. HACCP is a food safety and quality control analysis that is used to develop procedures to establish a preventative food safety program. In industry, a food safety program for a brewery would prevent unwanted and/or harmful pathogens, mycotoxins, chemical, biological, or material contamination of the beer. Although the process of brewing beer does not normally incur the risk of growing bacteria that is harmful to humans, such as *E. coli*, there are still concerns about chemical and biological agents producing off-flavors in the beer. While food safety is always a priority, the main focus of

this analysis will be for quality assurance purposes because the health risk due to bacteria is insignificant.

Normally, the entire production process would be under consideration. This would include the entire brewing process, ingredients, handling, storage, bottling, kegging, and canning. A full HACCP analysis would be an entire project on its own and would be redundant because Wachusett Brewery already has one. Because, we are only changing one aspect within the production process, a completely new analysis is not necessary. Instead, the HACCP analysis will be modified slightly and limited to fit within the confines of this project. Therefore, the control volume will only be the conditioning tanks and transfer equipment with a focus on the sanitation procedures. The sanitation standard operating procedure contains numerous variables that can alter the effectiveness of cleaning. Since there are several variables that could affect product quality, the critical control points under analysis were chosen to be appropriate for the scope of this project.

The basis of this MQP's HACCP plan derives from a Clemson University Professor, Doctor Felix Barron, who published a paper on specific application of HACCP for microbreweries, brewpubs, and other small breweries.[18] Using his reference guide and established HACCP principles, we will provide a simple hazard analysis for the sanitation of conditioning tanks. The HACCP plan incorporates several steps and principles in order to develop an effective safety protocol.

HACCP Terminology and Definitions

1. **Critical control point:** Any point or procedure in a food process where loss of control may result in an unacceptable health risk.
2. **Critical limit:** Prescribed tolerances established to control potential or actual hazards identified in a critical control point.
3. **HACCP:** Hazard Analysis Critical Control Points
4. **HACCP plan:** The written document describing the procedures to control food that should follow the HACCP principles.
5. **HACCP system:** The result of implementation of a HACCP plan.
6. **Hazard:** Any biological, chemical, or physical contaminant in the food that may cause an unacceptable health risk to the consumer.
7. **Monitoring:** A planned sequence of observations or measurements of critical limits to generate accurate records and to maintain food safety.
8. **Verification:** Methods, procedures, and tests used to determine if the HACCP system is in compliance with the HACCP plan.

Steps to Develop a HACCP Plan

Step 1: Select the product and the process to be analyzed
As previously mentioned, the HACCP plan will focus on the procedure for cleaning conditioning tanks after beer has been in them. Therefore the product is the beer itself and the process is cleaning and preparing the tanks for more beer. This particular stage of the brewing process was chosen because it is furthest downstream and is the last step before packaging. This will allow the brewers to be sure that everything upstream was properly cleaned if the beer during this final stage does not have any contamination. If the beer is not sterile at this stage, there is almost nothing that can be done to remedy the problem after it has been packaged. The only actions that can be done is to recall the beer if the spoilage is caught early enough and to dispose of it.

Step 2: Describe the product
The product is unconditioned beer. Since there is not a specific tank for each kind of beer, the tanks can experience a wide variety of beers with many different qualities. The fruit beers have more sugars and organic material whereas, the IPAs have oils from the hops. Different ales and lagers have varying compositions, but all have the potential to leave behind organic and inorganic material that can contaminate future batches of beer. Poor cleaning could lead to a tank with significant bacterial growth or other contaminates such as beer stone, which can lead to off-flavors and spoilage of the beer.

Step 3: Write a flow diagram
Being that this a modified HACCP plan, the flow diagram is not between units in the brewing process, but rather between different states of the conditioning tank. Each state is dynamic, so it is important to consider and monitor what is going in and out of the system.

> State 1 is where the tank is full of beer. This can be before or after the beer is conditioned, which represents the beginning and end state of the cycle. After the beer has been conditioned in the bright tanks, the beer is then removed and ready for packaging. Once the tank is emptied of the mass bulk of beer, then it is at State 2.
>
> State 2 is where the tank is empty, but has residual beer leftover. In addition to the residual beer, there is the possibility for other contaminates such as leftover yeast, organic matter, and other soils to be present. Bacteria can be present as well because the tank is open to the environment. The bright tanks cannot be returned to State 1, because it is unsanitary to add new beer to the tank, before it is clean and sterile. This means that all contaminates, be it physical, chemical, or biological, have to be removed from the system. The official start of the cleaning process is at State 3.
>
> State 3 is the initial rising of the tank with tepid and hot water. It serves two purposes because not only does it wash out a significant amount of solid and residue, it also heats up the tank. The temperature of the tank is increased stepwise until it is at the temperature in which the caustic rinse is optimal. The high temperature of the tank also kills bacteria and helps remove caked on soils. Once the temperature has reached the manufacturer's

recommendation for optimal caustic performance, the tank is ready to be brought to State 4.

State 4 is the caustic rinsing of the tank. This is a combination of caustic diluted in hot water and rinsed throughout the tank. The caustic reacts with the more difficult soils on the tank surface, kills bacteria, and neutralizes the pH (because beer is acidic). The caustic mix has surfactants and acidic components that maintains the slick surface of the tank to prevent buildup of material. After the caustic wash, the tank can be brought to the next step in the process, State 5.

State 5 is the post caustic rinse. It is a series of burst rinses to remove any residual caustic. Once the burst rinses are complete, the tank is now at State 6.

State 6 is the final processing step in the cleaning and sanitation procedure. A solution of iodophor and water is added to the tank to kill all bacteria and to ensure that it is sterile. After the iodophor rinse, the tank is drained and is now brought to State 7.

State 7 is simply where the tank is empty, clean, and sterile. It is closed off from the environment in order to prevent contamination before a new batch of beer is put into the tank. Once more beer is in the tank, the cycle is complete because the tank is back at State 1.

See *Figure 2* below for a summary and flow diagram of the states during the cleaning procedure.

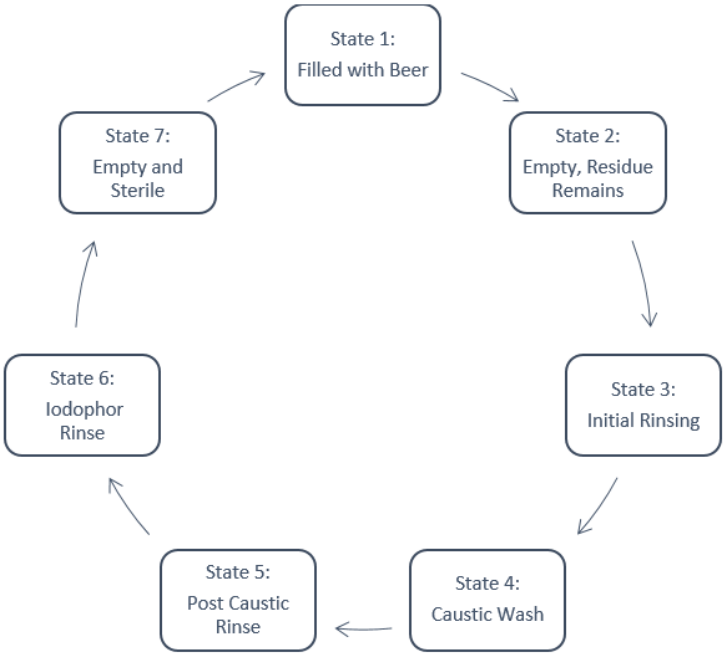


FIGURE 2: FLOW DIAGRAM FOR CLEANING OF BRIGHT TANKS

Step 4: Validate the flow diagram
The cleaning process and flow diagram have been validated by Seth, the Lead Cellar, Cullen, the head of Quality Assurance, and Howie, the Master Brewer. The old and new cleaning procedures were analyzed and developed by all parties.

Step 5: Application of the Seven Principles of HACCP
The seven principles of HACCP help develop assess, analyze, establish, and monitor quality, environmental, health, and safety considerations at critical points in the production process. In total, these principles are the basis of the HACCP plan and can be used to assist in developing new standard operating procedures (SOPs).

Principle 1: Assess the potential hazards (microbiological, chemical, or physical) at every step in the beer making process.

Excluding anything outrageous in terms of potential hazards, there are several common areas of concern. The main effort of the cleaning program is to prevent the growth of beer-spoiling bacteria. This microbiological hazard is normally difficult to detect, because it takes several days before the problem will manifest. By that time, the beer is out of the control of the brewery. Drinking skunked beer or beer with off-flavors is a detriment to the reputation of the brewery. Another potential hazard due to the presence of beer in the tank is the residue. The residue can lead to a buildup of soils that will also contribute unpleasant flavors.

Ironically, there is a hazard associated with trying to remove the biological and physical hazards. The caustic solution used to clean the tanks is hazardous at concentrated levels. This solution has to be diluted significantly before it is safe. Even at a safe level, the presence of residual caustic in the tank can lead to off-flavors as well. Overall, the biggest hazard is the presence of bacteria and caustic in the tank after it has completed the cleaning process.

Principle 2: Determine the critical control points (CCPs) necessary to control the hazards.

There are two critical control points that are necessary to control the hazards; the inside surface of the tank and the effluent wastewater. If the solution used for washing does not sufficiently clean the tank and remove all undesirables, then the cleanliness of the tank is no longer under control. On the other hand, if there is an overabundance of caustic leftover, control is lost as well. This loss of control over the effectiveness of the cleaning solution leads to a loss of control on the conditions on the inside surface of the tanks. Therefore, the concentration of caustic in the effluent wastewater and the bacterial contamination of both the wastewater and tank surface, are the two critical control points.

Principle 3: Establish the critical limits (CLs) for each CCP.

Setting the limit for residual caustic contamination is simple and well established. In the most ideal situation, the wastewater should be pH neutral. If the pure house water is not neutral, which in the case for Wachusett Brewery it is slightly basic, then the wastewater should be as close to the pH of house water as possible. The critical limit for the pH of the wastewater should not be higher than nine. If it is nine or higher, there is a likelihood that there is residual caustic in the tank. In addition, the basic water will cause other issues and costs when it is sewered.

Setting the critical limit for the bacteria content in the tank and in the wastewater is much harder to determine than the critical limit for pH. Before the MQP, the quality assurance team at Wachusett Brewery used plating to grow bacteria to see how many Colony Forming Units (CFUs) were present. If the plating resulted in more than five CFUs, then that would raise a warning flag. If the plate resulted in more than ten CFUs, then there was a serious issue with that batch of beer.

For ATP testing, the critical limits are slightly different. Industry standard dictates that the critical limits are between 30 and 100 RLUs, with 100 RLUs being the critical limit. The manufacturer's recommendation gives a lower and much narrower range of 10 to 30 RLUs for the critical limits. Case studies from other breweries have indicated a much smaller range and established that four RLUs is the critical limit. Other breweries and the manufacturer also recommended that Wachusett Brewery establish its own critical limits. This can be accomplished by taking the data of at least 100 samples over time to see what the range of values are. From there, the brewery can use a statistical analysis to establish a critical limit that best fits their company policy and quality assurance goals. From initial testing, it seems that the critical limit for the overall ATP result would be ten RLUs.

Principle 4: Establish procedures to monitor each CCP and their critical limits.

Fortunately, there are already effective measures to monitor the critical control points and their respective critical limits. With the new changes to the cleaning procedure and new testing methods, there are some adjustments needed. Chemical and physical monitoring is preferred over microbiological monitoring due to how much faster results can be obtained. While microbiological testing, such as plating, does not have to be phased out, the ATP testing can be used to supplement the quality control program. The procedure for ATP testing is discussed further in *Section 4.4.1*. For the pH of the water, a simple litmus strip test can be conducted quickly at the site of the tank to determine if the water is within the limit. For larger soils, a visual inspection can be performed to see if the tank looks clean or if there is anything out of the ordinary that warrants further investigation.

Principle 5: Establish corrective actions that need to be taken any time a deviation from critical limits occurs.

If at any time there is a deviation from the critical limit, then corrective actions must be taken to address the problem. Typically all that would be needed to rectify the problem would be to perform additional rinse cycles or redo the entire cleaning process on the offending tank. Additional corrective measures are up to the discretion of the cleaning staff.

Principle 6: Establish an effective record keeping system to document the HACCP plan.

In addition to the ATP luminometer, Hygenia's ATP testing system comes with SureTrend, a computer based software that is used as a record keeping and data analysis program. It allows Wachusett Brewery to track the results of each type of test at every location. The data analysis portion can be used to model the data and establish more fine-tuned critical limits. The biological testing records will be conducted and maintained in the same way as they had been before the project. All other record keeping will be the same as well, such as documenting pertinent information on the white boards attached to the tanks.

Principle 7: Establish procedures to verify that the HACCP system is working effectively.

To ensure that this HACCP system is working effectively, the standard biological testing should still be performed. The ATP testing should be used as a quick check to see if there are any glaring issues. Another way to verify that the ATP testing is effective is to create a standard curve to relate RLUs and CFUs. Also, the coordination between plating and ATP testing would ensure that the HACCP system is working as intended.

Using HACCP to Develop SOPs

Wachusett Brewery can use this HACCP analysis and the MQP to incorporate ATP testing into their regular quality assurance program. In addition, with the changes to the cleaning procedure, this document can be used to mitigate future risk due to the changes. Until the revised procedure has been conducted for a few months, the HAACP plan can be used as a guide to assess and prevent hazards while the new process is still in a trial period.

2.10 PROCESS VALIDATION

When analyzing a process to see if it is sufficient and operating properly, or to see what improvements are necessary, a process validation study is often performed. Process validation is an important aspect of a properly managed CIP system.[12] Three questions should be asked when performing validation on the process:

1. Is the cleaning system working as intended?
2. Has the process cleaned/sterilized to the level required?
3. Have residual chemicals been removed from the process equipment and process piping?

