THESIS

ASSESSMENT OF AIRBORNE MICROORGANISMS IN A CRAFT BREWERY

Submitted by

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ABSTRACT

ASSESSMENT OF AIRBORNE MICROORGANISMS IN A CRAFT BREWERY

Although pathogenic bacteria have little chance of surviving in beer due to its intrinsic antimicrobial hurdles, there are other microorganisms capable of surviving and spoiling beer. The quality of all food products including beer are not only affected by the integrity of the raw materials, and cleanliness of the equipment and packaging materials, but also by the purity of the environmental air surrounding the processing area. The purpose of this project was to examine the environmental microbial air quality within various areas of a craft brewery with special emphasis on potential beer spoiling bacteria.

First, samples inside the brewery and samples outside the brewery were collected to establish a baseline of data, identify areas of concern, and to examine the effect of seasonality. Those areas of concern then were sampled more often and also were sampled based on the risk of product contamination. The canning line within the brewery was identified as a specific area of concern. Bottling and canning lines in breweries often are considered non-closed production equipment and have the ability to become contaminated from outside sources including the environment.

The air was sampled 307 times over a period of 22 months using an automated impaction sieve sampler pulling 80 liters of air. Samples were plated both aerobically and anaerobically. The aerobic plates were used for a general cleanliness of the area while the anaerobic plates were included to examine for beer spoiling organisms. The standard (specification limit) used for the indication of a contaminated area was a plate with 40 colony forming units (CFU) or more per 80 liters of air sampled.

The results of this study revealed that testing for airborne microorganisms is highly recommended in the craft brewing industry due to the potential for the impurity of the environmental air surrounding the processing area. Seasonality had an effect on total number of aerobic airborne microorganisms with the spring months being approximately five times higher than other months. The canning line in the brewery was found to be contaminated with beer spoiling bacteria on average 75% of the time. Critical areas in the brewery, such as the bottling and canning lines, should be routinely tested for airborne microorganisms as they could lead to final product contamination. Routine microbial environmental air testing is a good indicator of overall brewery cleanliness.

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CHAPTER I

Introduction

Brewing beer is a complex process and critical steps during brewing, cellaring, and packaging need to be monitored for microbial contamination. If the equipment, raw materials, or yeast are microbiologically contaminated, the beer will not meet the brewer's expectations (Bamforth, 2002). Brewers typically are not worried about pathogen growth in beer. The poor nutrient status of beer, low pH, antiseptic action of hops, together with ethanol, restrict the range of bacteria that can grow in finished beers (Priest and Campbell, 2003). Most of the organisms of concern to the brewer will cause off-flavors or turbidity in the beer; these are beer quality related problems (Sakamoto and Konings, 2003).

One of the most important parts of the brewing process is the proper control of unwanted microorganisms (Stewart and Russell, 1998). Good quality assurance procedures are necessary for ensuring a high level of cleanliness, especially when there is no sterile filtration of beer or bottle/can pasteurization (Henriksson and Haikara, 1991). An increasing amount of microbiologically spoiled beers are caused by secondary contaminations even though overall brewery hygiene has greatly improved in recent years (Henriksson and Haikara, 1991). Sampling for cleanliness and increasing the awareness of hygienic conditions can enable the brewer to produce beer with longer

shelf life, fewer customer complaints, higher production rates, and lower rejection rates (Ligugnana and Fung, 1990).

Due to the inherent exposure to the environment during the filling process, bottling and canning lines in breweries often are considered open production equipment and have the ability to become contaminated from outside sources including the environment (Priest and Stewart, 2006). Microbes are in constant movement in the bottling and canning areas because they settle from the air onto surfaces and then are recirculated into the environment again by the turbulence of the air (Henriksson and Haikara, 1991). Trapping the organisms present in the air and growing them on the appropriate media should be important quality control parameters to brewers (Bamforth, 2002). Microbiological analysis of brewery environmental air can be used to determine if there are indicator or beer-spoiling organisms present (Henriksson and Haikara, 1991).

Microorganisms can vary in amount and types depending on seasonality. Environmental factors, such as humidity, temperature, and air speed can have a direct impact on the growth and survival of airborne microorganisms (Crozier-Dodson and Fung, 2002). Colorado has an arid climate with low humidity and seasonal shifts can produce wide temperature swings (Doesken et al., 2003).

Microbial environmental air quality in breweries to date has been minimally researched; there are only three peer-reviewed papers available with the most recent study done in 2001 (Henriksson and Haikara, 1991; Back, 1994; Odebrecht et al., 2001). None of these studies evaluated a U.S. craft brewery or looked at seasonality effects specifically in an arid climate with low humidity. A review of the literature confirmed a

need for further investigation of the microbial environmental air quality of a craft brewery and specifically a craft breweries' canning line.

CHAPTER II

Review of Literature

History of Brewing

No one knows when the first fermented beverage was produced, but most likely it was an accidental occurrence discovered by prehistoric peoples (Hornsey, 2003). Decomposing or damaged fruits and/or vegetables may have been exposed to the environmental air allowing bacteria, yeast, and molds present in the air to take residence in the sugar and carbohydrate rich inner areas of the produce and begin spontaneous fermentation. Spontaneous fermentation occurs when the sugars and carbohydrates are converted into alcohol and carbon dioxide by "wild" microorganisms that are present in the environment (Mussche, 1999).

The first evidence of brewing beer was from the Neolithic period (Nelson, 2005). From the evidence, it is not known whether grains were used or if other raw materials, such as honey or fruits, provided the sugar and carbohydrate sources (Meussdoerffer, 2009). Grain based brewing of beer most likely originated in Mesopotamia and Egypt, where grain cultivation first flourished (Meussdoerffer, 2009). Since these early times of brewing of beer, the importance of beer in society and history has been significant. Beer consumption has become common and widespread; it is the third most popular drink overall after tea and water (Nelson, 2005).

Importance of Craft Brewing

Craft brewing is defined as small, independent, and traditional; small meaning that the brewery produces 6 million barrels of beer or less per year (Brewers Association, 2011). Craft brewers typically are beer brewing innovators using traditional and non-traditional ingredients to develop unique twists to customary beer styles and creating brand new styles. Wild yeasts, strains of bacteria, spices, and fruits are some of the ingredients used by craft brewers.

The emergence of craft brewing in the United States has been a relatively new occurrence as of the last 30 years. In the early 80's, there were a total of 101 breweries in the U.S., with the top six breweries (Anheuser-Busch, Miller, Heileman, Stroh, Coors, and Pabst) controlling 92% of beer production (Raley, 1998). In 2010, craft brewers represented 4 to 9 percent of volume and 7.6 percent of retail dollars of the total U.S. beer category (Brewers Association, 2011). Craft brewing in the past 3 decades has spawned the creation of numerous microbreweries and brewpubs, raising the total number from under 100 to over 1700 (Figure 1) (Brewers Association, 2011). The legalization of homebrewing and brewpubs in 1979, lowered excise taxes for brewers selling less than 2 million barrels, and an increase in consumer preference for specialty beers were among some of the reasons for the increase in craft breweries (Tremblay and Tremblay, 2005).

Figure 1. U.S. Brewery Count from 1900 – 2010 (Brewers Association, 2010).

Craft brewing is a vital component of local economies. There have been an estimated 100,000 jobs created in the U.S. as a result of the growth of the craft brewing industry, including brewpubs and serving staff (Brewers Association, 2011). Colorado is home to over 120 established craft breweries, which contribute significantly to the state's economy (Colorado Brewers Guild, 2011).

Beer Production

Beer brewing is part art and part science. The main ingredients in traditional beer production are water, malted barley, hops, and yeast (Priest and Stewart, 2006). It is the manipulation of these ingredients, with variations in time and temperature that can determine the creation of tasty or terrible tasting beers. The brewer has a concept of the beer in mind, but must carefully plan and execute the intended brewing process in order to create the expected beer or beer style (Priest and Stewart, 2006). Even with the best laid brewing plans, if the equipment, raw materials, or yeast are microbiologically contaminated the beer will not meet the brewer's expectations (Bamforth, 2002).

Beer production is a complex process that can be divided into four stages: brewing, cellaring, packaging, and warehousing. Brewing is the first step and includes the

grinding of malted barley and mixing the barley with heated water (Priest and Stewart, 2006). The heating of the malt and water releases enzymes that break down the carbohydrates in the malt into simpler sugars, so yeast can utilize these simpler sugars and continue the fermentation process (Fix, 1999). The resulting liquid from the brewing process is called wort. After cooling, the wort is transferred to a vessel to begin the cellaring process. The cellaring process consists of adding yeast to the wort and at this step the fermentation process begins; the yeast metabolizes the sugars in the wort to produce carbon dioxide and alcohol (Priest and Stewart, 2006). After cellaring, the wort is now called beer. When fermentation is complete, the beer and yeast are separated and the beer is allowed to age (Fix, 1999). The beer at this stage is now considered bright beer and is ready to be packaged. After packaging in bottles, cans, or kegs, the beer will be transferred to a warehouse for storage until it is shipped to various outlets for distribution and consumption.

Brewing Microbiology

Microbiologically speaking, wort and beer have different characteristics that can create either a favorable or unfavorable environment for microorganisms. Oxygenated wort has a pH of approximately 5, is high in carbohydrates, and contains some available protein which makes it an ideal environment for the growth of yeast and bacteria (Menz et al., 2010a). Beer on the other hand, has a lower pH (~3.8 – 4.3), contains ethanol, hop bitter compounds, carbon dioxide, and has larger, more complex forms of carbohydrates and proteins to break down with little nutritive substances (Sakamoto and Konings, 2003). The poor nutrient status of beer, low pH, antiseptic action of hops, together with ethanol, restricts the range of bacteria that can grow in finished beers (Priest and

Campbell, 2003). Beer also is mostly anaerobic which generally favors the growth of microaerophilic and anaerobic organisms.