The measurement instruments associated with the system provide the answer to question one. These instruments include temperature gauges, fluid flow meters, pressure gauges, chemical concentration measurement devices, etc. Assuming the instruments are in good working condition and properly calibrated, their measurements can be used to ensure that the process is working according to the standard operating procedures from a technical and operational standpoint. For instance, if the flow rate data is showing consistent values lower than the targeted flow rates listed in the SOPs, then the problem is an operational one, most likely rooted in problems with a pump located upstream, blockage, or unintended sources of backpressure.

The answer to question two is obtained from analysis of the effluents, using analytical techniques such as GC (Gas Chromatography), ATP testing, and PCR techniques for estimating bacteria amounts.

The answer to question three is obtained by measuring the pH of the effluent streams. Additionally, efficient cleaning of the tanks in the process system requires that the interior surfaces be totally covered by the sprays introduced through the spray devices. The surface coverage is determined by the "Riboflavin Validation" process, a visual analysis based upon the response of the organic compound to ultraviolet light.

3. OBJECTIVES

The main goal of this MQP was to evaluate the current cleaning and sanitation techniques for the bright tanks at Wachusett Brewery and to validate their current methods with both analytical data and industry research. We also analyzed modified techniques that could result in reduced water usage, and subsequently associated operational costs. To accomplish this goal, we determined five objectives to be completed.

3.1 DEFINE CURRENT OPERATIONS AND PROCEDURES USED AT WACHUSETT BREWERY

Our first objective was to determine, in detail, the current operations and procedures used at Wachusett Brewery. This involved visiting the brewery and observing the procedures used for cleaning and sanitizing the bright tanks - essentially a series of "watch & learn" visits. We also sat down and discussed the process with the main employee who performs all of the cleaning and sanitizing tasks, and developed a detailed written procedure for their current cleaning methods. This involved noting flow rates, temperatures, times, and other important process variables.

3.2 COMPARE STANDARDS TO BREWING INDUSTRY

The second objective was to compare the cleaning procedures that Wachusett Brewery uses to other breweries and determine what industry standards may exist. These standards could include the passable limit for the ATP bioluminescence test, common chemicals used, and other quality assurance practices.

The practices from the other breweries and industry standards, will help guide us when developing a revised cleaning procedure for Wachusett Brewing Company.

3.3 DEVELOP A REVISED CLEANING PROCEDURE

The third objective was to determine areas in the procedure that could be changed to result in cost savings, increased efficiency, or sustainability. It could be possible that the Wachusett Brewery's procedures are done in excess, so we decided to propose a revised procedure that results in less water usage.

3.4 VALIDATE CURRENT AND REVISED PROCEDURES

Our fourth objective was to perform tests on both the current cleaning procedures and our revised procedures by using various tools. We performed multiple tests using ATP Bioluminescence, pH, Gas Chromatography, and PCR. This gave us a clearer picture based on analytical data as to whether or not the current procedures were sufficient, and if water usage could be reduced.

3.5 PROVIDE RECOMMENDATIONS FOR SUSTAINABILITY

Our fifth, and final objective is to provide any potential and final recommendations for cost savings, increased efficiency, and sustainability if needed, based on the conclusions arrived from objectives 1-4. The Wachusett Brewing Company was also interested in the feasibility of adding ATP testing to their current testing protocol. We chose to provide recommendations concerning these topics.

4. METHODOLOGY

The following steps were taken to accomplish our objectives listed in *Section 3*. From a process engineering standpoint, we first investigated the current operations to establish a baseline. Then, using suggestions and industry research, we developed a benchmarking matrix and ultimately an optimized procedure. Analytical testing methods were used to obtain relevant data.

4.1 CURRENT OPERATIONS AND PROCEDURES USED AT WACHUSETT BREWERY

By shadowing and interviewing the brewers and cellarers, we were able to identify the cleaning SOPs that were currently being used. We then transcribed our observations to a written document, as these procedures were traditionally passed on by apprenticeship.

4.2 COMPARE STANDARDS TO OTHER BREWERIES

Literature research and industry interviews were completed to investigate any standard cleaning practices, testing methods, and quality thresholds. This research was comprised of findings from textbooks, the Brewer's Association publications, and various journal sources. The interviews we performed were with other microbreweries that have recently implemented technologies that we were investigating.

4.3 REVISED CLEANING PROCEDURE

To determine how to revise the current cleaning procedure, we created a matrix to compare possible parameter changes, as seen in *Figure 3*. When looking at each specific parameter change, we listed the initial cleaning procedure that applies to it, or the "baseline scenario" and described the specific changes we wanted to make. From there, we analyzed each new procedure for its sustainability, benefits, possible investments required, and barriers we may encounter if

they were put into place. Based on these factors, we defined which parameters we wanted to change, see the "Conclusion" row in *Figure 3*.

Parameter							
Procedure Change	Fewer Initial Rinses	Initial Rinse Lengths	Recycle Initial Rinses	Length of Caustic Wash	Water for Caustic Wash	Fewer Final Rinses	Caustic Concentration
Baseline Scenario	1) Hot and cold 2x30 second rinses 2) Hot and half cold 2x30 second rinses 3) Hot only 2x30 second rinse	Initial rinses are 30 seconds each	1) Hot and cold together 2) Hot and half cold 3) Hot only	Caustic wash is 30 minutes	200 gal for 200 BBL tank. 100 gal for 100 BBL tank. 50 gal for 50 BBL tank	1) Hot only 2x30 second rinses 2) Hot and cold 2x30 second rinses 3) Cold and half hot 1x30 second rinse	1 gallon of caustic
Specific Changes	Reduce the number of initial rinses from 6 to 4	Change all rinses to 20 seconds each	Recycle with 80-100 gal for 2 minutes	Reduce the length of the caustic wash from 30 to 20 minutes	Reduce water volume by one half	Reduce the number of final rinses from 6 to 4	Reduce the amount of water
Sustainability Potential	High	High	Medium	Medium	Medium	Medium	Low
Benefits	Saves time and water	Saves time and water	Maximum use of thermal energy	Saves time	Saves water	Saves time and water	Saves water
Investment Required	None	None	Significant	None	None	None	None
Financial Viability	High	Medium	Low	Medium	Medium	Medium	Low
Barriers Infrastructure	None	None	None	None	Pump Cavitation	Equipment Failure Risk	Pump cavitation
Barriers Labor	None	None	Increased labor requirement	None	None	None	None
Barriers Financial	None	None	Increased labor cost	None	None	None	None
Barriers Policy	Current SOPs	Current SOPs	Current SOPs	Current SOPs	Current SOPs	Current SOPs	Current SOPs
Conclusion	Go	No Go	No Go	Go	Go	No Go	No Go
Comments	For 50 BBL tank, 1x30 second rinses are performed for each temperature as opposed to 2x 30 seconds for 100 and 200 BL tanks	Tank needs to be at 125 F. Ambient conditions may change the number of required rinses.	Does not actually save a significant amount of water.	None	None	Temperature of the tank needs to be less than 100 F. Cooling the hot tank too fast would cause tank collapse.	Caustic concentration parameters independent of tank size. Low levels of water caused cavitation in the pumps.

FIGURE 3: BENCHMARKING MATRIX

Our first option was to put the initial rinses on recycle. This would allow more of the thermal energy to be absorbed by the tank because of the longer residence time. The main concern was that if we were to put the initial rinses on recycle, then it would cause more work by the cellarers to hook and unhook the pump for recycling. As a result, we decided against making this change, but we did choose to focus on an idea provided to us by one of the employees. The idea was to decrease the number of initial rinses from six to four. This change can be seen in the first column in *Figure 3*. By decreasing the number of rinses, we are saving time and water without encountering any major barriers.

We also chose to look into the volume of water used in the caustic wash. By decreasing the amount of water and caustic, the same concentration can be used, but costs will still be reduced. We decreased the length of the caustic recycle wash as well, saving time. As a result, we decided to change the number of initial rinses as well as the volume of water. In addition, we also changed the amount of caustic and recycle time of the caustic cycle wash. The final revised procedures for the 50, 100, and 200 BBL tanks can be found in *Section 5.2*.

4.4 VALIDATE CURRENT AND REVISED PROCEDURES

After defining a new revised procedure, the rinse water samples and tank surfaces from both the original and new procedures were examined. Bioluminescence testing, PCR and Gel Electrophoresis, Gas Chromatography, and pH testing were used to obtain data to compare the effectiveness of both procedures.

4.4.1 ATP Experimental Procedure

Our MQP team received a 30-day trial to test the EnSure System with AquaSnap Total, AquaSnap Free, and UltraSnap Surface swabs. For the purposes of initial ATP testing, the pass/fail guidelines were kept at the default values that the unit came with, which were Pass: <10 RLU, Caution: 10-30 RLU, and Fail: >30 RLU. Testing plans and locations were programmed into the device after going through a one hour training session with a Hygiena representative.

For surface ATP sampling, when the cleaning procedure was finished for each bright tank, the UltraSnap swab was removed from the sealed test tube. A 4”x4” area was swabbed on the inside manway surface in a zigzag pattern, applying sufficient pressure to maximize contact between the swab and stainless steel surface. The swab was also rotated continuously throughout the sampling process. The swab was then placed back in the test tube.

For effluent water ATP sampling, the rinse water sample was shaken before testing. Before opening the swab device, it was forcefully flicked downward to release the extractant liquid from the dipper tip. The AquaSnap swab was removed from its sealed test tube, and dipped in the rinse water sample for one to two seconds. It was then placed back in the test tube. The tube was then flicked, and the bulb was broken and squeezed to release the solution into the test tube. Finally the two components were mixed together through a swirling motion. When the sampling procedure was done we utilized the EnSure luminometer to measure ATP amount. This procedure was the same for both UltraSnap and AquaSnap swab types.

4.4.2 PCR and Gel Electrophoresis

PCR was used to measure the presence of bacteria in the rinse water samples to ensure that each procedure was sanitary enough for brewing. These results were expected to fall in line with those found from the ATP testing. The *E. Coli* concentrations used for the standard curve can be found in *Table 1*. The same PCR procedure was used on the *E. Coli* as the procedure used for the brewery samples.

TABLE 1: *E. COLI* CONCENTRATIONS

Sample	Dilution Factor	CFU Count (CFU/ml)
A	100	6.0300E+03
B	1000	6.0300E+02
C	10000	6.0300E+01
D	30030	2.0080E+01
E	90090	6.6933E+00
F	270051	2.2329E+00
G	810373	7.4410E-01
H	2.43013E+06	2.4813E-01
I	2.43013E+07	2.4813E-02
J	2.43013E+08	2.4813E-03
K	2.43013E+09	2.4813E-04
L	2.43013E+10	2.4813E-05
M	2.43013E+11	2.4813E-06
N	2.43013E+12	2.4813E-07
O	2.43013E+13	2.4813E-08
P	2.43013E+14	2.4813E-09

The dilution factor means that the *E. Coli* ratio was one to whatever the dilution factor was. For example, a dilution factor of 100 means 1:100. Only *E. Coli* samples labeled B through O were used for gel electrophoresis. This is because sample A was expected to be too concentrated to compare with the brewery samples. The opposite was true for sample P, which was expected to be too dilute to be able to compare. These samples were omitted because only 20 wells were available on the gel electrophoresis. There were 14 *E. Coli* samples, three controls using material from a plating, one marker sample, and two Wachusett Brewery samples. The Wachusett Brewery samples were from the final rinse, one from each tank using the revised procedure.

PCR Procedure

1. Aliquot 5 mL of control or experimental sample and add in the same volume of glass beads
2. Vortex for 5 minutes
3. Centrifuge at full speed for 20 seconds
4. Transfer sample off of the beads into small containers that fit into the PCR
5. Create a master mix of GoTaq, water, and the template. For a 20 mL sample, the following should be used for each experimental sample being run
    a. 10 µl GoTaq
    b. 4 µl water
    c. 1 µl template
6. Vortex the master mix for one minute
7. Transfer 15 µl of the master mix into 5 µl sample.
8. Vortex this 20 µl sample for 30 seconds.

9. Centrifuge for 20 seconds if necessary
10. Place into PCR and change volume to 20 µl in PCR program
11. Run using 16S program
12. After PCR has run, samples can be stored in the 4°C refrigerator.

The program used for PCR was titled "16S" and was already preloaded into the machine. The following are the cycle conditions. The temperature and length held at each cycle are described, along with the number of times that the cycle was repeated.

16S Program

Cycle 1 (1x)

- 95 °C for 120 sec

Cycle 2 (30x)

- 95 °C 30 sec
- 55 °C 30 sec
- 72 °C 90 sec

Cycle 3 (1x)

- 72 °C for 120 sec

Gel Electrophoresis Procedure

1. Assemble gel electrophoresis system
2. Place two dividers with 10 well spacers on each (creating 20 wells) into kit
3. Mix 0.05g agarose in 50 ml of 1x TAE
4. Mix 1 µl of Ethidum Bromide into the total TAE solution
5. Heat in microwave until boiling
6. Pour mixture into assembly, but be sure ends are sealed.
7. After gel solidifies, remove rubber tubing that held gel in and remove dividers
8. Turn the assembly so the gel runs from negative to positive
9. Fill the assembly with TAE so that the gel is submerged
10. Carefully transfer each sample into separate wells, including marker and controls
11. Place cover over gel, inserting electrodes into the correct corners
12. Turn on the electrodes and let run until the samples travel to near the end of the gel
13. Turn off electrodes
14. Remove gel from assembly and image

4.4.3 Gas Chromatography

The gas chromatogram used was the Agilent Technologies 7820A GC located in the Unit Operations Lab in the basement of Goddard Hall. The GC akin to the one in the lab can be seen in *Figure 4*. The experiment was simply a characterization of the chemical composition of the water.

FIGURE 4: THE AGILENT TECHNOLOGIES 7820A GC AND ACCOMPANYING COMPUTER[19]

The GC uses a flame ionization detector to identify the components exiting the column. Schematics for the GC can be seen in *Figures 5* and *6*.

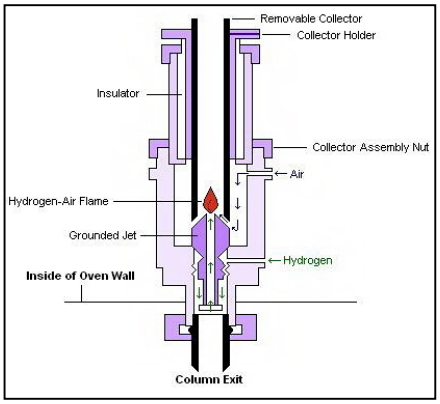


FIGURE 5: SCHEMATIC OF A TYPICAL FLAME IONIZATION DETECTOR[20]

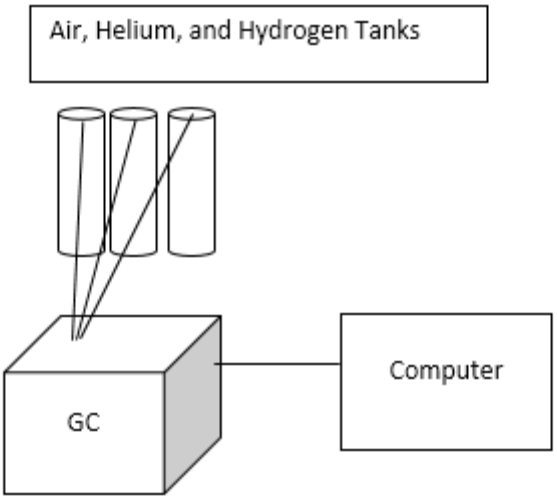


FIGURE 6: SCHEMATIC OF GAS CHROMATOGRAPHY LAB SET UP

Materials

- Dram vials (as needed)
- GC needle
- Kimwipe
- Nitrile gloves
- Pipet bulb
- 10 mL pipet (as needed)
- Permanent marker

Sample Preparation

1. Transfer 10 mL of liquid from bulk sample to a dram vial using the pipet. Make sure to use a clean pipet for each sample to prevent cross-contamination
2. Label each dram vial with appropriate designations

GC Preparation

1. Open the valves on the air, helium, and hydrogen tanks
2. Log into your User Profile on the Desktop Computer
3. On the Desktop, click the '7820A Remote Connection' icon
4. When the User Interface appears, select the 'Connection' tab and then click 'Connect"
5. When the GC Connection popup appears, click the 'Connect' button
6. On the User Interface, click the 'Oven' button and then click the 'On' button
7. Click the 'Back Inlet' button and then click the 'On' button
8. Click the 'Col #" button and then click the 2 button
9. Click the 'Front Det' button and then click the 'On' button
10. Using the arrow buttons, step down to the 'H2' input and then click the 'On' button
11. Using the arrow buttons, step down to the 'Air Flow' input and then click the 'On' button
12. Ensure that the system is on 'Constant Makeup'
13. Using the arrow buttons, step down to 'Makeup (He)' and then click the 'On' button
14. Back on the Desktop, click the 'Instrument Online' icon
15. Once all temperatures, pressures, and flow rates have reached their set point, the system is ready.
16. The GC will show its 'Ready Status' on the front LCD screen

Sample Characterization

1. Use the GC needle to transfer 2-3 μL of water sample out of the dram vial
2. Insert the needle into the GC inlet and push the plunger
3. Hit the 'Prep Run' Button
4. Once the light turns green and the display reads 'Ready', press the 'Start' button
5. After 3 minutes the report will be shown
6. Save the report to the desktop
7. Repeat characterization steps as needed

4.4.4 Ultraviolet Riboflavin Testing

To inspect the spray ball patterns for each bright tank and to ensure proper cleanability, we developed a UV Riboflavin Procedure that was tailored to Wachusett Brewery's bright tanks. This procedure was based off of two main sources.[15, 16] VDMA represents over 3,100 mostly medium-sized companies in the capital goods industries, making it the largest industry association in Europe. Below is the proposed procedure. Unfortunately due to time constraints, we were unable to physically perform this experiment, however the proof of concept is provided.

Wachusett Brewery UV Riboflavin Testing Procedure:

Purpose

To test Clean in Place (CIP) spray patterns in stainless steel process vessels for completeness of spray coverage. The test involves using a florescent substance for examination of cleanability of components. The tanks may contain an agitator, vortex breaker, nozzles, man-way, fittings, projections, etc., which could create spray "shadows." These shadowed areas must all be cleaned.

Materials

1. Riboflavin Powder-98%, Identification number: CAS #83-88-5
2. Hydroxyethyl cellulose
3. Long wavelength UV lamp in a suitable fixture to insert into various tanks
4. Sufficient water source to perform tests
5. Riboflavin solution hand atomizer, capable of producing a fine mist spray
6. Spray device with water
7. Calibrated pressure gauge
8. Flow meter
9. Deionized water

Dye Preparation

1. Mix 0.2 grams of riboflavin with one liter of deionized water in a clean container.
2. Add 5.0 g hydroxyethyl cellulose (optional, increases viscosity and layer thickness).
3. Transfer an appropriate amount of dye solution to a suitable hand atomizer or spray bottle.

Set-up

1. Before entering tank, observe all confined space safety regulations.
2. Verify that the tank is clean and has been rinsed and dried previously.
3. Verify that cleaning devices, spray balls, spray wands or bayonets are properly installed.

Pre-test Procedure

1. Visually inspect the interior of the tank under illumination by the UV lamp.
2. Record any observations.

Test Procedures:

Dye Application

1. Enter tank and spray the entire interior surface of the tank with dye, including the manway cover area, upper headspace, nozzle projections, and tri-clamp connections up to cap/clamp areas.
2. Exit tank without touching any sprayed surface.
3. Inspect the interior of the tank, illuminated with UV lamp to ensure complete coverage (dye will fluoresce with a bright green color under UV illumination).
4. Re-spray any areas that do not have complete coverage.
5. Ensure that all equipment is installed to measure pressure and flow rate.
6. Clean the tank by following the conditions that exist in the customer's facility
    a. Wachusett Brewery: Perform a series of six burst rinses for 30 seconds, which is close to the usual post-rinse procedure performed at Wachusett Brewery.
7. Document the set-up conditions, flow rate, pressure, and time.

Inspection

1. Inspect tank interior after cleaning. Illuminate with the UV lamp to observe any residual dye. Riboflavin does not fluoresce if dry. If drying does occur, lightly spray with water to cause fluorescence again.
    a. Acceptance Criteria: An acceptable test is one where no fluorescence is visible under UV illuminationon for any interior tank surface, nozzle, manway, fitting, projection, etc., during the inspection process.
2. If fluorescence is detected, the design criteria and/or location for the spray ball(s) and /or bayonets must be reviewed and modified prior to repeating test steps again.
3. Document any pertinent results.

Post-test Procedure

1. When all testing has been completed, clean tank by manually spraying tank interior with water.
2. Drain and seal all port openings.
3. Rinse tank with water and collect a 200 ml to 500 ml rinse sample from the drain valve (performed under routine cleaning operations).
4. Examine rinse sample under UV illumination in a darkened area. If no fluorescence is detected, record results and tank is completely clean.
5. If fluorescence is detected, repeat manual cleaning steps until a passing sample is obtained.

4.4.5 PH

The pH of the post-caustic rinse cycles were measured with a pH probe. Standard buffer solutions of pH (4, 7, and 10), were made before each pH testing session by mixing the buffers in powder form with water. These buffers were used to calibrate the pH meter before any sample measurements were taken. The pH values of the samples were measured during two major testing sessions; on the day after the normal cleaning procedure samples were taken and the day after the revised cleaning procedure samples were taken.

5. RESULTS AND DISCUSSION

The results obtained from ATP Bioluminescence, PCR and Gel Electrophoresis, Gas Chromotography, and pH values were used help analyze both the original and revised procedures. Overall, the data is consistent enough to determine that the revised procedure is just as effective as the original procedure. Knowing this information, we then moved forward with a cost analysis to determine savings if the new procedure is put into effect.

5.1 STANDARDIZED INITIAL PROCEDURES

One of our first main objectives was to determine the current operations and procedures used at Wachusett Brewery. After the beer is transferred for bottling, the bright tanks start off with residual beer, as well as foam, scum rings, or yeast if the bright tank contained unfiltered beer. The tank is usually filled with CO_2. The CO_2 pressure is first relieved and allowed to vent. The front manway of the tank is opened and CO_2 escapes through that exit. It is essential to get all CO_2 out of the tank before adding in caustic, so no reactions will take place between the caustic and CO_2. This could result in a loss of pressure and implosion if the tank is not open to the atmosphere.

Next, all parts of the tank, such as the pressure relief valve, extra valves, sample ports, and manway gaskets are taken off. These pieces will have a similar wash as the tank. They are washed with caustic, hot water, and iodine in a bucket placed next to the tank.

The bright tanks also have a glycol refrigeration system used to circulate glycol for cooling purposes. The glycol cooling system is turned off before the cleaning process begins. The cleaning process starts with burst rinses of water by hooking up a hose from the house water source to the top of the bright tank containing the CIP spray balls. Manipulating the hot and cold valve position on the house water supply controls the temperatures of the burst rinses. For instance, hot and half cold indicate that the hot water valve is fully open, and the orientation of the cold-water valve is 50% closed. After the burst rinses are performed, the bright tanks are filled with either 50, 100, or 200 gallons of hot water, depending on tank size. The tank supply hose is connected to a portable low-horsepower pump, and the concentrated caustic solution is added to the water. The dimensions of the drain hose, which acts as the pump supply, are 2” x 25’. The dimensions of the supply hose to the tank is 1½” x 50’. The valve on the pump supply hose is closed so it does not drain when transferring between the pump and water source, and vice versa. Additionally, water is allowed to reach the pumps before starting it. This will “prime” the pump and will help avoid any cavitation.

Once these steps are completed, the pump is turned on, then the caustic solution is pumped through the spray balls, onto the tank inner surface, through the bottom tank outlet, and back to the pump inlet. Images and specifications of the spray balls are provided in *Appendix C, D, F*. The caustic solution is on recycle for a set amount of time. The tubes are then transferred back to the house water source for another set of burst rinses to cool the bright tanks. The bright tanks are filled with water again, and iodophor is added to the water. The iodophor is also recycled through the tank for a set amount of time, and it acts as a sanitizer. All sanitized loose parts are then reassembled.

The thermocouple that indicates temperature of the fluid inside the tank is located in the bottom of the bright tank. A photo of the thermocouple interface can be seen in *Appendix E*.

The following is an in-depth description of the process, originally written by a Wachusett Brewery employee who performs the cleaning and sanitation processes.

CB7 (200 BBL):

1. Hot and cold together for 2x30 second bursts
2. Hot and ½ cold for 2x30 second bursts
3. Hot only for 30 seconds until CB reaches 120°F
4. 5 minutes hot is equivalent to 205 gallons (water level is rising during that time)
5. 2 gallons of caustic are added
6. Pump: 60 hz at 112 gpm or 50 hz at 90 gpm
7. Recycle for 30 min at 154°F
8. Hot only-30 second bursts
9. Hot and cold together at 70 gpm
10. Cold and ½ hot at 55 gpm
11. Cold only at 43 gpm
12. Cold only: 30 sec bursts until CB less than or equal to 100°F
13. Set glycol to 32°F
14. Pumps Microdyne with 2 minutes cold ~ 86 gallons
15. Pump for 1 minute on recycle
16. Drain out
17. Fill parts bucket with iodine solution

CB5 (100 BBL):

1. Hot and cold together, 2x30 second rinses at 52 gpm
2. Hot and ½ cold, 2x30 seconds at 44 gpm
3. Hot only 2x30 second rinses at 36 gpm
4. Tank is greater than or equal to 125°F
5. 2 ½ min hot ~ 90 gallons
6. Add 1 gallon caustic when tank is at 152°F
7. Circulate for 30 minutes at 63 gpm
8. Drain out
9. Hot only 2x30 second rinses at 36 gpm
10. Hot and cold 2x30 second rinses at 52 gpm
11. Cold and ½ hot 1x30 second rinse
12. Tank less than 100°F, set to 32°F
13. 2 min cold ~76 gallons and 2 pumps iodophor

CB4 (100 BBL):

1. Hot and cold, 2x30 second bursts at 55 gpm
2. Hot and ½ cold, 2x30 second bursts at 48 gpm
3. Hot only for 30 seconds at 37 gpm, tank is at 123°F

4. 2 ½ min hot fill to ~95 gallons, add 1 gallon caustic
5. Circulate for 30 minutes at 70 gpm until 147°F and the line pressure is 40 PSI
6. Drain out
7. Rinse hot only for 2x30 second bursts at 37 gpm
8. Hot and cold for 2x30 second bursts at 55 gpm
9. Cold and ½ hot, 1x30 second burst at 47 gpm
10. Cold only for 30 seconds at 39 gpm
11. Fill with cold for 2 minutes ~79 gallons and add 2 pumps Microdyne (52 ml)

5.2 REVISED PROCEDURES

Proposed Revised Procedures

All changes to the normal procedure are italicized

CB7 (200 BBL):

1. Hot and cold together, 2x30 second rinses
2. *Skip hot and half cold step*
3. Hot only 2x30 second rinses
4. Tank at recommended temperature
5. *Fill tank for 2.5 minutes hot ~100 gallons*
6. *Add 1 gallon caustic when tank is at temperature*
7. *Circulate for 20 minutes*
8. Drain out
9. Hot only 2x30 second rinses
10. Hot and cold 2x30 second rinses
11. Cold and ½ hot 1x30 second rinse
12. Tank less than 100°F, set glycol to 32°F
13. Iodophor sanitization

CB5 and CB4 (100 BBL):

1. Hot and cold together, 2x30 second rinses
2. *Skip hot and half cold step*
3. Hot only 2x30 second rinses
4. Tank at recommended temperature
5. *Fill tank for 1 ½ minutes hot ~50 gallons*
6. *Add ½ gallon caustic when tank is at temperature*
7. *Circulate for 20 minutes*
8. Drain out
9. Hot only 2x30 second rinses
10. Hot and cold 2x30 second rinses
11. Cold and ½ hot 1x30 second rinse
12. Tank less than 100°F, set glycol to 32°F
13. Iodophor sanitization

CB1 (50 BBL):

1. Hot and cold together, 1x30 second rinse
2. *Skip hot and half cold step*
3. Hot only, 1x30 second rinse
4. Tank is to recommended temperature
5. *Fill 45 seconds hot*
6. *Add 1/4 gallon caustic when tank is at temperature*
7. *Circulate for 20 minutes*
8. Drain out
9. Hot only, 1x30 second rinse
10. Hot and cold, 1x30 second rinse
11. Cold and ½ hot 1x30 second rinse
12. Tank less than 100°F, set glycol to 32°F
13. Iodophor sanitization

Finalized Revised Procedure

When revised procedure was executed, it was determined that for the 50 BBL tank, the amount of water added during *Step 5* was insufficient and resulted in pump cavitation at the end of the recycle period. It is our suggestion to increase the water fill time to 60 seconds. Cost Analysis is based off of 60 seconds.