Beer production utilizes beneficial microorganisms. The most beneficial organism is yeast used for the fermentation of wort (Bamforth, 2002). Yeasts are beneficial because they are widely used for production of beer, wine, spirits, foods, and a variety of biochemicals (Stewart and Russell, 1998). There are two main types of yeast for most beers brewed worldwide, ale yeast (*Saccharomyces cerevisiae)* and lager yeast (*Saccharomyces carlsbergensis*); there are hundreds of subspecies of these two types of yeast (Priest and Campbell, 2003). In the presence of oxygen, the yeast take up the dissolved sugars, nutrients, and free amino nitrogen in the wort to support growth and proliferation (Stewart and Russell, 1998). Under anaerobic fermentation conditions, the yeast generate several end products: ethanol and carbon dioxide, as well as other volatile and non-volatile compounds (Fix, 1999). In some sour and wood aged beers, there are other types of wild yeasts (i.e., *Brettanomyces bruxellensis*) and bacteria (i.e., *Lactobacillus* spp.) used in the production to produce the sour and/or spicy notes (Fix, 1999).

Spoilage Microorganisms

Food and beverage processors need to pay careful attention to monitoring the microbial environment within their facilities to prevent the contamination of their product. There are several types of microorganisms that can cause the spoilage of beer. One of the most important parts of the brewing process is the proper control of unwanted microorganisms (Stewart and Russell, 1998).

Most of the organisms of concern to the brewer will cause off-flavors or turbidity in the beer; these are beer quality related problems (Sakamoto and Konings, 2003). The gram-positive lactic acid bacteria are the group of bacteria likely to cause a significant threat to beer (Priest and Campbell, 2003). The two main lactic acid spoilage microorganisms in beer, *Lactobacillus* species and *Pediococcus* species, are able to survive in beer because they are both facultative anaerobes (Fix, 1999). These spoilage bacteria are considered the most hazardous. In Germany between 1980 and 1990, 58-88% of microbial beer-spoilage incidents were caused by lactobacilli and pediococci (Back, 1994).

Lactobacillus and *Pediococcus* are Gram positive organisms. *Lactobacillus* can cause a silky turbidity with some lactic acid sourness and/or diacetyl (buttery) off flavors (Priest and Campbell, 2003). *Pediococcus* can cause ropiness turbidity with off-flavors of lactic acid (sourness) and diacetyl (Priest and Campbell, 2003).

Acetic acid bacteria and *Enterobacteriaceae* are Gram negative beer spoiling bacteria. Acetic acid bacteria oxidize ethanol into acetic acid resulting in producing sour off flavors and turbidity issues (Priest and Stewart, 2006). Acetic acid bacteria are aerobes so spoilage of beer should be minimal since beer should be stored with limited access to air (Priest and Stewart, 2006). The enterobacteria are facultative anaerobes, but are inhibited by ethanol and low pH (Priest and Stewart, 2006). Enterobacteria can produce dimethyl sulfide (DMS) which imparts a parsnip like, sulfury flavor to beer (Boulton and Quain, 2001).

Brewers need to be concerned about wild yeasts in the brewery. Wild yeasts can come from raw materials, cross-contamination within the brewery, or the air (Priest and Stewart, 2006). Wild yeasts can cause problems for beer quality, causing turbidity, creating phenolic or spicy off flavors, and out competing or killing beneficial production yeasts (Priest and Campbell, 2003).

Food Safety

Pathogenic bacteria in food have been linked to numerous foodborne illness outbreaks. In 2011 the annual estimate of disease due to contaminated food consumed in the United States was calculated to be 47.8 million illnesses, 127,839 hospitalizations, and 3,037 deaths (Scallan et al., 2011). Several antimicrobial intrinsic and extrinsic hurdles occur during fermentation including production of ethanol and carbon dioxide, lowered pH and nutrient levels, hop additions, and pasteurization that make beer a hostile environment for the growth of pathogens (Menz, 2010b).

While pathogenic bacteria typically are not a concern to brewers, there are other microbes that need to be monitored for quality purposes (Boulton and Quain, 2001). Good quality assurance procedures are necessary for ensuring a high level of cleanliness, especially when there is no sterile filtration of beer or bottle/can pasteurization (Henriksson and Haikara, 1991). Sampling for cleanliness and increasing the awareness of hygienic conditions can enable the brewer to produce beer with longer shelf life, fewer customer complaints, higher production rates, and lower rejection rates (Ligugnana and Fung, 1990).

Hazard Analysis and Critical Control Points (HACCP)

One of the most rigorous and widely accepted preventative programs for the safe production of foods and beverages is known as Hazard Analysis and Critical Control Points (HACCP). HACCP involves implementing a series of preventative measures throughout production to control and limit potential hazards and reduce the risks of foodborne illness in consumers (Barron, 1996). Setting up a HACCP plan comprises seven steps: step 1. conducting the hazard analysis, step 2. determining critical control points (CCPs), step 3. defining critical limits for CCPs, step 4. establishing the monitoring system, step 5. setting up the corrective actions, step 6. verifying the effectiveness of the system, and step 7. documenting all procedures and records (Erzetti et al., 2009). The intention of HACCP is to produce zero defects; although unattainable, a well-executed HACCP plan does help minimize the number of unsafe products (Kourtis and Arvanitoyannis, 2001).

Potential hazards, or CCPs, can be things that cause harm to the consumer and may be biological, chemical, or physical in nature (Sadeghi, 2010). As risk is being evaluated, hazards that have low or no risk associated with them are identified and often are considered quality control points (Rush, 2006). Quality control points become part of the brewery's quality assurance management program as they are critical to the integrity of the food product (Bamforth, 2002). Many breweries now integrate HACCP and quality assurance management systems to create a holistic approach to assure product safety and quality (Jackson, 2000). Although beer is generally safer that other food products because of its intrinsic antimicrobial properties, identification of potential hazards and

establishment of preventative and corrective actions is vital (Kourtis and Arvanitoyannis, 2001).

Importance of Cleanliness of Equipment

Cleanliness of equipment and raw materials is key to the production of beer. The brewer must pay careful attention to the cleanliness of equipment and quality of materials to assure that no beer spoiling organisms are introduced into the product (Priest and Stewart, 2006). The quality of all food products including beer not only are affected by the integrity of the raw materials, the cleanability of the equipment, and the packaging materials, but also by the purity of the environmental air surrounding the processing area (Al-Dagal and Fung, 1990).

The presence of undesirable microorganisms can interfere with the process of fermentation by competing with production yeast and producing off-flavors and/or turbidity problems (Bamforth, 2002). If contamination leads to customer complaints and product recalls; the consequences can be very expensive, time consuming, and damage the reputation of the brewer (Priest and Stewart, 2006). Environmental microbial air testing can be used to assess for possible bacterial contaminants.

Most breweries use a cleaning in place (CIP) regime to assure that the brewing equipment and cellaring vessels are clean and will not contaminate the finished beer. A properly designed brewery CIP regime consists of a rotation of caustic and acid cleanings followed by thorough rinsing and use of hypochlorite or paracetic acid-based sterilizers (Bamforth, 2002). The CIP regime of closed production equipment is proven to be one of

the most efficient and effective ways of limiting spoilage microorganisms and assuring the safety and integrity of food products (Rice, 2011).

Bottling and canning lines in breweries often are considered non-closed production equipment and have the ability to become contaminated from outside sources including the environment (Priest and Stewart, 2006). Microbes are in constant movement in the bottling and canning areas because they settle from the air onto surfaces and then are recirculated into the environment again by the persistent turbulence of the air (Henriksson and Haikara, 1991). In a canning line, the open container is exposed to the environmental air for several seconds or milliseconds, depending on the speed of the canning line, prior to a lid being attached. This environmental air exposure has the potential to lead to final product contamination.

Microbiological analysis of brewery environmental air can be used to determine if there are indicator or beer-spoiling organisms present (Henriksson and Haikara, 1991). The presence of microorganisms in the air can be indicative of poor cleaning of equipment and poor filtering of environmental air (Henriksson and Haikara, 1991). Trapping the organisms present in the air and growing them on the appropriate media should be important quality control parameters to brewers (Bamforth, 2002). There have been many cases where exposed product has become microbiologically contaminated due to unfiltered air and negative air pressure in food plant areas (Graham et al., 2011). Many food production facilities and dairies use microbiological environmental air sampling for this purpose (Ligugnana and Fung, 1990).

Air Sampling

Air in a food processing plant or brewery can contain different types of microorganisms at varying levels of intensity depending on the types of activities within the plant (Al-Dagal et al., 1992). The microorganisms in the air can vary greatly in size, from 0.1 µm to greater than 100 µm in diameter (Lutgring et al., 1997). The Food and Drug Administration advises that environmental air contamination may be a vector for microbial contamination of product (Ren and Frank, 1992). There currently are no limits set or regulations required for microbial environmental air sampling in food plants (Devico, 2002).

There are several ways to test the microbiological environmental air quality in food plants and breweries. Two of the most common methods used are: active impaction sampling and passive settle plates (Holah, 2009). Active impaction samplers are automated samplers that pull a set volume of air through a perforated sieve and microorganisms collect on an agar petri dish, agar strip, or liquid medium contained inside the sampler (Zorman and Jersek, 2007). Passive settle plates are open agar petri dishes that are placed throughout the area for a set amount of time and rely on the microorganisms in the air to settle on the plate (Sullivan, 1979). This method of air sampling is referred to as sedimentation sampling and does not give an accurate assessment of the number of microorganisms in the air, only the organisms of sufficient size and weight which tend to fall to the surface of the agar petri dish (Holah, 2009).

Seasonality

The amount and types of microorganisms present in the air can be affected by environmental factors such as humidity, temperature, and air speed (Crozier-Dodson and Fung, 2002). In warm climates as compared to cold climates, the levels of indoor airborne microorganisms have been found to be higher, which indicates the effect of temperature (Shale and Lues, 2007). Higher relative humidity is conducive to microbial growth and survival (Lutgring et al., 1997).

Colorado is a mid-latitude interior continental state with the highest average elevation in the U.S. (Colorado Climate Center, 2011). In general, the climate is cool and dry. There are large seasonal swings in temperature although overall humidity is low (Doesken et al., 2003).

Scope of Study

Microbial environmental air quality in breweries to date has been minimally researched; there are only three peer-reviewed publications available with the most recent study done in 2001. None of these studies evaluated a U.S. craft brewery. A review of the literature confirmed a need for further investigation of the microbial environmental air quality of a craft brewery and specifically a craft breweries' canning line.