5.3 CHEMICAL INFORMATION

The following information was indicated on the front and sides of the caustic drum:

HLC-5200 GB, Liquid Alkaline Heavy Duty Cleaner, Industrial Use Only

HLC-5200 GB produces no foam and provides caustic alkalinity and powerful chelation. It will remove food soils, light mineral deposits and is highly effective in removing beer stone in brewery operations. Equipment exposed for extended periods to hot HLC-5200 GB solution emerge with a "polished" appearance.HLC-520 GB is safe on stainless steel if used correctly.

Soak Tank Cleaning: Dilute HLC-5200 GB *to 1:100(1:50 typical) and heat to 140-150F. Immerse soiled parts and equipment for up to one hour followed by optional light brushing. All surfacesmust bethoroughly rinsed with potable water.*

CIP Applications: Dilute HLC-5200 GB at ½ to 2 fl. Oz. per gallon of water for removal of light to heavy soils. For best results heat up to 150F. Do not use on aluminum, tin or zinc-plated surfaces. Pitting or surface deterioration may result. Mix only with water

Ingredients: Sodium hydroxide(1310-73-2), Potassium hydroxide(1310-58-3), water(7732-18-5), sodium gluconate(527-07-01), phosphonic acid(6419-19-8)

See *Appendix A and B* for the supplemental MSDS sheet provided on the side of the caustic chemical drum.

5.4 ATP BIOLUMINESCENCE TESTING

The following assumption was made for the data in *Figure 7*, *8*, and *9*. Both the original procedure and modified procedure were performed on the same tanks. However, each tank was differing in size. Because the tanks follow the same procedure to scale, it is assumed that the RLU results are independent to the size of the tank. Thus, the average is compiled of RLU results from more than one sized tank.

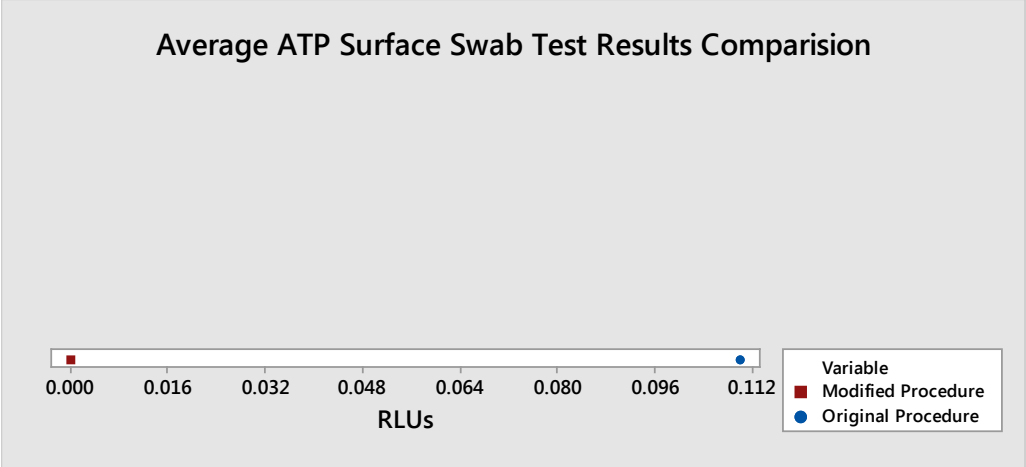


FIGURE 7: TEST- ATP SURFACE SWAB. A COMPARISON OF AVERAGE RLUS PRODUCED FROM ORIGINAL PROCEDURE AND MODIFIED PROCEDURE

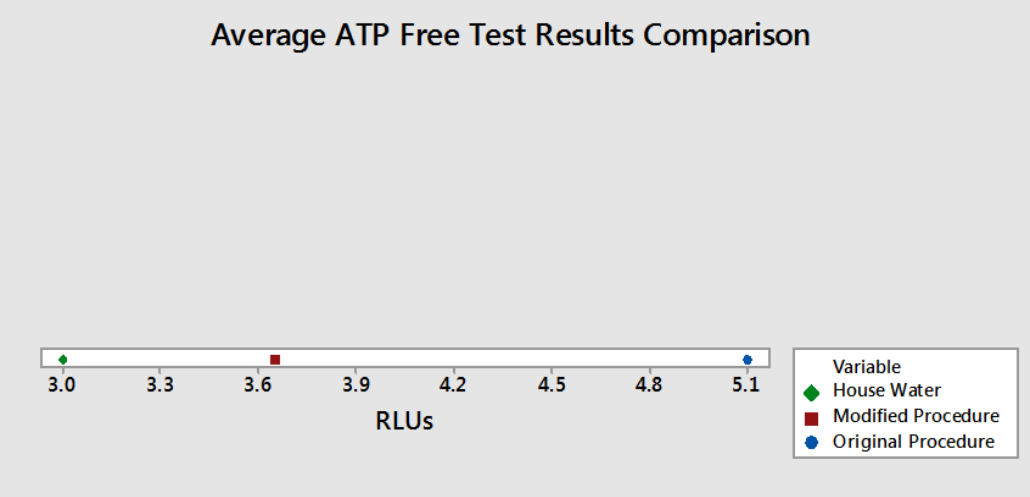


FIGURE 8: TEST- ATP FREE. A COMPARISON OF AVERAGE RLUS PRODUCED FROM HOUSE WATER, ORIGINAL PROCEDURE, AND MODIFIED PROCEDURE

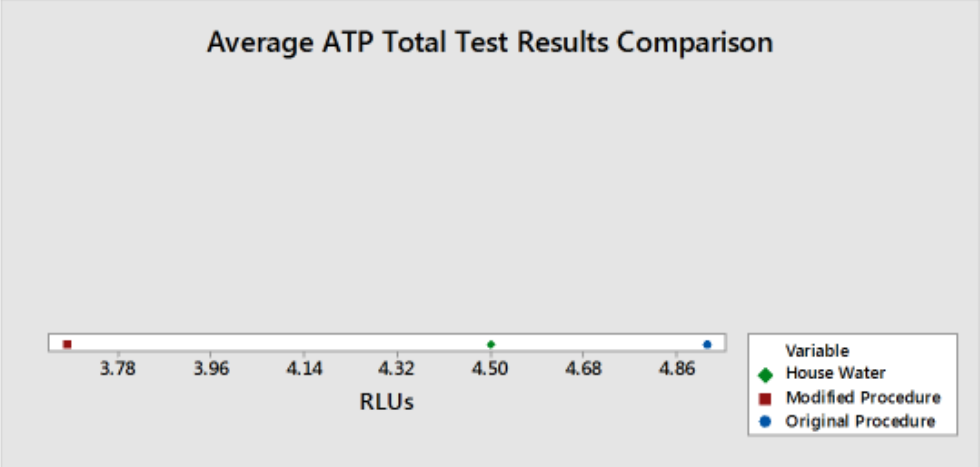


FIGURE 9: TEST- ATP TOTAL. A COMPARISON OF AVERAGE RLUS PRODUCED FROM HOUSE WATER, ORIGINAL PROCEDURE, AND MODIFIED PROCEDURE

As seen in *Figure 7*, the average RLUs produced from Hygenia's swab test is zero for the modified procedure and just below 0.112 for the original procedure. *Figure 8* and *Figure 9* also shows that the modified procedure averaged nearly 1.5 less RLUs than the original procedure.

5.3 PCR AND GEL ELECTROPHORESIS

The results obtained from the gel electrophoresis were inconclusive. Only the marker and a few of the *E. Coli* controls were visible. This was likely caused by the extremely low concentrations of bacteria in the Wachusett Brewery samples. Due to the inability to find any detectable amounts of bacteria, qPCR, which is capable of quantifying the bacteria while also growing the amount of DNA, would not be beneficial. The concentrations would be too low to be detected by qPCR, as they were too low to be seen after PCR. Based on the image in *Figure 10*, it was determined that the concentration of bacteria is likely less than any of the *E. Coli* concentrations used. This cannot necessarily be pinpointed, as the results from the gel electrophoresis were inconclusive.

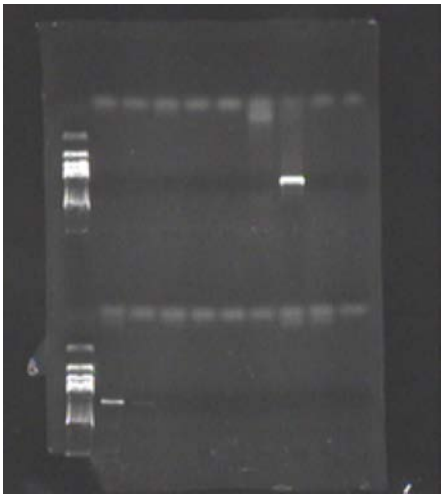
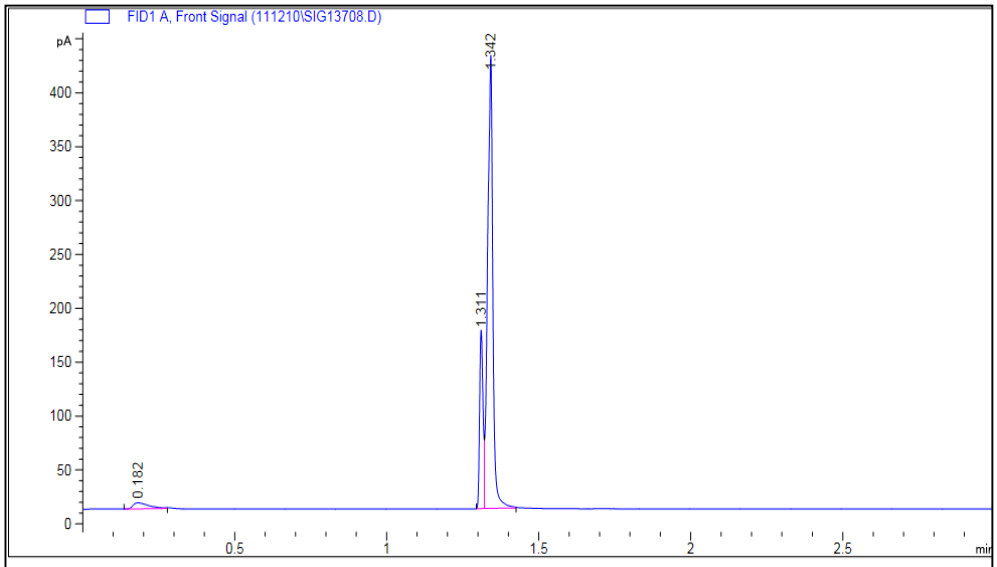


FIGURE 10: GEL ELECTROPHORESIS IMAGE

5.4 GAS CHROMOTOGRAPHY

As previously mentioned, gas chromatography (GC) was used to model the changes in organic chemical concentration in the waste water. Since it was not possible to determine the exact composition of the wastewater, the focus was placed more on the presence of material rather than what it was. The MQP did try to make assumptions as to the identity of the peaks using a reference sample. As seen below in *Figure 11*, it is safe to assume that peaks at 1.3-1.4 minutes can be attributed to the sodium gluconate in the caustic. It is also important to note the strength of the signal. For the reference sample, the strength of the signal is 400 pA. This sample is not pure caustic, but rather it is pure caustic diluted in 500 mL of deionized water. If it was pure caustic, the signal strength would be over 10,000 pA. Because this sample should be the most concentrated sample of caustic, all others should have a much weaker signal. Effluent water samples were taken at each interval of the cleaning process and analyzed with the GC. Here only the beginning and end samples are presented to show an abridged model but with the same overall result.