Objectives of this study were to:

- Obtain a baseline quantification of a craft brewery's microbial environmental air population
- Examine the effect of seasonality on the microbial environmental air population

- Survey the microbial environmental air specifically in the canning line of a craft brewery

CHAPTER III

Materials and Methods

Sampling Location

The research was conducted at New Belgium Brewing Company Inc.; a regional craft brewery located in Fort Collins, Colorado and employs approximately 400 people. The brewery was founded in 1991 and the brewery distributes beer in 26 states throughout the continental U.S. In 2010, the brewery produced 650,000 barrels of beer. New Belgium packages beer in glass bottles, stainless steel kegs, and aluminum cans.

The canning line was installed in 2008 in order for the brewery to begin canning some of its products. The size of the canning line is small and runs at a slow speed. There was a concern regarding the exposed surface area of the beer and the possibility of contamination from the surrounding microbial environmental air. Several micro positive can finished product results and the fact that there is no pasteurization after canning were additional reasons for air sampling in the beer canning line.

Air Sampling

The environmental air throughout various locations, inside and outside at New Belgium Brewing Company Inc., 500 Linden Street, Fort Collins, CO, and specifically the MASTERCAN 9/1 can filler (SBC/BC International, Monteccio Emilia, Italy), was evaluated for airborne microbial organisms, including mold, bacteria, and yeast. The can filler dispenses beer into 12 ounce aluminum cans at approximately 60 cans per minute

using 9 filler heads. Three locations within the filler were evaluated: 1) approximately 38 cm above the can seamer, 2) approximately 61 cm above the can filler, 3) suspended midway, approximately 76 cm, in the filler. These three locations were sampled on a weekly basis for 22 months depending on production scheduling; there were times when the canning line would not be running due to maintenance and lack of demand for canned beer. Additional locations near the can filler (can conveyor, can accumulation table, west can/keg hall, east can/keg hall, middle of can/keg hall, top of can depalletizer, outside top of can filler, and can storage area) were sampled periodically for comparison. Sample collection locations were selected to represent distinct areas within the brewery and particularly in the vicinity of the canning line. A total of 156 environmental air samples were collected from May 2009 to February 2011.

Environmental air was sampled by impaction on solid media using a bioMérieux Inc. airIDEAL sampler (Marcy l'Etoile, France). The airIDEAL is an impactor type of air sampler in which air is aspirated through a grid perforated with a pattern of 286 calibrated holes. The resulting air streams containing microbial particles were directed onto an agar surface of microbiological media. Eighty liters of environmental air were pulled through the sampler for each collection after a thirty second countdown of the sampler to allow for standard environmental conditions. There is no published standard recommended sample threshold for environmental brewery air sampling. However, EcoLab (Boufford, 2003) has developed the standard of <40 colony forming units (CFU)/2 minute sampling time, or 80 liters of air sampled, as normal and generally acceptable air in food production plants.

Microbiological Analysis

Each sampling location was analyzed for both aerobic and anaerobic microbes. Two types of culture media were used: selective media MRS (de Man, Rogosa, and Sharpe) manufactured by Oxoid Limited (Basingstoke, Hampshire, United Kingdom) and non-selective WLN (Wallerstein Laboratory Nutrient Agar) manufactured by Oxoid Limited (Basingstoke, Hampshire, United Kingdom).

MRS media was prepared in the microbiology laboratory at New Belgium Brewing Company according to the manufacturer's directions. The MRS agar was supplemented with maltose (5 g/liter) and acidified with HCL to a pH of 4.5. The additions of maltose and HCL have been found to aid in the recovery of brewing specific bacteria (Priest and Stewart, 2006). According to the Difco Manual (1999), *Lactobacilli* MRS agar is recommended for use in the isolation, enumeration, and cultivation of *Lactobacillus* species. Cycloheximide (Actidione) solution (0.1% manufactured by Oxoid) (Basingstoke Hampshire, England)) was added to MRS (0.1ml/100mls) to inhibit yeast growth in MRS media (Priest and Campbell, 2006).

WLN agar media was prepared following the manufacturer's directions. According to the Difco Manual (1999), WLN medium is used for cultivating yeasts, molds, and bacteria encountered in brewing and industrial fermentation processes. The selective media, MRS, was anaerobically incubated with carbon dioxide at 25-28°C for 7 days using a VWR Scientific Vacuum Oven (Radnor, PA). The non-selective media, WLN, was aerobically incubated at 28°C for 3 days using a VWR Aerobic Incubator (Radnor, PA). Positive (yeast/bacteria) and negative controls for each type and batch of media were conducted for quality control purposes.

Environmental air quality is normally expressed as microbial counts per volume of air, e.g. CFU/liter. After the incubation period, the colony forming units were counted and recorded. Microbial counts were performed using standard guidelines adapted from *The Compendium of Methods for the Microbiological Examination of Foods* (Swanson et al., 1992). Counts above 250 CFU per plate were marked as too numerous to count (TNTC) and spreader colonies that exceeded 50% of the plate were marked "spreaders." TNTC and "spreader" plates were not included in the statistical analysis.

Statistical Analysis

Three hundred and seven samples were collected from the brewery and canning line. The following variables were examined: locations, dates, aerobic colony forming units (CFU), and anaerobic CFUs. Seasonal impacts on microbial populations also were assessed. The sampling design divided eight locations into twenty-nine sub-locations (Table 1). The packaging canning line location's data was analyzed separately from the other locations. All locations were sampled both aerobically and anaerobically within five minutes of each other.

Analysis was conducted using SAS/STAT® software version 9.2, SAS Institute Inc. (Cary, North Carolina). Aerobic results were transformed onto the $\log_{10}$ scale for analysis. Means then were back-transformed and expressed as CFU per 80 liters of air sampled. Because over half of the anaerobic results were zeros, they were transformed into zeros and ones (for positive results) and logit transformed; logit transformation is the inverse of the sigmoidal logistic fuction. Means were back-transfomed and expressed as percentage of positives. Zero sites meant that those sites were most likely clean of beer

spoiling bacteria. Analysis of variances were run with one or two factors (location and/or date) on the $\log_{10}$ or logit transformed data. Means and 95% confidence intervals were computed for the back-transformed data.

Anaerobic results were transformed into zeros and ones and expressed as percentage of positives. Zero sites meant that those sites were most likely clean of beer spoiling bacteria. A series of t-tests were performed with 95% confidence intervals.

CHAPTER IV

Results and Discussion

Aerobic Results All Locations

Mean counts of aerobic colony forming units (CFU) for all locations are shown in Figure 2. Data is color coded to indicate main location groupings with each bar representing a sub-location. A red line indicates the specification limit at 40 CFU/80 liters of air sampled (Boufford, 2003).

The overall range extended from 3 CFU/80 liters to 158 CFU/80 liters. Aerobic mean counts of exterior sampling sub-locations ranged from 31 to 69 CFU/80 liters. Brewhouse aerobic mean counts had the highest average at 79.5 CFU/80 liters and the means of the two sub-locations ranged from 76 to 83 CFU/80 liters. Fermentation cellar aerobic mean counts exhibited a wide range from 18 to 98 CFU/80 liters. Aerobic mean counts from sub-locations sampled in the microbiology laboratory had the lowest average at 4.5 CFU/80 liters with the smallest range from 3 to 6 CFU/80 liters. Warehouse aerobic counts were relatively high and ranged from 38-61 CFU/80 liters. The five sub-locations sampled in the yeast cellar were fairly consistent with aerobic mean counts ranging from 27-40 CFU/80 liters. The ten packaging sub-locations sampled had the widest range of aerobic mean counts from 11-158 CFU/80 liters.

Forty–eight percent of the sub-locations were over 40 CFU/80 liters. The packaging canning line area drain had the highest overall count at 158 CFU/80 liters. The sub-location with the lowest overall count at 3 CFU/80 liters was the microbiology laboratory hood.

Overall there was quite a bit of variability among sample locations. The exterior and canning line drain samples were high which is to be expected as these areas are where temperature and humidity come into play. Also, critical areas where strict hygiene is important such as the microbiology laboratory and the yeast cellar were on average slightly lower.

Aerobic Results by Sample Date

Figure 3 shows mean aerobic CFU counts by sample date. Mean aerobic counts ranged from 3 to 437 CFU/80 liters. Sample locations varied by date. The colored bars represent meteorological seasons (Hopkins, 2005): orange for spring (March, April, and May), red for summer (June, July, and August), green for autumn (September, October, and November), and blue for winter (December, January, and February).

Mean aerobic CFU counts varied by time of year with seasonal means ranging from 32 to 437 CFU/80 liters. Spring samples had the widest range from 16 to 437 CFU/80 liters with the highest average of 185 CFU/80 liters. Summer samples had the narrowest range from 14 to 70 CFU/80 liters and an average of 40 CFU/80 liters. Autumn samples ranged from 3 to 77 CFU/80 liters with the lowest average of 32 CFU/80 liters. Winter samples ranged from 11 to 128 CFU/80 liters with an average of 42 CFU/80 liters.

Spring time total aerobic count samples were approximately five times greater than other seasons. Temperature and humidity are typically elevated during the spring months. According to Henriksson and Haikara (1991), the higher the humidity and temperature, the higher the amounts of airborne microbes. Variations of sample date could be attributed to physical changes within the brewery such as doors being propped open, construction or work being performed, and heating and ventilation changes.

The baseline quantification of a craft brewery's microbial environmental air population was obtained from the aerobic results by sample date. From September 4, 2009 through October 6, 2010 the aerobic counts from this data create an annual number of airborne microorganisms to which future data can be compared (refer to Figure 3). Extreme deviations from this baseline data should be examined in order to find a root cause to explain the abnormalities.

Anaerobic Results All Locations

Figure 4 represents the percentage of anaerobic positives for all locations sampled. For example, the graph shows that the packaging canning line seamer will be positive for beer spoiling bacteria 86% of the time it is sampled. Anaerobic positive percentages ranged from 22 to 86% of the time. The packaging canning line location will test positive for anaerobic beer spoiling bacteria on average 75% of the time due to the constant motion of the air in the area. The yeast cellar location will test positive for anaerobic beer spoiling bacteria on average 40% of the time. These two locations are areas within a craft brewery where hygiene is critical and regular microbial air testing should be implemented.

Subset Canning Line Location Results

Figure 5 shows the mean aerobic counts for the canning line subset locations. Mean aerobic counts ranged from 11 to 49 CFU/80 liters. The sub-location of the can filler bottom had the lowest value at 11 CFU/80 liters and the canning line seamer had the highest value at 49 CFU/80 liters. The canning line is a critical area because there is no pasteurization downstream. The chance for microbial contamination is high due to the constant motion of the air in this area, the humidity of the environment, and the exposed surface area of the beer.

Figure 6 shows the percentage of anaerobic positives for the canning line subset locations. Anaerobic positive percentages range from 66-86% of the time. The canning line seamer had the highest percentage for testing positive for anaerobic beer spoiling bacteria 86% of the time. Based on these results, the canning line was cleaned more often throughout a day, routine swab samples of the machinery were taken, and HEPA filters were changed. Similar to the aerobic results, the canning line seamer becomes the area of concern for the craft brewer.

CHAPTER V

Conclusions and Recommendations for Further Study

The results of this study revealed that testing for airborne microorganisms is highly recommended in the craft brewing industry due to the potential for the impurity of the environmental air surrounding the processing area. This study was designed to examine the microbial air quality within certain areas of a craft brewery to establish baseline values, identify areas of concern, and examine the effect of seasonality on microbial populations. By looking for areas of concern, the canning line was identified as a main area that microbial organisms could pose the greatest risk. The air in the canning line is in constant movement and can cause microbes to settle onto equipment surfaces therefore possibly contaminating finished product.

Establishing the environmental microbial air population baseline in the brewery highlighted the importance of regular testing and the importance of a robust hygiene program. The entire process, from brewing, cellaring, packaging to warehousing, needs to be routinely tested for airborne microorganisms and specifically beer spoiling bacteria. Packaging of beer, particularly canning, is a vulnerable area where undesirable airborne microorganisms can potentially spoil beer because of the constant motion of the air, the humidity of the environment, and the exposed surface area of the beer. Also, there is no pasteurization downstream of the canning process. The canning line was predicted to be contaminated with beer spoiling bacteria on average 75% of the time. By sampling for

the cleanliness and periodically monitoring the microbial environment throughout the beer process, the brewer can produce a product that will retain a longer shelf life as well as avoid customer complaints.

Air temperatures and humidity change with the seasons. Airborne microbial populations also fluctuate in numbers and types during seasons. This study showed that seasonality had an effect on total number of aerobic airborne microorganisms with the spring months being approximately five-fold higher than other seasons.

With respect to the safety of the consumer, the incidence of pathogenic bacteria that are linked to food illness is greatly reduced due to the antimicrobial intrinsic and extrinsic hurdles that occur during the fermentation process. But even though the fermentation process will mitigate the growth of microbes in the beer, there are certain bacteria that can still be a major concern for the brewer. *Lactobacillus* and *Pediococcus* are two organisms that will cause beer turbidity, off flavors, and spoiling. These organisms are the main bacteria that require routine monitoring because they are considered most hazardous to the beer process.

With the importance of concentrating on the testing for unwanted beer spoiling bacteria, air sampling for these microbes is crucial for craft breweries. This study showed that using active impaction sampling methods for airborne microorganisms throughout the production of beer is beneficial for quality purposes. This method requires sampling 80 liters of air in various areas throughout the facility and then using the appropriate microbiological media to determine and monitor the types and amounts of

airborne bacteria present. Areas of concern can then be identified and an appropriate regimen of air testing and hygiene can be implemented.

By promoting cleanliness throughout the production facility and constant monitoring of air samples for airborne microorganisms, the craft brewer will be able to produce beer that will be less affected by spoilage. Keeping the airborne microbial population in check is important not only for the integrity of the beer but also because craft breweries are a vital part of local economies. Even with the most well thought out brewing plans, if the equipment, raw materials, or yeast are microbiologically contaminated, the beer will not live up to consumer's expectations.

Recommendations for Further Studies

- Determine if there is a relationship between microbial contaminated finished product and microbial air testing results.
- Research the possible link between equipment cleaning procedures and microbial air testing.
- Look for possible connections of microbial air bacteria with customer complaints of finished product.
- Examine seasonal airborne bacteria fluctuations in a craft brewery located in a non-arid and higher humidity climate.

Table 1. *Brewery locations and sub-locations sampled.*

Location	Sub-location
Brewhouse	BH2 hallway
	BH2 double doors
Exterior (outside)	Acid alley
	BH2 double doors
	Wood cellar rolling door
	Truck bays - warehouse
Fermentation Cellar	Cellar 1
	Cellar 2
	Cellar 3
Keg storage	Empties
Lab	Micro lab
	Micro lab hood (neg control)
Warehouse	Truck bays
	Wood cellar
Yeast Cellar	Prop room - middle of room
	Prop room - north wall
	Yeast storage- middle of room
	Yeast storage - near hallway door
	Yeast storage - west wall
Packaging – Canning Line	Can palletizer - top
	Can filler - outside box - top
	Can conveyor
	Can filler - top
	Can filler - bottom
	Can seamer
	Canning line hall -west wall
	Canning line hall - east wall
	Canning line hall - middle of room
	Canning line/kegging line drain

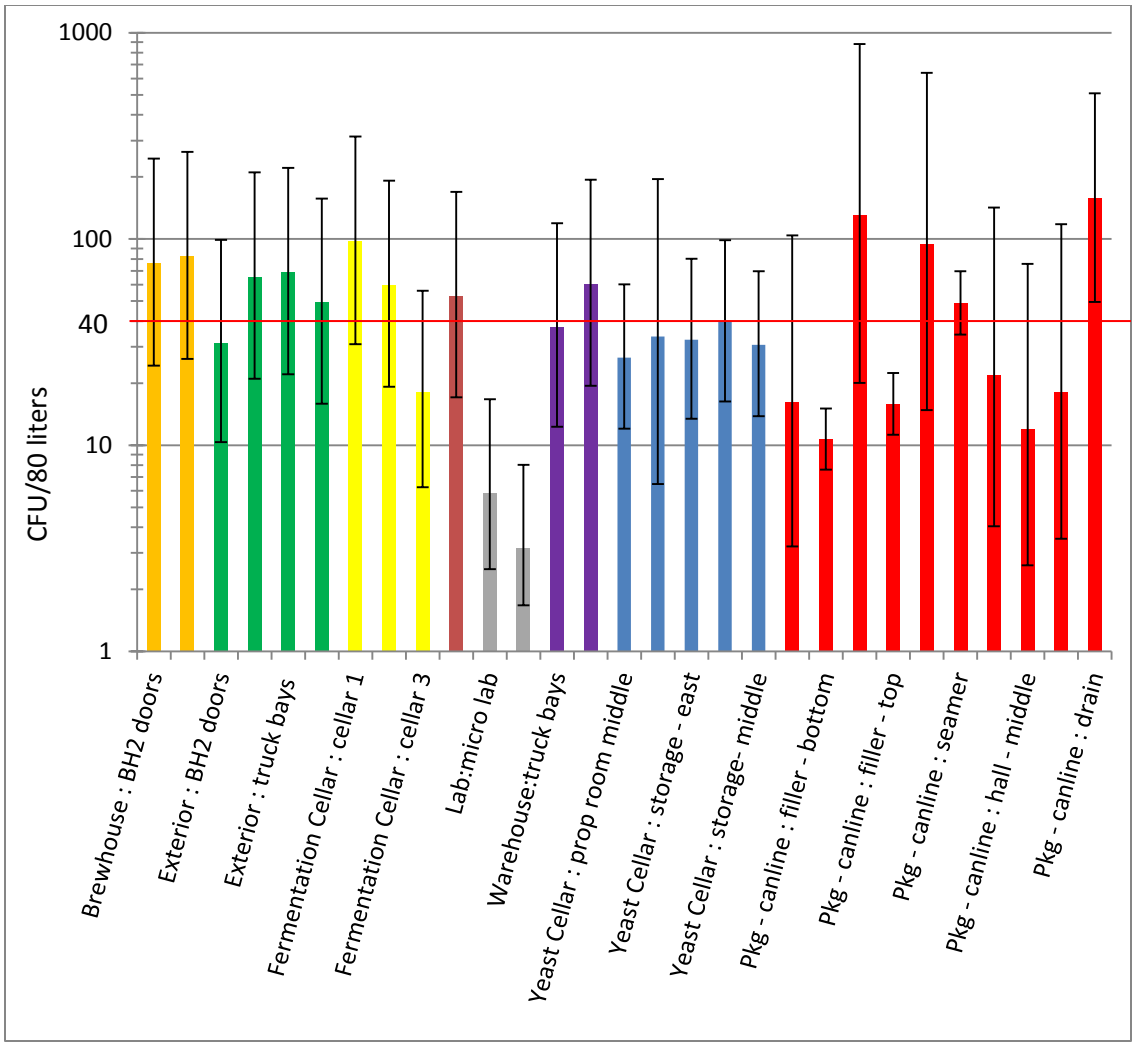

Figure 2. *Means for aerobic CFUs for all sub-locations.*

Colored bars represent main locations. Error bars indicate the standard error of the mean.