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

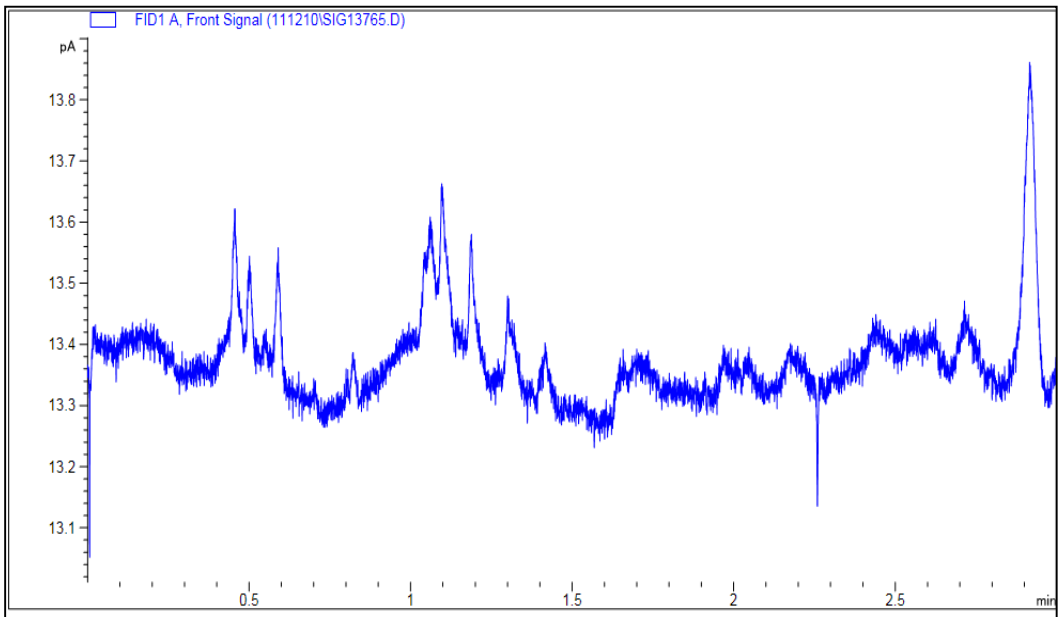

Signal 1: FID1 A, Front Signal

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [pA*s]      [pA]        %
----|-------|----|-------|----------|----------|--------|
   1   0.182 BB    0.0524   20.18330    5.61172  3.11423
   2   1.311 BV    0.0124  129.73515  164.19672 20.01779
   3   1.342 VB    0.0187  498.18069  418.46622 76.86798

Totals :                   648.09915  588.27465
```

FIGURE 11: GC REPORT FOR DILUTE CAUSTIC SOLUTION REFERENCE SAMPLE

Rinse 1 for the original procedure is the first burst rinse out of the set that heats up the tank and removes preliminary beer residue. At this stage, the only organic material should just be the beer, and at very low concentrations. The effluent water should be chemically similar to the house water and the concentration of chemical is negligible. The absence of organic chemicals is evident in *Figure 12* below, where the signal is primarily noise and no peaks are found. This type of report, when signals are all noise, is assumed to have such low concentrations of organic material that it is chemically clean. After the caustic cycle is finished, the GC report for those samples should look similar to *Figure 12*.

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========================================================================
                        Area Percent Report
========================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found
```
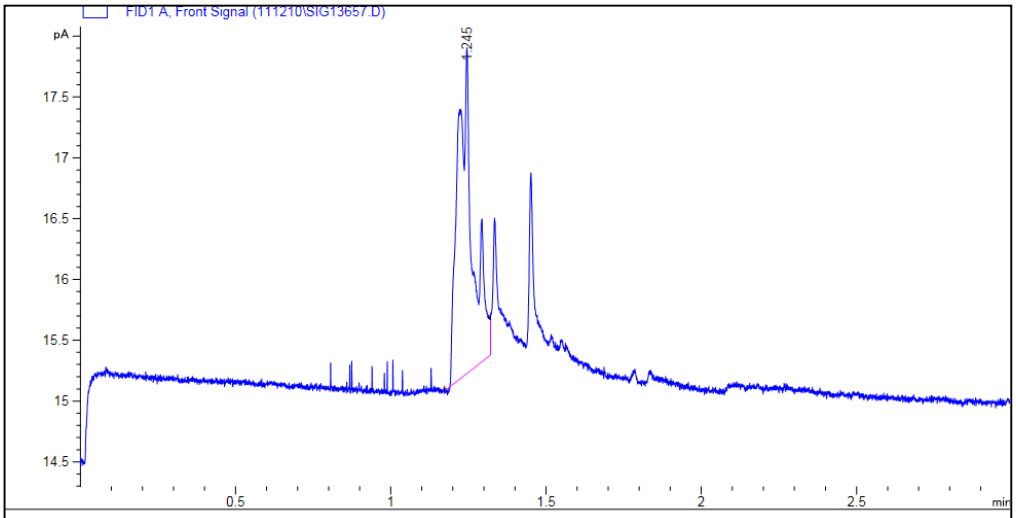

FIGURE 12: GC RESULTS FOR TANK CB1 RINSE 1 FOR THE ORIGINAL PROCEDURE

Once organic chemicals are introduced to the tank during the caustic wash, there should be definitive signals similar to *Figure 12* above. *Figure 13* shows the GC report for effluent water 10 minutes into the caustic wash cycle. It has the same characteristic peaks as the caustic reference sample. However, the signal is much weaker, only 18 pA. The lower signal strength compared to the reference sample is valid because the dilutions are not the same. But both signals are at 1.2-1.3 minutes indicating that they are likely the same chemical. The other peaks that are not definitive signals can be attributed to noise or chemicals that are the product of the caustic reacting with material in the tank, but are not concentrated enough to produce a signal.

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A, Front Signal

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [pA*s]      [pA]        %
----|-------|----|-------|----------|----------|--------|
   1   1.245 BB    0.0506    8.81492    2.61102 1.000e2

Totals :                     8.81492    2.61102
```

FIGURE 13: GC RESULTS FOR TANK CB1 10 MINUTE CAUSTIC WASH FOR THE ORIGINAL PROCEDURE

Ideally, at the end of the final rinse, the composition of the wastewater should be identical to the house water and the first burst rinse. There would be a negligible concentration of chemicals and the GC report would not show any signals. Under these conditions, the water and the tank can be considered clean. *Figure 14* shows the GC report for the final rinse under the original procedure. Because the signal is all noise, the sample fits the aforementioned criteria to be considered clean. In addition, because this is the final condition in which Wachusett Brewery considers the tank to be clean, we can use this profile for the revised procedure as the standard of cleanliness.

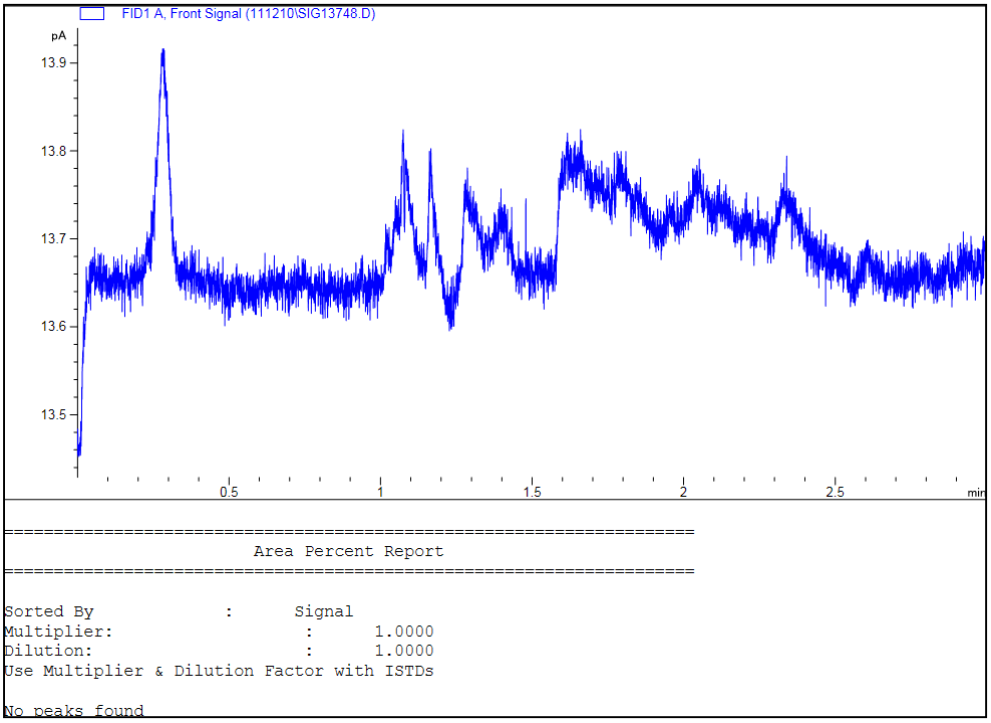
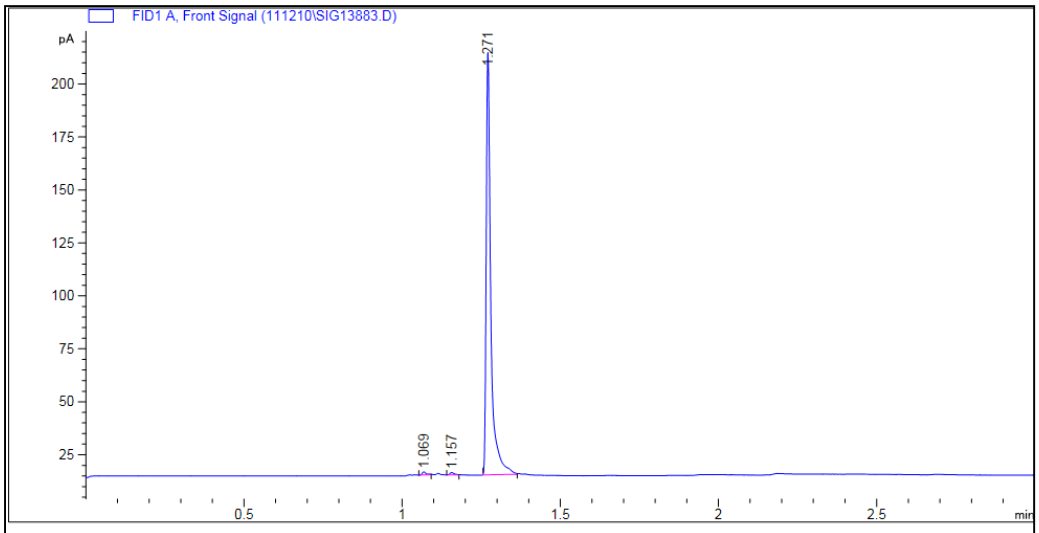


FIGURE 14: GC RESULTS FOR TANK CB1 FINAL RINSE FOR THE ORIGINAL PROCEDURE

Under the revised procedure, it is expected that the concentration of chemicals would be higher than the original procedure because there is a significant reduction in water that is not proportional to the reduction in caustic. This is evident in *Figure 15* below where the signal strength for the initial caustic rinse is about 200 pA. Again, it can be said with confidence that the signal is due to caustic in the effluent water because it registered around 1.2-1.3 minutes. While there are two other peaks present, the identity of the chemicals cannot be determined with certainty. However, it can be assumed that it is attributed to either ancillary chemicals from the beer, sample contamination, or measurement error. Regardless, the strength of the two signals are so small they can be deemed irrelevant.




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

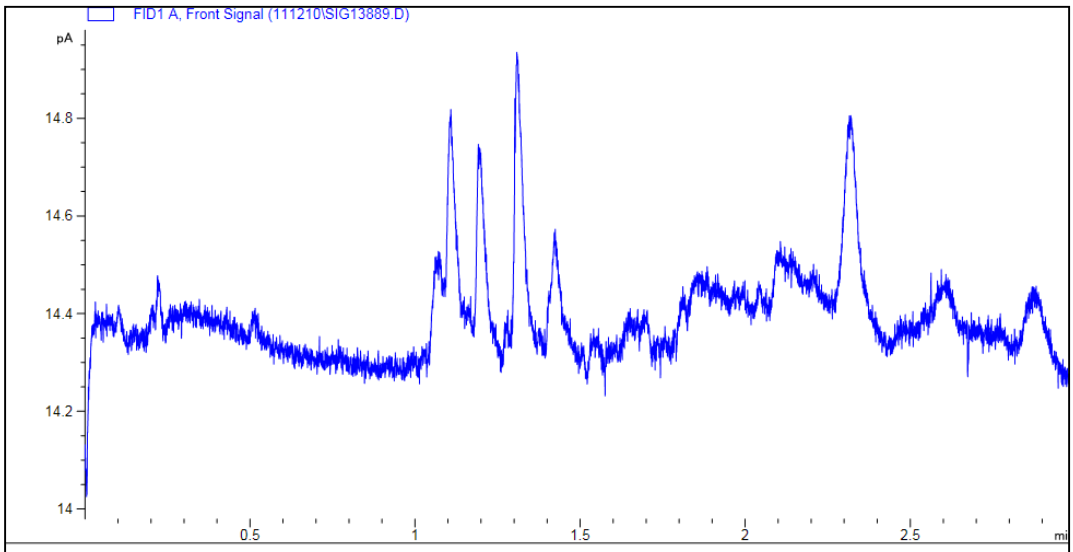

Signal 1: FID1 A, Front Signal

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [pA*s]      [pA]        %
----|-------|----|-------|----------|----------|--------|
   1   1.069 BB    0.0134    1.25014    1.42713  0.60276
   2   1.157 BB    0.0143    1.07860    1.13696  0.52005
   3   1.271 BB    0.0153  205.07297  198.17482 98.87718

Totals :                    207.40171  200.73890
```

FIGURE 15: GC RESULTS FOR CB1 INITIAL CAUSTIC RINSE FOR THE REVISED PROCEDURE

Lastly, and most importantly, is the GC report for the final rinse under the revised procedure. If this report has signals that only show noise, then the assumptions that the tank is clean and the condition of the tank is basically the same end result as the original procedure are valid. *Figure 16* below is the GC report for the final rinse under the revised procedure. Because the signals are all noise, the assumptions for the sample are met and the tank is clean. This substantiates the idea that as long as the end results are the same, the revisions to the original procedure do not negatively affect the outcome.