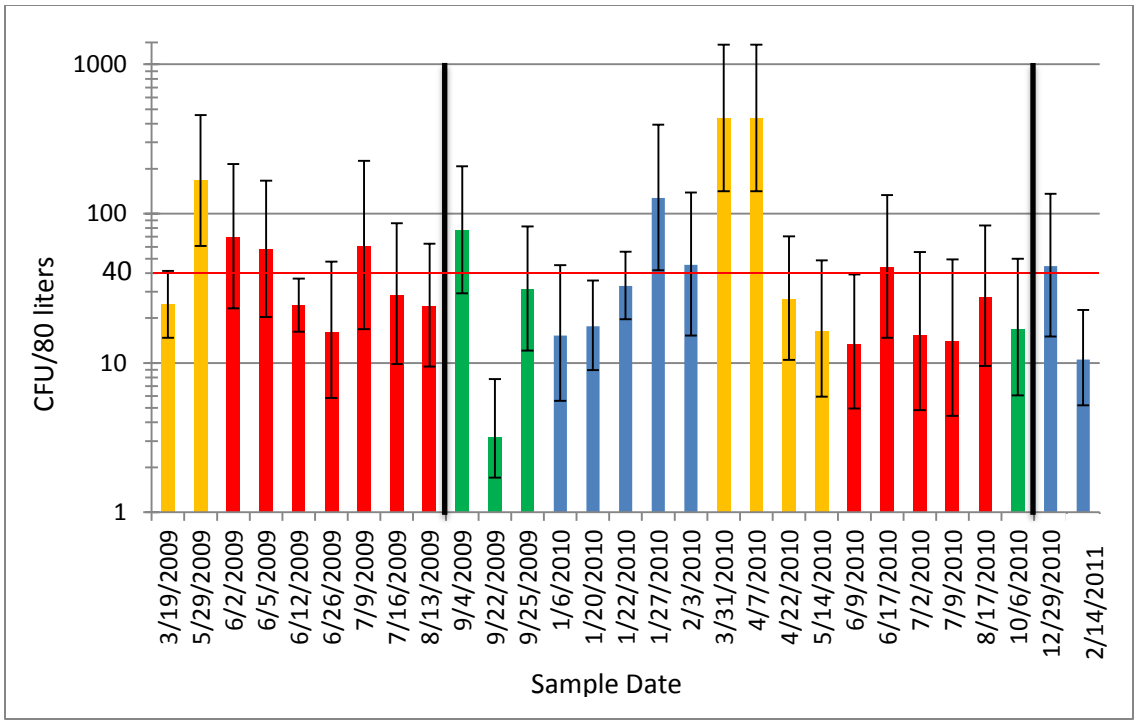

Figure 3. *Mean aerobic CFUs by sample date.*

Colored bars represent meteorological seasons: (spring = yellow, summer = red, autumn = green, blue = winter). Vertical black lines represent the annual baseline aerobic quantification. Error bars indicate the standard error of the mean.

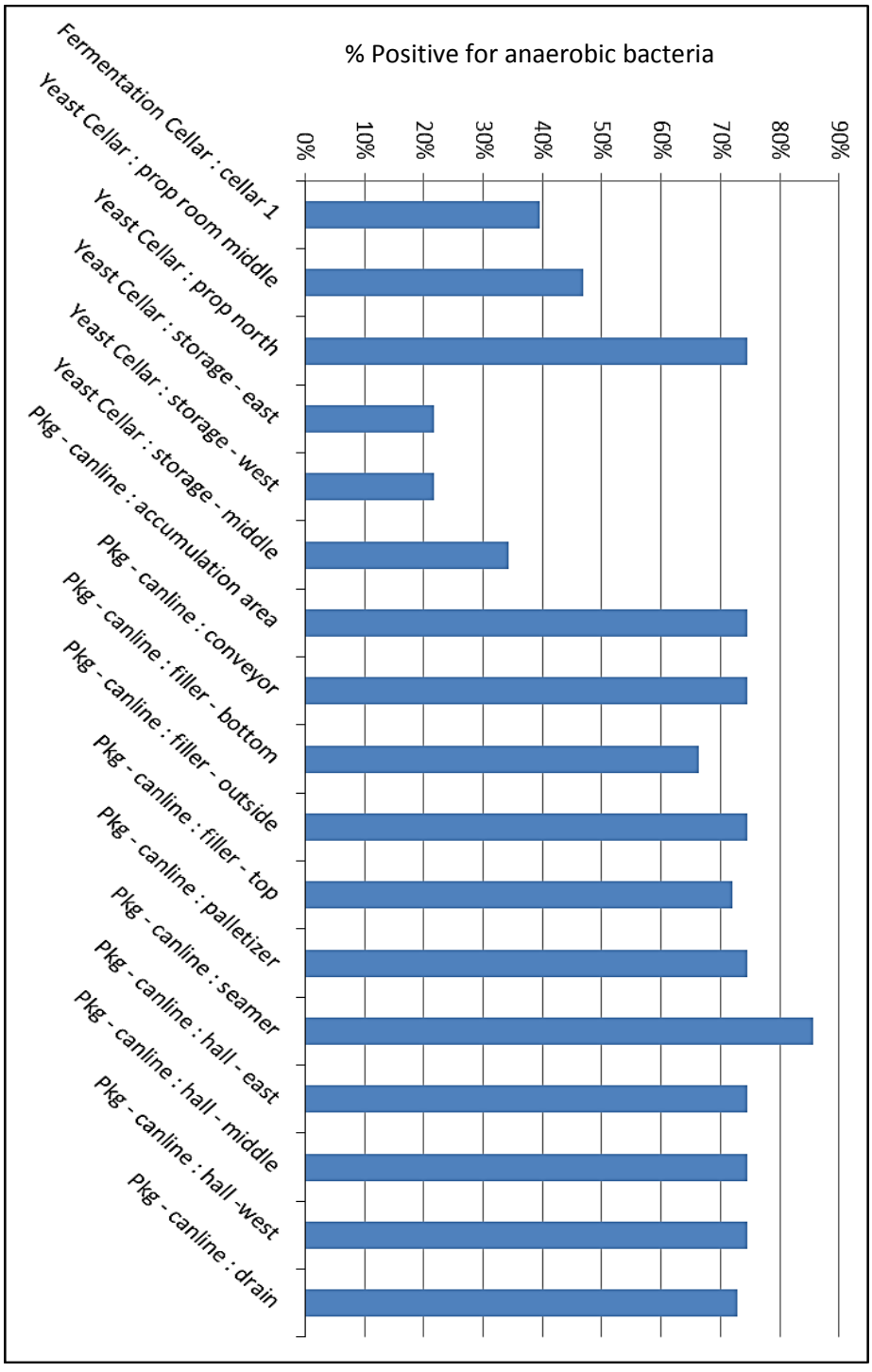

Figure 4. *Percentage of anaerobic positives for all locations.*

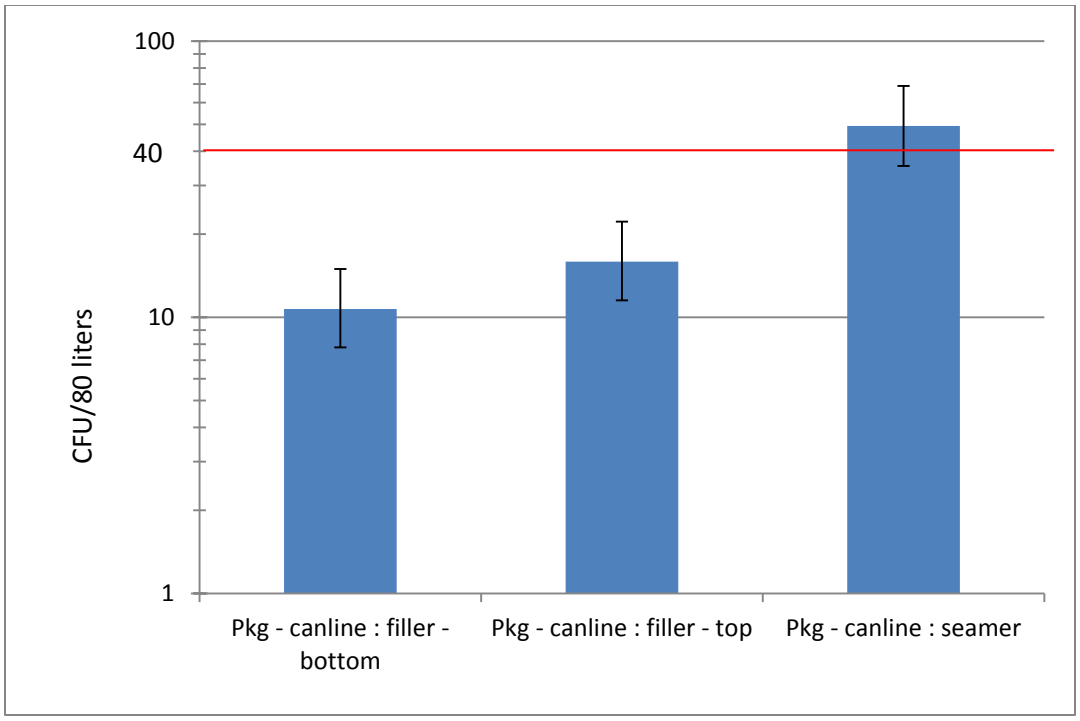

Figure 5. *Aerobic mean CFUs for canning line subset.*

Error bars indicate the standard error of the mean.