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found
```

FIGURE 16: GC RESULTS FOR CB1 FINAL RINSE FOR THE REVISED PROCEDURE

5.5 PH

The following assumption was made for the data in *Table 2*. Both the original procedure and modified procedure were performed on the same tanks. However, each tank was differing in size. Because the tanks follow the same procedure to scale, it is assumed that the pH of the rinse water is independent to the size of the tank. Thus, the average is compiled of the pH results from more than one sized tank. The absolute difference is based on the assumption that obtaining the same pH for the house water and the final rinse water is the best case scenario.

TABLE 2: AVERAGE PH OF RINSE WATER SAMPLES FOR THE ORIGINAL AND MODIFIED PROCEDURE

Procedure	Average pH from Final Rinse Water	Average pH of House Water	Absolute Difference
Original	7.69	7.41	0.28
Modified	8.13	7.99	0.14

As seen in *Table 2*, the absolute difference produced by the modified procedure is less than the original procedure.

5.6 COST ANALYSIS

A cost analysis was performed to identify the overall savings if the revised procedure is put into place. The amount of water used for each tank was measured with flow meters and a timer. It was assumed that each time a particular type of tank was cleaned, the same amount of water was used. The cleaning schedule for each tank was provided by Wachusett Brewery to determine how many times per year it is cleaned. The price per gallon of water, including treatment, is provided by Stephen Kmiotek through water costs at Dow Chemical. Finally, the cost of the modified procedure and the original procedure are compared.

TABLE 3: PRICE PER GALLON OF WATER FOR 200 BBL TANK

200 BBL Tank		
Water setting	**Flow Rates (gpm)**	**$/ gallon**
Hot only	41	$ 0.03
Hot & Cold	70	$ 0.03
1/2 Hot & Cold	55	$ 0.03
Cold Only	43	$ 0.03

TABLE 4: ORIGINAL PROCEDURE- COST OF WATER PER WASH FOR 200 BBL TANK

Original Procedure (200 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	1	70	$ 1.86
Hot & 1/2 Cold	1	55	$ 1.46
Hot only	1	41	$ 1.09
Hot only	5	205	$ 5.43
Hot only	1	41	$ 1.09
Hot and Cold	1	70	$ 1.86
1/2 Hot and Cold	0.5	27.5	$ 0.73
Total:		**509.5**	**$ 13.50**

TABLE 5: MODIFIED PROCEDURE- COST OF WATER PER WASH FOR 200 BBL TANK

Modified Procedure (200 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	1	70	$ 1.86
Hot & 1/2 Cold	0	0	$ -
Hot only	1	41	$ 1.09
Hot only	2.5	102.5	$ 2.72
Hot only	1	41	$ 1.09
Hot and Cold	1	70	$ 1.86
1/2 Hot and Cold	0.5	27.5	$ 0.73
Total:		**352**	**$ 9.33**

TABLE 6: PRICE PER GALLON OF WATER FOR 100 BBL TANK

100 BBL		
Water setting	**Flow Rates (gpm)**	**$/ gallon**
Hot only	36	$ 0.03
Hot & Cold	52	$ 0.03
1/2 Hot & Cold	44	$ 0.03
Cold Only	38	$ 0.03

TABLE 7: ORIGINAL PROCEDURE- COST OF WATER PER WASH FOR 100 BBL TANK

Original Procedure (100 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	1	52	$ 1.38
Hot & 1/2 Cold	1	44	$ 1.17
Hot only	1	36	$ 0.95
Hot only	2.5	90	$ 2.39
Hot only	1	36	$ 0.95
Hot and Cold	1	52	$ 1.38
1/2 Hot and Cold	0.5	22	$ 0.58
Total:		**332**	**$ 8.80**

TABLE 8: MODIFIED PROCEDURE- COST OF WATER PER WASH FOR 100 BBL TANK

Modified Procedure (100 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	1	52	$ 1.38
Hot & 1/2 Cold	0	0	$ -
Hot only	1	36	$ 0.95
Hot only	1.5	54	$ 1.43
Hot only	1	36	$ 0.95
Hot and Cold	1	52	$ 1.38
1/2 Hot and Cold	0.5	22	$ 0.58
Total:		**252**	**$ 6.68**

TABLE 9: PRICE PER GALLON OF WATER FOR 50 BBL TANK

50 BBL Tank		
Water setting	**Flow Rates (gpm)**	**$/ gallon**
Hot only	36	$ 0.03
Hot & Cold	52	$ 0.03
1/2 Hot & Cold	44	$ 0.03
Cold Only	38	$ 0.03

TABLE 10: ORIGINAL PROCEDURE- COST OF WATER PER WASH FOR 50 BBL TANK

Original Procedure (50 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	0.5	26	$ 0.69
Hot & 1/2 Cold	0.5	22	$ 0.58
Hot only	0.5	18	$ 0.48
Hot only	1.5	54	$ 1.43
Hot only	0.5	18	$ 0.48
Hot and Cold	0.5	26	$ 0.69
1/2 Hot and Cold	0.5	22	$ 0.58
Total:		**186**	**$ 4.93**

TABLE 11: MODIFIED PROCEDURE- COST OF WATER PER WASH OF 50 BBL TANK

Modified Procedure (50 BBL)			
Step	**Time (min)**	**Water used (gallons)**	**Total Price**
Hot & Cold	0.5	26	$ 0.69
Hot & 1/2 Cold	0	0	$ -
Hot only	0.5	18	$ 0.48
Hot only	1	36	$ 0.95
Hot only	0.5	18	$ 0.48
Hot and Cold	0.5	26	$ 0.69
1/2 Hot and Cold	0.5	22	$ 0.58
Total:		**146**	**$ 3.87**

TABLE 12: CAUSTIC USAGE AND PRICING

	Caustic Usage (gallon)	Price ($/Gallon)
Original (200 BBL)	2	$ 12.00
Revised (200 BBL)	1	$ 12.00
Original (100 BBL)	1	$ 12.00
Revised (100 BBL)	0.5	$ 12.00
Original (50 BBL)	0.5	$ 12.00
Revised (50 BBL)	0.25	$ 12.00

TABLE 13: TOTAL SAVING PER YEAR BY USING THE MODIFIED PROCEDURE

Tank Type	Savings ($) / wash	Number of Tanks	Washed/ Month	Savings ($) / year
200 BBL	$ 16.17	2	4	$ 1,552.68
100 BBL	$ 8.12	3	4	$ 1,169.28
50 BBL	$ 4.06	2	4	$ 389.76
Extra 100 BBL	$ 8.12	2	2.5	$ 487.20
Total				**$ 3,598.92**

5.7 DISCUSSION: PROCESS VALIDATION & OPTIMIZATION

The purpose of process validation was to determine if the new revised procedure was as sufficient as the existing procedure. We also wanted to ensure that it was effective with decreasing the cost of cleaning. After transcribing the original procedure to text and proposing a revised procedure, we investigated both processes using various analytical tests. These tests included ATP bioluminescence testing, PCR and gel electrophoresis, gas chromatography, ultraviolet riboflavin testing, and pH testing. The data provided by these tests helped bolster the feasibility of using this new process in the near future.

Based on our trial period with the ATP bioluminescence testing device, it was found that the values for all swab types were under 10 RLUs. This is below the critical limit that we set from HACCP and from Hygenia's recommendations based on standards from food industries. As seen in *Figures 7-9*, it is clear that the modified procedure results in tanks with less residual ATP compared to the original procedure, thus supporting the use of the modified procedure.

PCR and gel electrophoresis were used both to measure the overall amount of bacteria in each rinse water sample and to later correlate this data with RLUs. According to *Figure 10*, the data from PCR was inconclusive. This is because there were not any visible bands in the gel that would represent bacteria. The bands are produced from various weights of the DNA in the gel. If there was no DNA present, bands would not appear. We are not able to use this information to assume that there is no bacteria in the sample, because our *E. Coli* controls did not produce any bands either. As a result, we determined that PCR was not a worthwhile tool for identifying the presence of contamination at Wachusett Brewery.

Gas chromatography served multiple purposes. Not only was it used to determine if the end state of both procedures were similar, it also helped to model the process to identify the changes in chemical composition over time. While the initial samples for the revised procedure had higher concentrations of caustic than the original procedure, the final rinse was still clean. This opens up the possibility for further alterations of the procedure than can lead to additional sustainability measures. Overall, GC successfully validated the inherent procedure and showed potential opportunities for improvement. The chemical characterization and analysis of the revised procedure indicated that the same results can be replicated using much less water and chemicals, which saves time, money, and increases the utility value of the cleaning process.

Our pH values were used to identify the possible caustic hazard in the latent rinse water. As seen in *Table 2*, it is evident that the modified procedure produced a final pH closer to the average house water pH as compared to the original procedure. These results support that the revised procedure is comparable to the original procedure in terms of effective removal of caustic.

Not only did our analytical tests conclude that the revised procedure is as effective as the original procedure, but it is also more efficient in terms of cost. By performing a cost analysis on the water and chemical usage expenses, there is a potential to save an average of $9.11 per wash. This would ultimately save the Wachusett Brewing Company nearly $3,600 per year. In addition to the direct monetary savings, there is a utility savings by using the modified procedure. As the tanks are cleaned concurrently, there will be an overall time savings benefit, which correlates to a decreased need for labor associated with cleaning.

It should be noted that there are several sources of error and assumptions made during testing. Sampling water is not always consistent because there is a lag time between when the water is in contact with the tank surface and when the sample is taken out from the drainage hose. Sampling times were not exact from tank to tank because the time reference was determined from a clock in the production area, and not a precise timer. There is also a chance that the dipping times for the swabs were too short for the ATP AquaSnap swabs. This could account for the Total Swabs having a lower RLU value than the Free Swabs. This occurred for a few of our AquaSnap samples. The measurement noise of the Hygiena EnSure unit was +/- 5 RLU, so the RLU values that we obtained could be slightly off.[5]

6. CONCLUSIONS & RECOMMENDATIONS

The overall results indicate that the revised procedure is as equally effective as the original procedure in terms of meeting the quality thresholds set by Wachusett Brewery. The use of the revised procedure would improve sustainability by saving time, money, labor, energy, and water.

Our overall research of cleaning methods and chemicals used both in the brewing industry and case studies show that there are various acceptable methods for cleaning with no set standard. Therefore, we decided to focus on Wachusett Brewery's specific needs and equipment by performing analytical tests rather than trying to change Wachusett Brewery's procedures to align with other breweries.

Our experience with Hygiena regarding training and customer support was very positive.[21] The phone number on their website led directly to a human service representative, and not an automated system. The representative we initially spoke with was happy to answer our questions through email correspondence, and eventually helped us to coordinate a 30-day trial of the EnSure system. The Hygiena representative also guided us and the Wachusett Brewery employees through a one hour training session to gain familiarity with the testing unit and the analysis software. Additionally, Hygiena's testing units were previously tested by third party researchers and surpassed other units of similar retail value regarding linearity, sensitivity, repeatability, and accuracy[22]. Overall, we were very impressed by the help and the customer service that Hygiena provided.