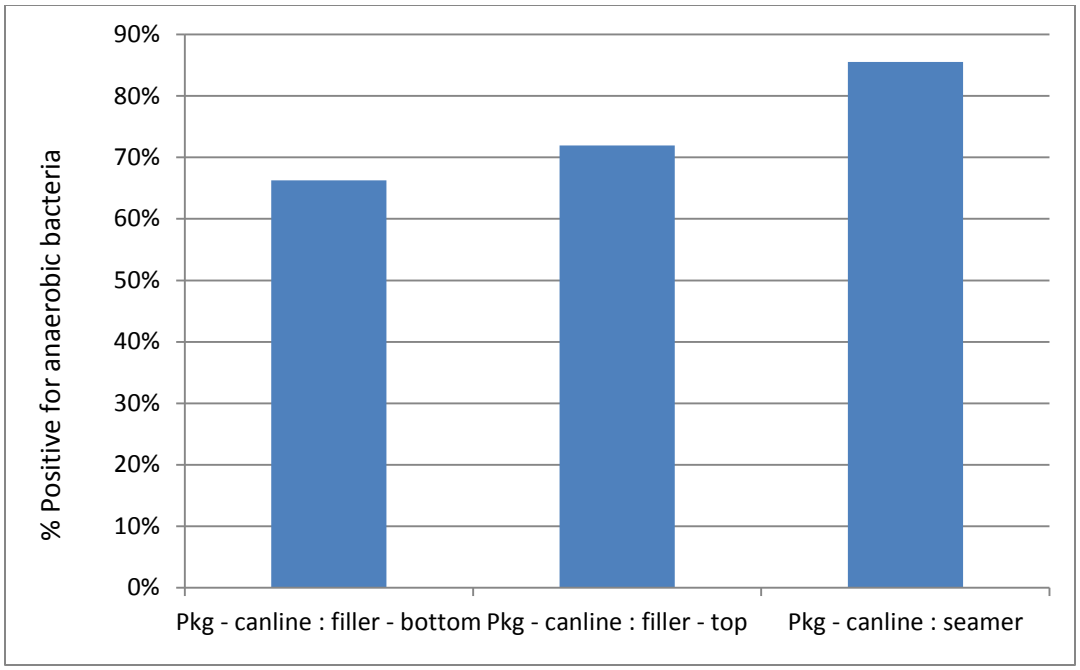

Figure 6. *Percentages of anaerobic positives for canning line subset.*

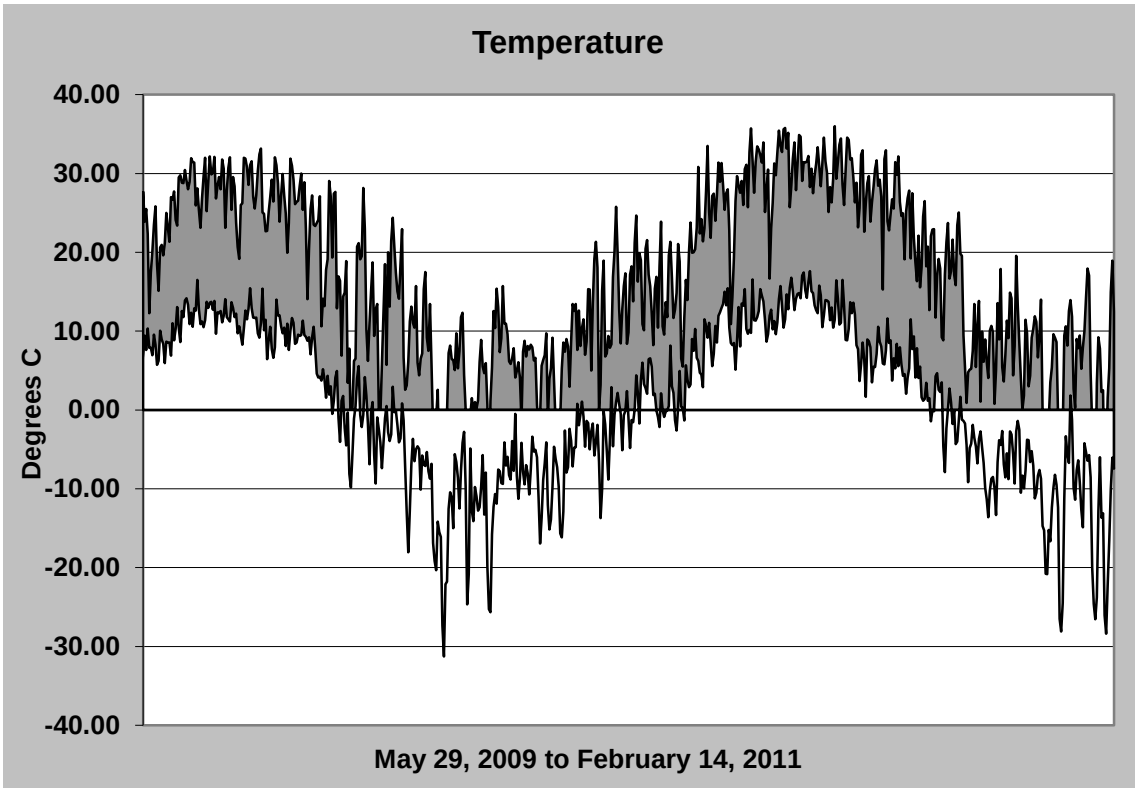

Figure 7. *Temperature from May 29, 2009 to February 14, 2011.Data obtained from Northern Colorado Water Conservancy District weather station located approximately 8 km from the brewery.*

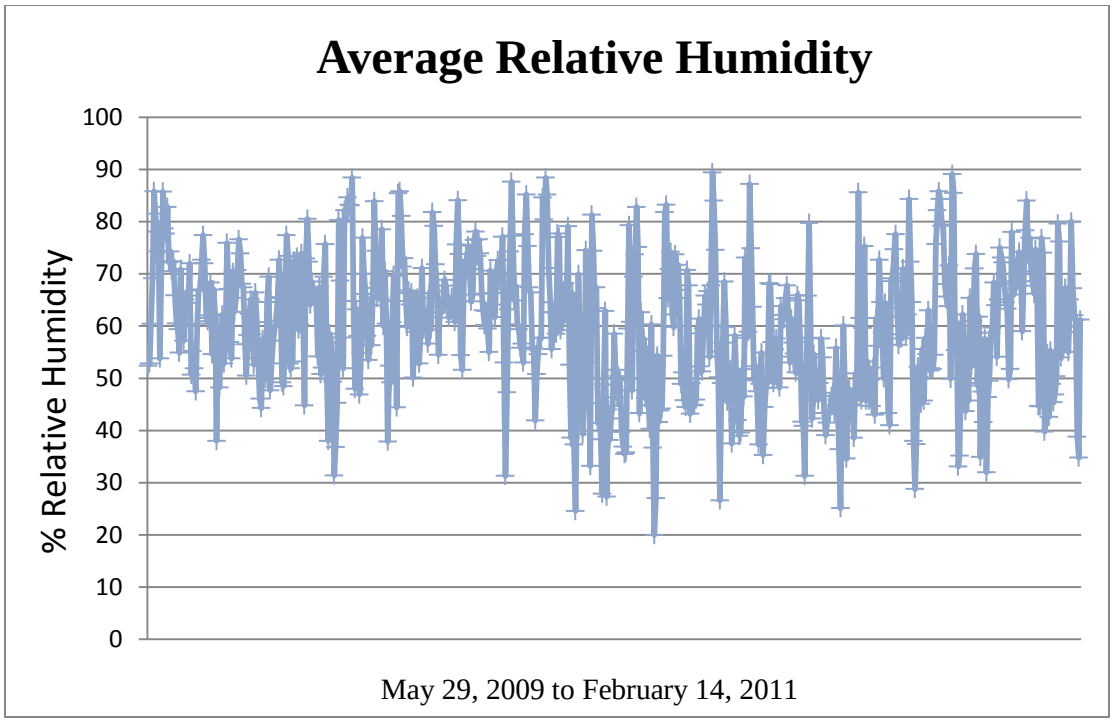

Figure 8. *Average relative humidity from May 29, 2009 to February 14, 2011. Data obtained from Northern Colorado Water Conservancy District weather station located approximately 8 km from the brewery.*

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APPENDICES

APPENDIX A

Microbial Air Testing Data

Std >40 CFU/2 min or 80L air

location	sublocation	3/19/2009 aerobic	5/29/2009 aerobic	6/2/2009 aerobic	6/5/2009 aerobic	6/12/2009 aerobic	6/26/2009 aerobic	7/9/2009 aerobic	7/16/2009 aerobic	8/13/2009 aerobic
Brewhouse	BH2 hallway	spreader				33				
	BH2 double doors	30				26				
Yeast Cellar	prop room - middle of room	16				23				22
	prop room - north wall					22				
	yeast storage- middle of room	30				71				14
	yeast storage - near hallway door					32				22
	yeast storage - west wall					55				26
Fermentation Cellar	cellar 1	26				64				
	cellar 2	18				20				
	cellar 3	24				3				
pkg - canline	can palletizer - top				160					
	can filler - outside box - top				221					
	can conveyor		75							
	can filler - top		52	12	12	19	18	16/spreader	10	
	can filler - bottom		183	105	105	0	1	5	5	
	can seamer		87	44	24	29	16	tntc/spreader	59	
	can accumulation table		90/spreader							
	canline hall -west wall		85							
	canline hall - east wall		103							
	canline hall - middle of room		54							
	can storage - empty/dry storage							31/spreader		
	canline/kegline drain	78				94				
Keg storage	empties	27				52				
Wood cellar	climbing wall	63				51				
Warehouse	truck bays	24				37				
Lab	micro lab	6				1				
	micro lab hood (neg control)	0				0				
Exterior (outside)	acid alley	32				49				
	BH2 double doors	24				53				
	wood cellar rolling door	35				79				
	truck bays - warehouse	47				50				

Std >40 CFU/2 min or 80L air										
		9/4/2009	9/22/2009	9/25/2009	1/6/2010	1/20/2010	1/22/2010	1/27/2010	2/3/2010	3/31/2010
location	sublocation	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic
Brewhouse	BH2 hallway						30			
	BH2 double doors						spreader			
Yeast Cellar	prop room - middle of room	31		27		7	14			
	prop room - north wall									
	yeast storage- middle of room	57		32		13	9			
	yeast storage - near hallway door	56		29		11				
	yeast storage - west wall	spreader		22		10				
Fermentation Cellar	cellar 1						spreader			
	cellar 2						cont.media			
	cellar 3						23			
pkg - canline	can palletizer - top									
	can filler - outside box - top									
	can conveyor									
	can filler - top		1		4	10		60	8	spreader
	can filler - bottom		0		4	6		28	20	spreader
	can seamer		0		21	44		217	87	spreader
	can accumulation table									
	canline hall -west wall									
	canline hall - east wall									
	canline hall - middle of room									
	can storage - empty/dry storage									
	canline/kegline drain						spreader			
Keg storage	empties						45			
Wood cellar	climbing wall						30			
Warehouse	truck bays						24			
Lab	micro lab						3			
	micro lab hood (neg control)						4			
Exterior (outside)	acid alley						79			
	BH2 double doors						9			
	wood cellar rolling door						18			
	truck bays - warehouse						62			

Std > 40 CFU/2 min or 80L air		4/7/2010	4/22/2010	5/14/2010	6/9/2010	6/17/2010	7/2/2010	7/9/2010	8/17/2010	10/6/2010
location	sublocation	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic
Brewhouse	BH2 hallway									
	BH2 double doors									
Yeast Cellar	prop room - middle of room		50							
	prop room - north wall									
	yeast storage- middle of room		18							
	yeast storage - near hallway door		24							
	yeast storage - west wall		13							
Fermentation Cellar	cellar 1									
	cellar 2									
	cellar 3									
pkg - canline	can palletizer - top									
	can filler - outside box - top									
	can conveyor									
	can filler - top	spreader		4	7	21	3	5	11	4
	can filler - bottom	spreader		5	0	41			3	1
	can seamer	spreader		22	43	15	33	17	73	73
	can accumulation table									
	canline hall -west wall									
	canline hall - east wall									
	canline hall - middle of room									
	can storage - empty/dry storage									
	canline/kegline drain									
Keg storage	empties									
Wood cellar	climbing wall									
Warehouse	truck bays									
Lab	micro lab									
	micro lab hood (neg control)									
Exterior (outside)	acid alley									
	BH2 double doors									
	wood cellar rolling door									
	truck bays - warehouse									