Concerning the actual equipment, the EnSure unit and the associated software was very intuitive and easy to use. The EnSure featured a simple readout, where you can program specific plans and testing locations that fit the needs of the facility. When you are ready to test at a location, it was simple to choose that specific labeled location within the device, insert the swab into the unit, and get a result 15 seconds later. When testing was complete, the unit was able to upload the labeled results into the included SureTrend software. This software not only keeps track of the raw data, but can also generate scatter plots to see performance over time or pie charts to compare specific periods of time, as seen in *Appendix G*.

We recommend that Wachusett Brewing Company incorporate ATP Testing on a regular basis. The AquaSnap Total swabs and UltraSnap Surface swabs should be used to measure the total ATP amount in the last post-caustic rinse cycle and the bright tank manway surfaces, respectively, each time the bright tanks are cleaned. It also is important to note that, although the use of ATP testing may alleviate the need to perform as many cell culture tests, ATP testing is meant to be a "spot check" for cleanliness, and is not meant to solely replace all other testing methods.

We also recommend that Wachusett Brewery set custom pass/fail RLU limits in the future that are tailored to their quality standards. Hygiena recommends that pass/fail limits be determined by the facility and documented as to how they were determined. "The default limits of 10 (Pass) and 30 (Fail) RLUs on the luminometer are based on years of food & beverage processing experience and third party studies. Hygiena recommends that users validate these recommendations and adjust them to meet the needs of each facility's unique needs".[23] Hygiena has their own recommended procedure for determining custom pass/fail limits, and we recommend that Wachusett Brewery follows this procedure below:

1. **Identify** control points in the facility. For our MQP, the control points were the manway surface and last rinse water sample for each bright tank. However, Wachusett Brewery can expand and include more control points, such as bottle fillers, heat exchanger tubes, etc.
    a. Control points can be programmed into the luminometer before testing so that results are saved with the control point location name, date, and time of test.
2. **Clean** surfaces to the desired level of cleanliness.
    a. This may include a total production line breakdown.
    b. Future cleanings should be held to this level of clean as a standard.

3. **Perform** an ATP test at each control point, taking 5-10 replicate tests.
    Use one of two methods:
    a. Perform tests over several days.
    b. Or for control points with sufficient surface area, perform multiple tests from different spots at that test location.
4. **Calculate** the lower and upper RLU limits
    a. **Lower RLU limit**: Calculate the average RLU for each location based on the 5-10 test results. The average result will be the lower RLU limit.
    b. **Upper RLU limit:** There are two options for determining the upper limit
        i. Multiply the lower limit by three
        ii. Or determine the standard deviation from the test results, multiply the standard deviation by three, and add this to the lower limit.

Wachusett Brewery can also take 1-2 months of data, with possibly 50+ independent data points, and base the custom limits off of that average and standard deviation. A higher sample size will also ensure a set of data that follows a normal bell-curve.[22]

We also recommend that Wachusett Brewery performs a UV Riboflavin test, as outlined in *Section 4.4.4*, for each bright tank that has a unique spray ball design. The absence of any fluorescence after a normal rinse procedure will indicate that the spray balls exert a spray pattern that covers the entire tank surface and are effective at rinsing the tanks.

6.1 LEGACY

Throughout the project, our group faced a number of hurdles due to academia clashing with business. A number of lessons were learned while confronting these challenges. In order to ensure that future generations do not make the same mistakes, the following is advice on how we overcame each obstacle.

Determining the Project

One of the larger challenges we faced at the inception of our project was determining what exactly Wachusett Brewery wanted for a project. We went in with a preconceived notion of what we were going to accomplish. This caused us to create an agenda and make assumptions. With our assumptions made, our first two meetings were difficult because we came in with a detailed plan, but never asked what exactly the company wanted from us. This lack of communication led to forming a less than ideal relationship with our sponsors. With the brewery always in full operation while we were at the plant, it was often difficult to sit with everyone to make specific goals. This leads us to our next lesson learned.

There is No Such Thing as a Free Lunch

In order to realign Wachusett Brewery's and our vision for the project, we determined it would be best to go to a third party location. We decided to take out our sponsors for lunch because everyone is happy when they are eating and it allowed our sponsors to get away from their day-to-day work without feeling too guilty. During this meeting, we were able to present a lot of our findings and what we were hoping to gain from the project. This also gave our sponsors an opportunity to set goals for us as well. This was a major turning point in our project and we would recommend all sponsored MQPs to start with a business lunch.

Money and Compliments Always Make Businesses Happy
In order to gain traction with our ideas, it was vital for us to put a positive spin on our plans. Any company will want to pursue an idea that will save them money and has little overhead cost. In order to move forward, we were fortunate enough to find a company that would allow a 30 day free trial of their ATP testing device. Wachusett Brewery was more than willing to give this a try because if the device did not meet expectations it would incur no cost to them. However, if (and when) the device did work, it would save them money long-term. It was also important to not insult their current processes. In the eyes of the company, you may be viewed as a young college student who knows nothing about their business, and they will probably be right. It is vital to go into the project asking questions and trying to learn rather than to criticize. Also, if the data shows that their current methodology is not adequate, it is important for them to hear why it will benefit them to change, rather than "you could be better" or "you're wrong".

You May Not Always Be the Priority
Combining academia and business can always be a challenge. Another important thing to realize is just because you are doing a project with a company does not mean you are the priority. There may be times when it takes a week or two to get an email response. It also may take longer to set up a tests or data collection. This is simply because the company is in full operation and your sponsors have full time jobs they need to complete as well. So it is important to be patient.

With time, we were able to overcome each obstacle that we faced. However, we hope that future projects will consider our advice and learn from our mistakes, as we wish we knew this information going into our project.

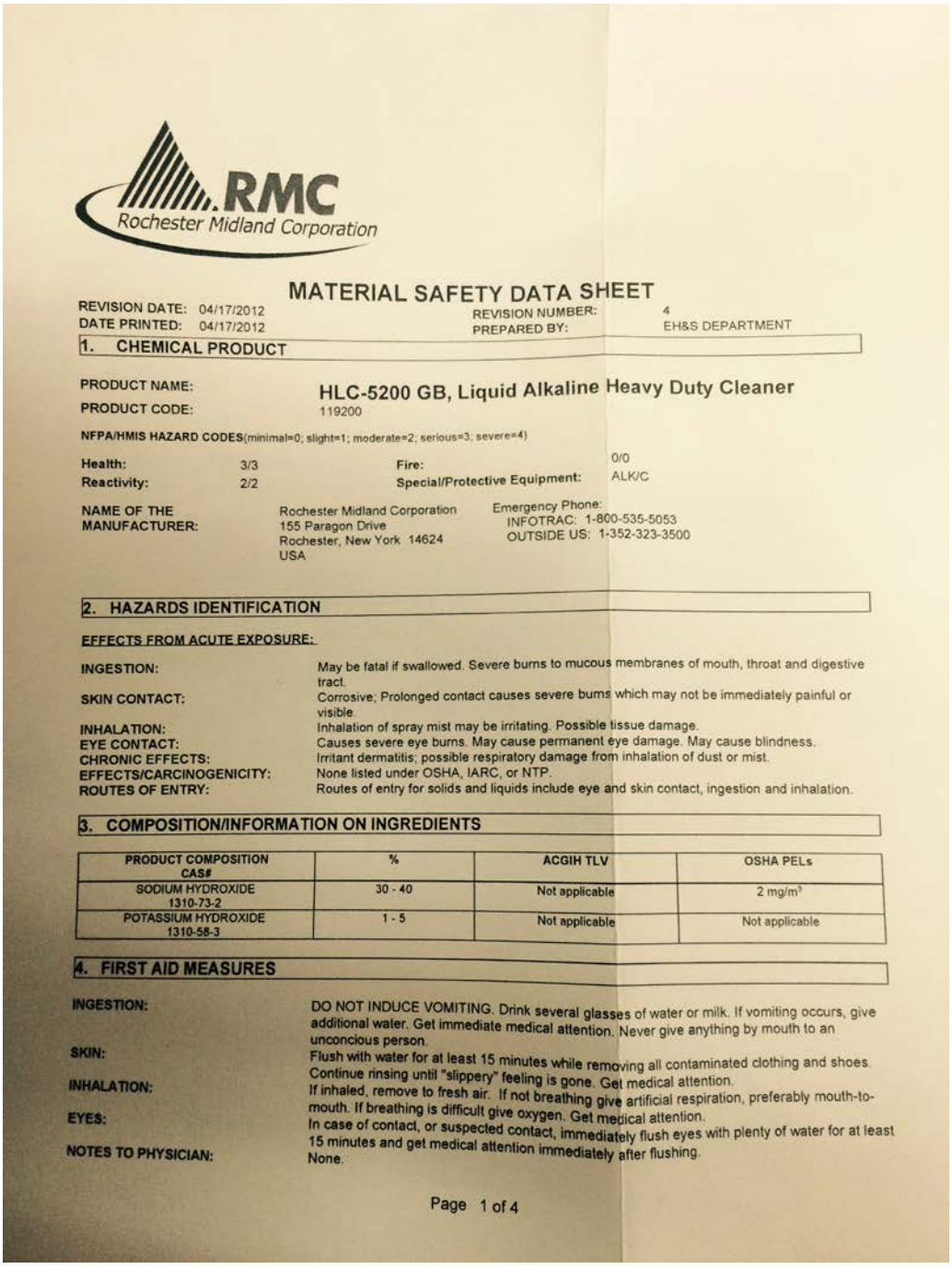
6.2 MQP CONTINUATION
As our experience with Wachusett Brewery was very positive, we expect that future MQPs will work with them as well. A few things our project group would like to see accomplished in the future is an evaluation of the cleaning procedure of the bottling line, an assessment of the cleaning procedure of the fermentation tanks, as well as a review of the data collected from Hygenia's ATP Ensure testing device.

Once Wachusett Brewery was able to get their hands on the ATP testing device, they expressed a lot of interest in testing their bottling line and fermentation tanks. It is our belief that an evaluation of their other equipment may also be advantageous to Wachusett Brewery. The ATP testing device could be used in a similar fashion as we used it, to determine if they are able to use less water and chemicals while still being able to obtain similar results within their quality thresholds.

It may also be constructive for a group to review the data collected from the ATP testing device on the bright tank. Given our short time with the device, we were only able to collect a limited number of data points. It may be worthwhile to determine a data driven pass/fail range of RLUs for the bright tank as well as their other equipment.

APPENDICES

APPENDIX A: MSDS OF CAUSTIC USED AT WACHUSETT BREWERY



RMC
Rochester Midland Corporation

MATERIAL SAFETY DATA SHEET

REVISION DATE: 04/17/2012
DATE PRINTED: 04/17/2012
REVISION NUMBER: 4
PREPARED BY: EH&S DEPARTMENT

1. CHEMICAL PRODUCT

PRODUCT NAME: HLC-5200 GB, Liquid Alkaline Heavy Duty Cleaner
PRODUCT CODE: 119200

NFPA/HMIS HAZARD CODES(minimal=0; slight=1; moderate=2; serious=3; severe=4)

Health: 3/3
Reactivity: 2/2
Fire: 0/0
Special/Protective Equipment: ALK/C

NAME OF THE MANUFACTURER: Rochester Midland Corporation
155 Paragon Drive
Rochester, New York 14624
USA

Emergency Phone:
INFOTRAC: 1-800-535-5053
OUTSIDE US: 1-352-323-3500

2. HAZARDS IDENTIFICATION

EFFECTS FROM ACUTE EXPOSURE:

INGESTION: May be fatal if swallowed. Severe burns to mucous membranes of mouth, throat and digestive tract.
SKIN CONTACT: Corrosive; Prolonged contact causes severe burns which may not be immediately painful or visible.
INHALATION: Inhalation of spray mist may be irritating. Possible tissue damage.
EYE CONTACT: Causes severe eye burns. May cause permanent eye damage. May cause blindness.
CHRONIC EFFECTS: Irritant dermatitis; possible respiratory damage from inhalation of dust or mist.
EFFECTS/CARCINOGENICITY: None listed under OSHA, IARC, or NTP.
ROUTES OF ENTRY: Routes of entry for solids and liquids include eye and skin contact, ingestion and inhalation.

3. COMPOSITION/INFORMATION ON INGREDIENTS

PRODUCT COMPOSITION CAS#	%	ACGIH TLV	OSHA PELs
SODIUM HYDROXIDE 1310-73-2	30 - 40	Not applicable	2 mg/m^3
POTASSIUM HYDROXIDE 1310-58-3	1 - 5	Not applicable	Not applicable

4. FIRST AID MEASURES

INGESTION: DO NOT INDUCE VOMITING. Drink several glasses of water or milk. If vomiting occurs, give additional water. Get immediate medical attention. Never give anything by mouth to an unconcious person.