Std >40 CFU/2 min or 80L air										
		12/29/2010	12/29/2010	12/29/2010	2/14/2011	2/14/2011	2/14/2011	Mean	3/19/2010	5/29/2009
location	sublocation	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	anaerobic	anaerobic
Brewhouse	BH2 hallway							31.50	0	
	BH2 double doors							28.00	0	
Yeast Cellar	prop room - middle of room							23.75	0	
	prop room - north wall							22.00		
	yeast storage- middle of room							30.50	1	
	yeast storage - near hallway door							29.00		
	yeast storage - west wall							25.20		
Fermentation Cellar	cellar 1							45.00	2	
	cellar 2							19.00	1	
	cellar 3							16.67	3	
pkg - canline	can palletizer - top							160.00		
	can filler - outside box - top							221.00		
	can conveyor							75.00		77
	can filler - top	32	22	13	4	3	3	14.08		6
	can filler - bottom	6	12	10	0	2	1	23.61		19
	can seamer	67	97	76	9	26	34	50.54		9
	can accumulation table							n/a		34
	canline hall -west wall							85.00		5
	canline hall - east wall							103.00		14
	canline hall - middle of room							54.00		17
	can storage - empty/dry storage							n/a		
	canline/kegline drain							86.00	7	
Keg storage	empties							41.33	4	
Wood cellar	climbing wall							48.00	1	
Warehouse	truck bays							28.33	0	
Lab	micro lab							3.33	0	
	micro lab hood (neg control)							1.33	0	
Exterior (outside)	acid alley							53.33	2	
	BH2 double doors							28.67	0	
	wood cellar rolling door							44.00	0	
	truck bays - warehouse							53.00	0	

Std > 40 CFU/2 min or 80L air		6/2/2009	6/5/2009	6/12/2009	6/26/2009	7/9/2009	7/16/2009	8/13/2009	9/4/2009	9/22/2009
location	sublocation	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic
Brewhouse	BH2 hallway			33						
	BH2 double doors			0						
Yeast Cellar	prop room - middle of room			14				0	1	
	prop room - north wall			1						
	yeast storage- middle of room			18				0	1	
	yeast storage - near hallway door			0				0	1	
	yeast storage - west wall			2				0	0	
Fermentation Cellar	cellar 1			1						
	cellar 2			0						
	cellar 3			0						
pkg - canline	can palletizer - top		6							
	can filler - outside box - top		9							
	can conveyor									
	can filler - top	0	1	2	7	2	n/a			n/a
	can filler - bottom	3	3	1	1	3	n/a			n/a
	can seamer	0	8	8	1	19	n/a			n/a
	can accumulation table									
	canline hall -west wall									
	canline hall - east wall									
	canline hall - middle of room									
	can storage - empty/dry storage					0				
	canline/kegline drain			4						
Keg storage	empties			0						
Wood cellar	climbing wall			0						
Warehouse	truck bays			0						
Lab	micro lab			0						
	micro lab hood (neg control)			0						
Exterior (outside)	acid alley			0						
	BH2 double doors			0						
	wood cellar rolling door			0						
	truck bays - warehouse			0						

Std >40 CFU/2 min or 80L air		9/25/2009	1/6/2010	1/20/2010	1/22/2010	1/27/2010	2/3/2010	3/31/2010	4/7/2010	4/22/2010
location	sublocation	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic
Brewhouse	BH2 hallway				0					
	BH2 double doors				0					
Yeast Cellar	prop room - middle of room	0		0	1					4
	prop room - north wall									
	yeast storage- middle of room	0		0	0					0
	yeast storage - near hallway door	0		0						0
	yeast storage - west wall	0		0						0
Fermentation Cellar	cellar 1				0					
	cellar 2				0					
	cellar 3				0					
pkg - canline	can palletizer - top									
	can filler - outside box - top									
	can conveyor									
	can filler - top		0	n/a		n/a	7	8	n/a	
	can filler - bottom		0	n/a		n/a	1	21	n/a	
	can seamer		1	n/a		n/a	26	178	n/a	
	can accumulation table									
	canline hall -west wall									
	canline hall - east wall									
	canline hall - middle of room									
	can storage - empty/dry storage									
	canline/kegline drain				2					
Keg storage	empties				0					
Wood cellar	climbing wall				0					
Warehouse	truck bays				2					
Lab	micro lab				0					
	micro lab hood (neg control)				0					
Exterior (outside)	acid alley				0					
	BH2 double doors				0					
	wood cellar rolling door				0					
	truck bays - warehouse				0					

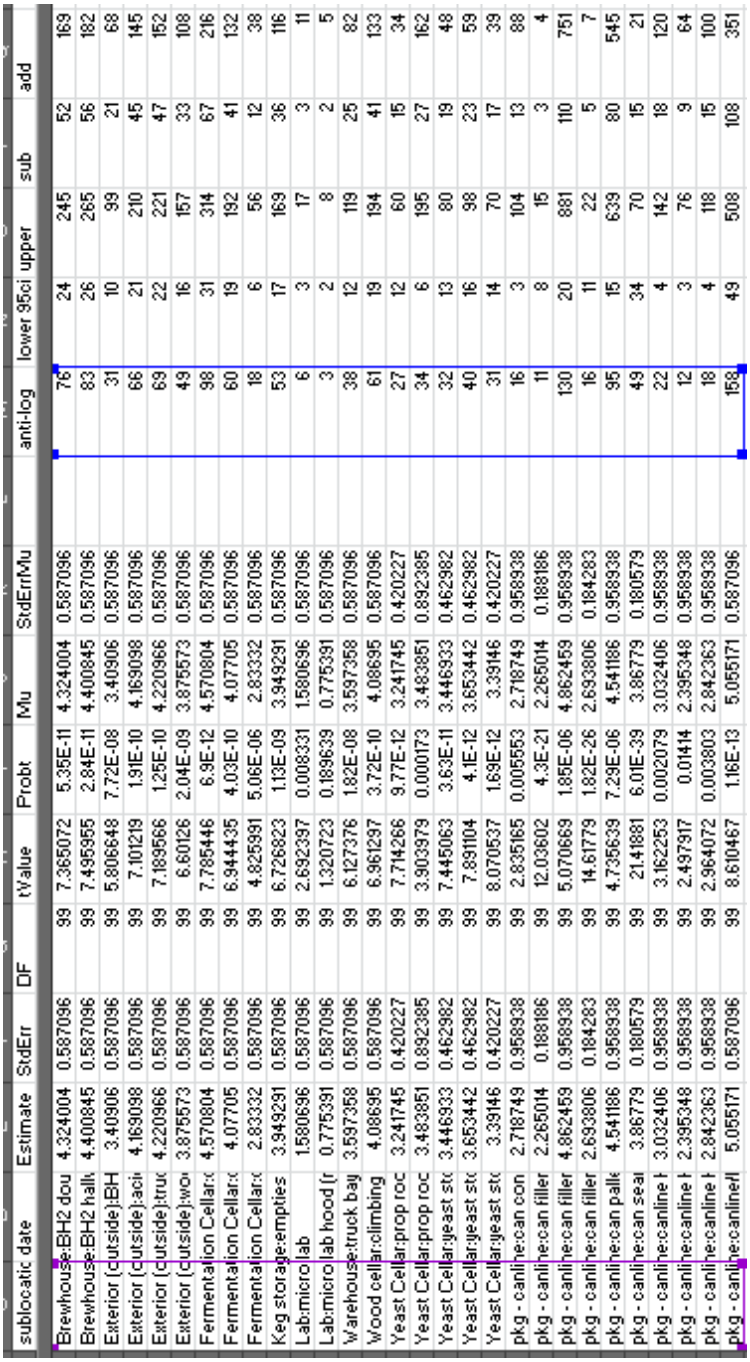
Std > 40 CFU/2 min or 80L air		5/14/2010	6/9/2010	6/17/2010	7/2/2010	7/9/2010	8/17/2010	10/6/2010	12/29/2010	12/29/2010
location	sublocation	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic	anaerobic
Brewhouse	BH2 hallway									
	BH2 double doors									
Yeast Cellar	prop room - middle of room									
	prop room - north wall									
	yeast storage- middle of room									
	yeast storage - near hallway door									
	yeast storage - west wall									
Fermentation Cellar	cellar 1									
	cellar 2									
	cellar 3									
pkg - canline	can palletizer - top									
	can filler - outside box - top									
	can conveyor									
	can filler - top	0	0	1	4	5	0	1	0	5
	can filler - bottom	10	0	3			0	1	0	1
	can seamer	1	3	3	10	28	0	16	19	15
	can accumulation table									
	canline hall -west wall									
	canline hall - east wall									
	canline hall - middle of room									
	can storage - empty/dry storage									
	canline/kegline drain									
Keg storage	empties									
Wood cellar	climbing wall									
Warehouse	truck bays									
Lab	micro lab									
	micro lab hood (neg control)									
Exterior (outside)	acid alley									
	BH2 double doors									
	wood cellar rolling door									
	truck bays - warehouse									

Std > 40 CFU/2 min or 80L air						
		12/29/2010	2/14/2011	2/14/2011	2/14/2011	
location	sublocation	anaerobic	anaerobic	anaerobic	anaerobic	mean
Brewhouse	BH2 hallway					11.00
	BH2 double doors					0.00
Yeast Cellar	prop room - middle of room					2.50
	prop room - north wall					1.00
	yeast storage- middle of room					2.50
	yeast storage - near hallway door					0.17
	yeast storage - west wall					0.33
Fermentation Cellar	cellar 1					1.00
	cellar 2					0.33
	cellar 3					1.00
pkg - canline	can palletizer - top					6.00
	can filler - outside box - top					9.00
	can conveyor					77.00
	can filler - top	2	2	1	0	2.45
	can filler - bottom	0	0	0	0	3.35
	can seamer	8	0	2	0	16.14
	can accumulation table					34.00
	canline hall -west wall					5.00
	canline hall - east wall					14.00
	canline hall - middle of room					17.00
	can storage - empty/dry storage					0.00
	canline/kegline drain					4.33
Keg storage	empties					1.33
Wood cellar	climbing wall					0.33
Warehouse	truck bays					0.67
Lab	micro lab					0.00
	micro lab hood (neg control)					0.00
Exterior (outside)	acid alley					0.67
	BH2 double doors					0.00
	wood cellar rolling door					0.00
	truck bays - warehouse					0.00