SKIN: Flush with water for at least 15 minutes while removing all contaminated clothing and shoes. Continue rinsing until "slippery" feeling is gone. Get medical attention.
INHALATION: If inhaled, remove to fresh air. If not breathing give artificial respiration, preferably mouth-to-mouth. If breathing is difficult give oxygen. Get medical attention.
EYES: In case of contact, or suspected contact, immediately flush eyes with plenty of water for at least 15 minutes and get medical attention immediately after flushing.
NOTES TO PHYSICIAN: None.

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APPENDIX B: MSDS 2 OF CAUSTIC USED AT WACHUSETT BREWERY

HLC-5200 GB, Liquid Alkaline Heavy Duty Cleaner

5. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT:	(F): None (C): None
METHOD:	None
FLAMMABLE LIMITS IN AIR	
LOWER (%):	No data
UPPER (%):	No data
SENSITIVITY TO MECHANICAL IMPACT(Y/N):	NO
SENSITIVITY TO STATIC DISCHARGE:	Sensitivity to static discharge is not expected.
SUITABLE EXTINGUISHING MEDIA:	Product will not burn.
FIRE FIGHTING PROCEDURES:	Corrosive material. Avoid exposure to mist and splashes. Fire-fighters should wear self-contained breathing apparatus and full protective clothing when fighting chemical fires.

6. ACCIDENTAL RELEASE MEASURES

SPILL PROCEDURES:	
SMALL SPILLS:	Reclaim as much as possible. Flush residue with water.
LARGE SPILLS:	Dike to contain. Pick up with absorbant material. Put in suitable container for disposal. Flush remainder with water.
PERSONAL PRECAUTIONS:	Not applicable
ENVIRONMENTAL PRECAUTIONS:	Not applicable
METHODS FOR CLEANING UP:	Not applicable

7. HANDLING AND STORAGE

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE:	DANGER: Concentrated, caustic material. Avoid contact with eyes, skin and clothing. Do not breathe dusts or mists. Store indoors. Store only in original container and keep closed. Store in a cool, dry area. Store in a well ventilated area.
OTHER PRECAUTIONS:	Do not reuse container. Empty containers may retain product residue, follow MSDS/label precautions even after container is emptied. Contact with certain food sugars can release hazardous amounts of carbon monoxide gas in enclosed vessels. Read and follow label instructions.
SPECIFIC USE(S):	Not applicable.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

PROTECTIVE EQUIPMENT:

EXPOSURE CONTROLS:	Use in a well ventilated area.
RESPIRATORY PROTECTION:	Use approved NIOSH respiratory protection if TLV/PEL exceeded or if over exposure is likely.
PROTECTIVE GLOVES:	Nitrile, neoprene, or other appropriate gloves are recommended to minimize hand skin contact.
EYE PROTECTION:	Goggles and face shield are recommended to minimize eye contact.
OTHER PERSONAL PROTECTION EQUIPMENT:	Appropriate protective clothing as needed to prevent skin contact. Liquid may penetrate leather shoes and cause delayed burns. Eyewash fountains and safety showers must be easily accessible. It is the responsibility of the end user of this product to determine level of PPE required that is consistent with safe use of this product.
VENTILATION:	General mechanical and/or local exhaust as needed to meet exposure limits if mist in air. Corrosion resistant equipment recommended.

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AND ODOR:	Clear brown liquid. Mild odor.

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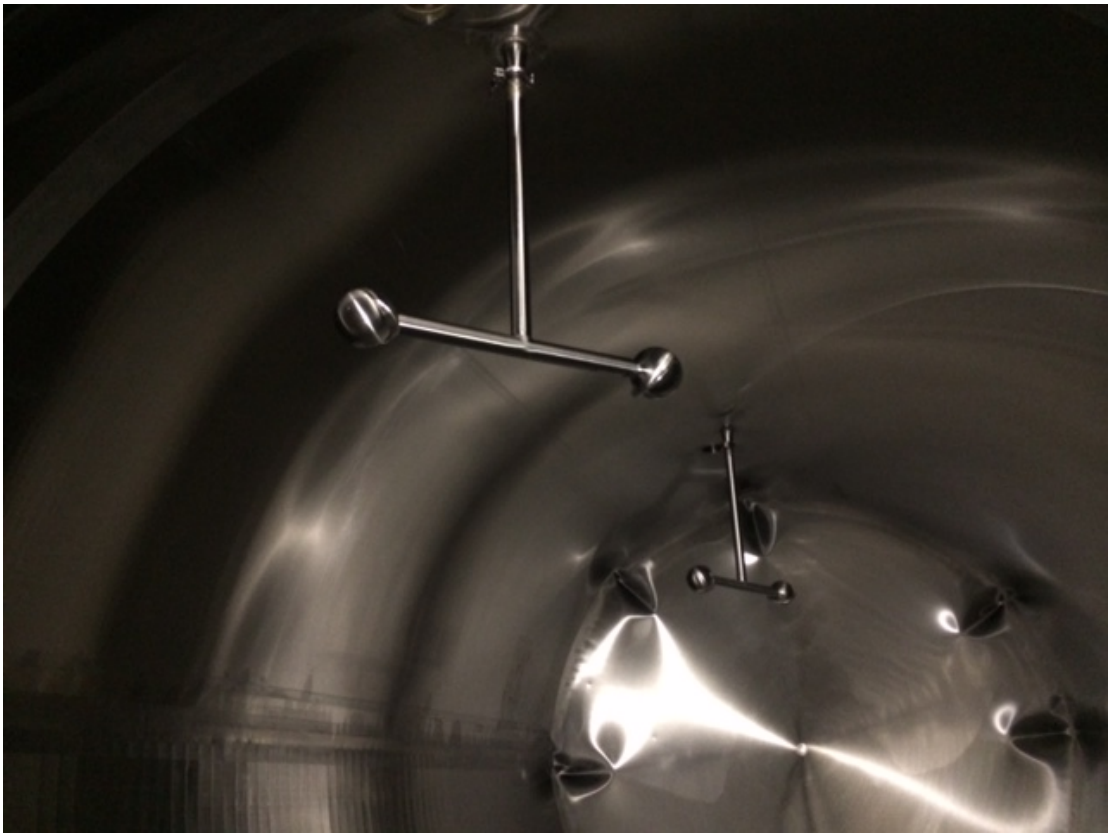
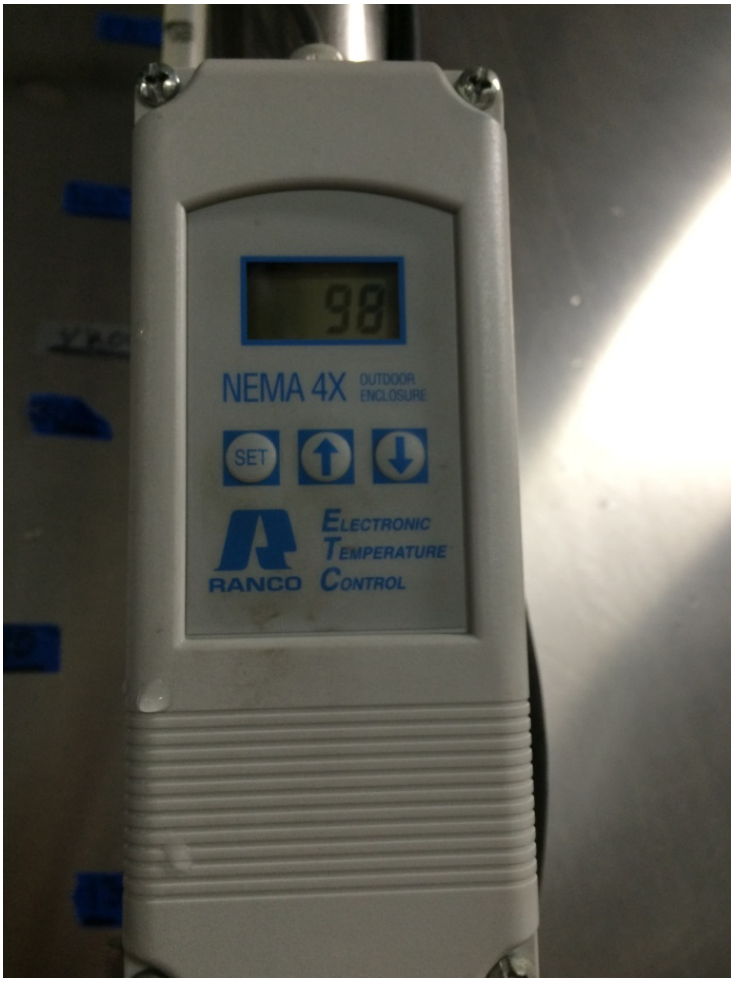
APPENDIX C: BRIGHT TANK INTERIOR

APPENDIX D: ADDITIONAL PHOTO OF BRIGHT TANK INTERIOR



APPENDIX E: BRIGHT TANK TEMPERATURE DISPLAY



APPENDIX F: SPRAY NOZZLE DATA SHEET

Spray Nozzles for Brewery Applications

VESSEL TO BE CLEANED	CLEANING SCALE	TYPE OF SOIL	TANK CLEANING PRODUCT		
			Large Tanks 11–30 ft. dia. 300–800 BBL's	Medium Tanks 5–10 ft. dia. 50–280 BBL's	Small Tanks 2–3 ft. dia. 55 gals
Brew Kettles, Calandrias, Hop Backs	Difficult	Protein, Scale	577/579, 5TM / M20	569	
Hot Liquor Tanks	Difficult	Scale	577/579, 5TM / M20	569	
Hot Wart Receivers	Difficult	Scale	577/579, 5TM / M20	569	
Fermenters, Uni Tanks, Conditioning Tanks	Intermediate	Protein	577/579, 5TM / M20	569, 573, 583	
Mash Tuns	Easy	Carbohydrate, protein		569	
Lauter Tuns	Easy	Carbohydrate, protein		569	
Serving Tanks, Bright Beer Tanks	Intermediate	Protein		5MI, 569, 583	
Carboys	Easy	Scale			500, 566
Kegs	Intermediate	Scale			5MI, 566

DESCRIPTION	SERIES	MATERIAL	CONNECTION	SPRAY ANGLES	FLOW RANGE	PRESSURE
 **Mini Whirly** • Free-spinning • Very Inexpensive • Fits in small openings	**500**	PVDF	1/2" female BSPP	180° up 180° down 360°	4–8 gpm	20–60 psi
 **Mini Whirly** • Free-spinning • Excellent impingement • Very durable	**566**	316L SS	3/8" male NPT 3/8" female NPT 3/4" slip-on	180° up 180° down 360°	4–8 gpm	20–60 psi
 **Spinner** • Free-spinning • Fits in small openings • Operates in any position	**5MC/5MI**	316L SS	3/4" female NPT 1/2" female NPT	180° down 360°	9–21 gpm	20–60 psi
 **Whirly** • Free-spinning • Well balanced spray head • Flat fan nozzles for impact	**569**	316 SS	3/4" female NPT 3/4" slip-on 1" slip-on 1" Tri-clamp 1-1/2" slip-on	270° up 270° down 360°	11–55 gpm	20–60 psi
 **PTFE Whirly** • Free-spinning • Meets 3A sanitary guidelines • Operates in every position	**573 / 583**	PTFE	3/4" female NPT 3/4" slip-on 1" female NPT 1" slip-on 1-1/2" Tri-clamp	270° up 270° down 360°	19–38 gpm	20–60 psi
 **Gyro** • Free-spinning • Heavy duty and submergeable • PTFE bearings easily replaceable	**577 / 579**	316L SS	1" female NPT 2" female NPT 3" female NPT	180° up 180° down 270° up 360°	35–98 gpm 85–208 gpm 177–306 gpm	20–60 psi
 **Tank Cleaning Machine** • Gear driven for controlled rotation • High cleaning performance • Low maintenance, no lubricants	**5TM/M20**	316L SS	1-1/2" male NPT 1-1/2" female NPT Flange	360°	4-110 gpm	40–100 psi

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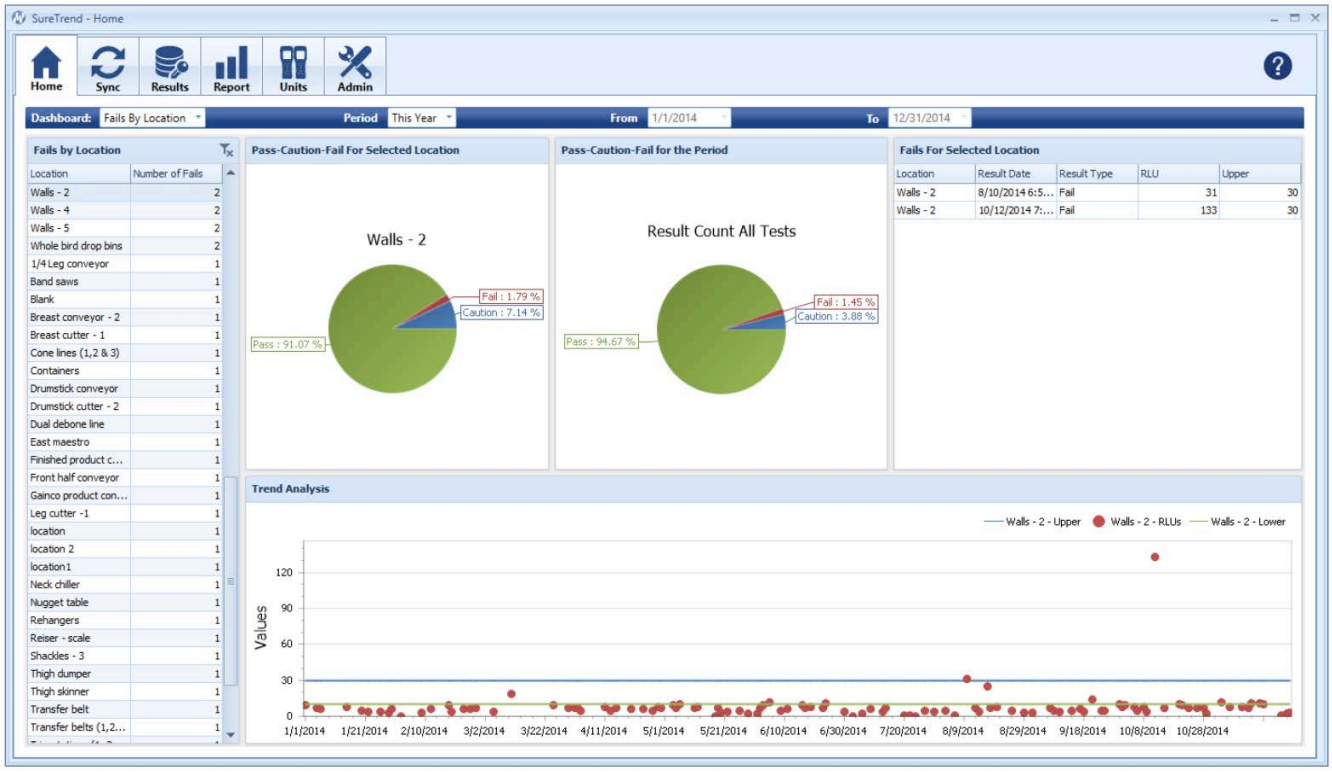
APPENDIX G: EXAMPLE OF SURETREND SOFTWARE CAPABILITIES



Appendix H: Magic Hat Brewery Interview Questions

1. Which testing system and swab types do you use from Hygiena?

We use the SystemSure testing device and Ultrasnap swabs.

2. Which locations do you test for around the brewery? We have rinse water cycles and a final sanitizing wash of iodophor solution. Do you test sanitizing solutions and your rinse water as well as surface testing?

We currently test inside the filler: filler tubes, capper, rinsers on the bottling line, we test the filling spear on the keg line as well as all tanks that have been cleaned. We do not test solutions or rinse water (we have in the past).

3. How did you set pass/fail testing limits, or are you still following the standard recommended by Hygiena (<10RLU is a pass, 10-20 RLU is caution, >30 RLU is fail)?

We set the pass/fail limits at <10RLU on the filler except for on equipment which tend to give us false positive readings like the capper, cleaned tanks the limit is <3RLU if higher it will be recleaned and checked.

4. How is your overall experience with the Hygiena ATP testing units?

The units are easy to calibrate and use for all employees needing to use them, they tend to test accurately though the units themselves are fairly slow compared to the Charm units (I have used both systems before).

5. Any advice for someone who is new to the testing devices?

Keep a steady hand when swabbing, if you get a reading you do not feel is accurate make sure you test a different spot if possible and do not hesitate to make someone clean/sanitize again if you feel your reading is accurate. Certain chemicals can give you an inaccurate reading, know what these are and adjust accordingly in testing methodology.

6. What other testing methods do you usually use besides ATP Testing (cell culture testing on beer, PCR, etc.)?

Microbiological plating, PCR is coming soon for microbial identification.

APPENDIX I: HIGHLAND BREWERY INTERVIEW QUESTIONS

1. Which testing system and swab types do you use from Hygiena?

- 3M atp swabs what they used to use
- super snaps (dry swab) aqua snaps (liquids)
- snaps need to be stored cold- which is one drawback
- hygenia, not losing sample as you push it through membrane
- thinks they used aquasnap total, prefers hygiena

2. Can you give us an overview of your cleaning and sanitation procedures?

Fermenters and bright tanks: Initial rinses, Caustic cycle, water rinse, acid cycle, water rinse, sanitizer- paracetic acid.

- Initial rinse, caustic cycle heated to 130, done in bursts
- Half hour on each, 10 on sanitizer

- Ensure CO2 purged out vessel because it could react with sodium hydroxide
- May not even need to use acid
- Acid can break down protein and organic buildup. Caustic is ineffective against beerstone buildup.
- Run all of house water through carbon filter, already have soft water

3. Which equipment do you test in the brewery? We have rinse water cycles and a final sanitizing wash of iodophor solution. Do you test sanitizing solutions and your rinse water as well as surface testing?

- Fermenter cellar, after acid and before sanitation, open up and test in manway with supersnaps
- Also test last rinse water with aquasnaps
- Do bright testing in a similar way
- Chose manway because of ease of access
- Rinse water used because it was homogenized

4. How did you set pass/fail testing limits, or are you still following the standard recommended by Hygiena (<10RLU is a pass, 10-20 RLU is caution, >30 RLU is fail)? (relative leg unit)

- 4 is a fail for them RLU
- redo CIP if over a 4 RLU
- chosen because of what they were used to seeing, seeing 0s every time
- 2-3 SD should fall with 99-96% anything outside of that you should pay attention to

5. How is your overall experience with the Hygiena ATP testing units?

- More sensitive than other companies used

Their fine. Wouldn't say phenomenally better- price point is better. RLU- not directly comparable between units-more sensitive.

6. Any advice for someone who is new to the testing devices?

- Use micro results, visual inspection, AND ATP all together
- If ATP is high, don't even bother with micro, just redo CIP and then retest with everything

Depends on how many tanks you have. Curious about the software. Don't rely on ATP alone and rely on micro results to verify. Rely more on experience, inspections, and rely most on growth plates.

7. What other testing methods do you usually use besides ATP Testing (cell culture testing on beer, PCR, GC, etc.)?

trial of PCR kits-biotech diagnostics
Beer spoiler tester kit through biotech diagnostics

- They do memory filtration, analysis, and ID
- Don't use molecular methods unless they have concerns, send it out to a third party

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[4] Deapo, Chris. "Magic Hat Brewery Interview." Telephone interview. 21 Jan. 2015. For more information see *Appendix I*.

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