APPENDIX B

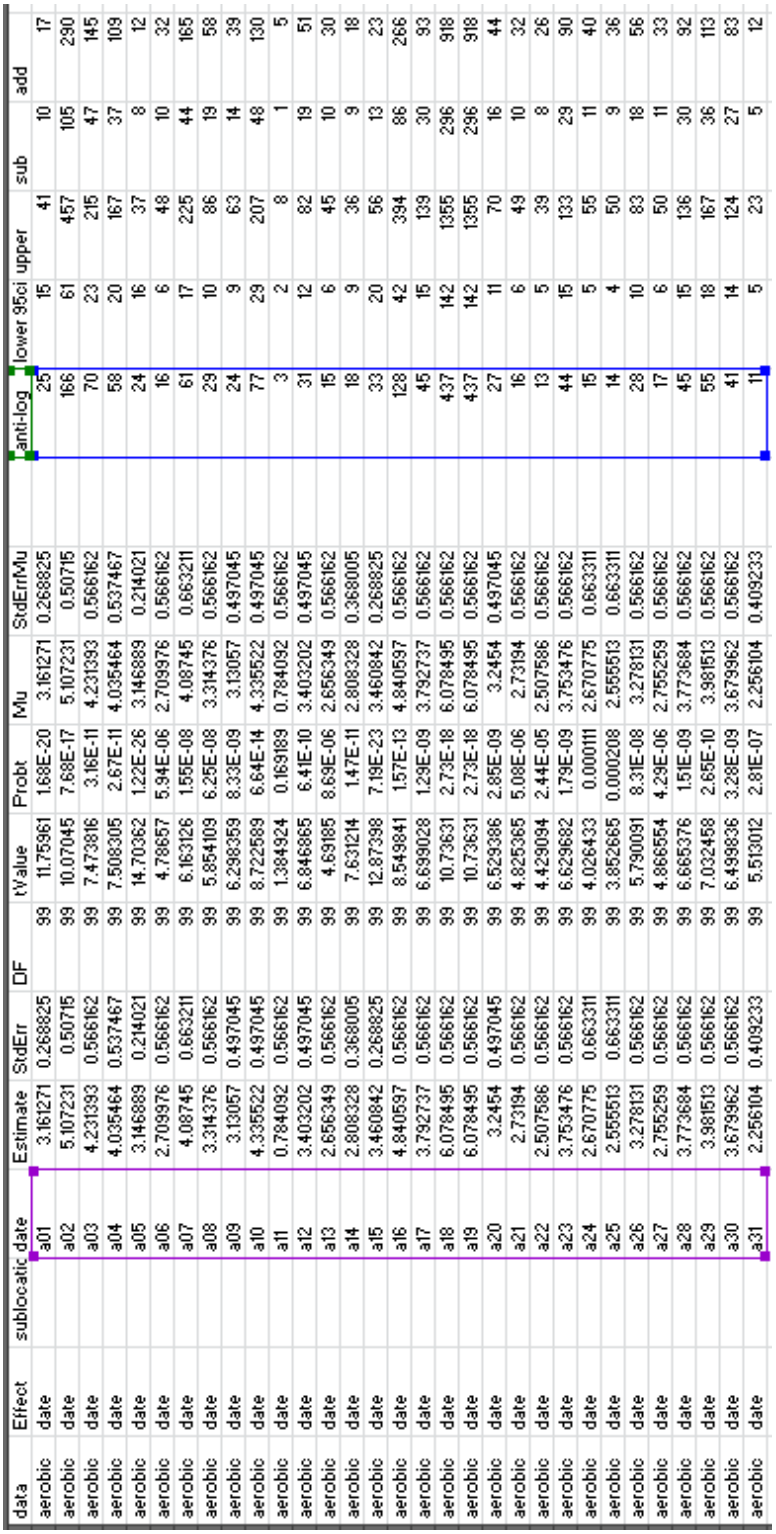
Statistical Analysis

Aerobic All Locations Data.



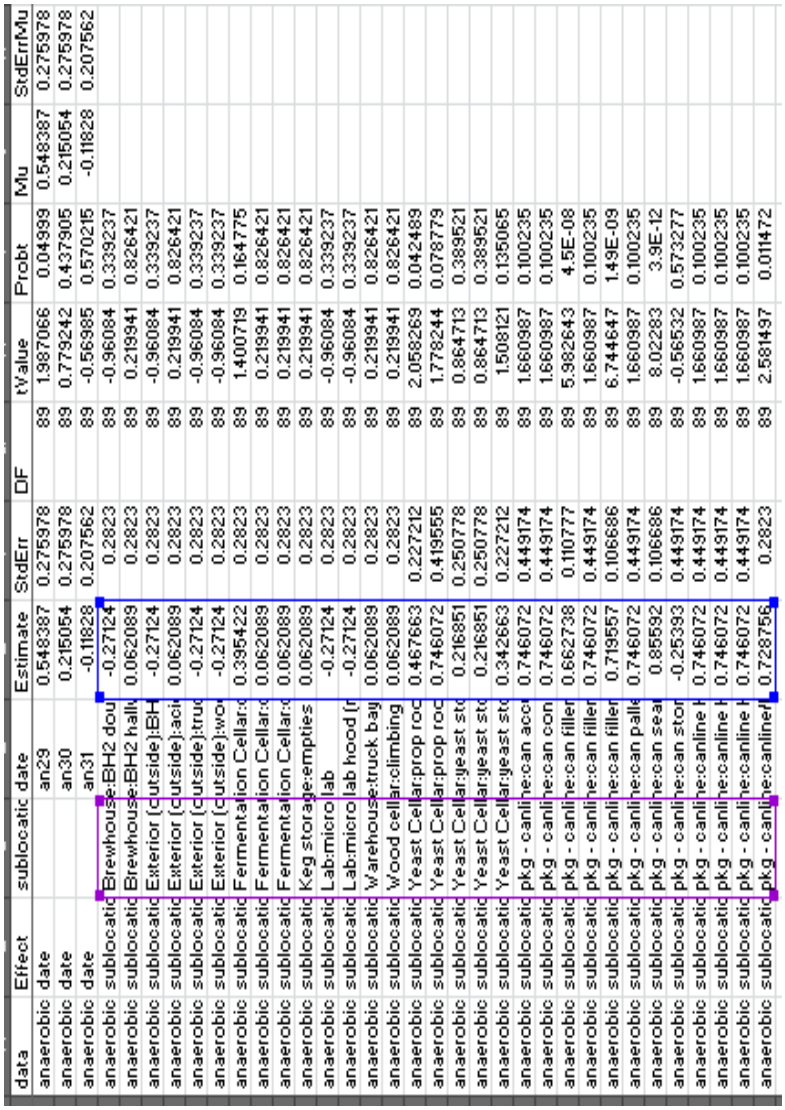
sublocatic date	Estimate	StdErr	DF	tValue	Probt	Mu	StdErrMu	anti-log	lower 95ci	upper	sub	add	
Brewhouse:BH2 dou	4.324004	0.587096	99	7.365072	5.35E-11	4.324004	0.587096	76	24	245	52	169	
Brewhouse:BH2 hall	4.400845	0.587096	99	7.495955	2.84E-11	4.400845	0.587096	83	26	265	56	182	
Exterior (cutside):BH	3.40906	0.587096	99	5.806648	7.72E-08	3.40906	0.587096	31	10	99	21	68	
Exterior (cutside):aci	4.1690098	0.587096	99	7.101219	1.91E-10	4.1690098	0.587096	66	21	210	45	145	
Exterior (cutside):truc	4.220966	0.587096	99	7.189566	1.25E-10	4.220966	0.587096	69	22	221	47	152	
Exterior (cutside):wo	3.875573	0.587096	99	6.60126	2.04E-09	3.875573	0.587096	49	16	157	33	108	
Fermentation Cellar:c	4.570804	0.587096	99	7.785446	6.9E-12	4.570804	0.587096	98	31	314	67	216	
Fermentation Cellar:c	4.07705	0.587096	99	6.944435	4.03E-10	4.07705	0.587096	60	19	192	41	132	
Fermentation Cellar:c	2.83332	0.587096	99	4.825991	5.06E-06	2.83332	0.587096	18	6	56	12	38	
Keg storage:empties	3.949291	0.587096	99	6.726823	1.13E-09	3.949291	0.587096	53	17	169	36	116	
Lab:micro lab	1.5806696	0.587096	99	2.692397	0.008331	1.5806696	0.587096	6	3	17	3	11	
Lab:micro lab hood (r	0.775391	0.587096	99	1.320723	0.189639	0.775391	0.587096	3	2	8	2	5	
Warehouse:truck bay	3.597358	0.587096	99	6.127376	1.82E-08	3.597358	0.587096	38	12	119	25	82	
Wood cellar:climbing	4.086695	0.587096	99	6.961237	3.72E-10	4.086695	0.587096	61	19	194	41	133	
Yeast Cellar:prop roc	3.241745	0.420227	99	7.714266	9.77E-12	3.241745	0.420227	27	12	60	15	34	
Yeast Cellar:prop roc	3.483851	0.892385	99	3.903979	0.000173	3.483851	0.892385	34	6	195	27	162	
Yeast Cellar:yeast sto	3.446933	0.462982	99	7.445063	3.63E-11	3.446933	0.462982	32	13	80	19	48	
Yeast Cellar:yeast sto	3.653442	0.462982	99	7.891104	4.1E-12	3.653442	0.462982	40	16	98	23	59	
Yeast Cellar:yeast sto	3.39146	0.420227	99	8.070537	1.69E-12	3.39146	0.420227	31	14	70	17	39	
pkg - canline:can con	2.718749	0.958938	99	2.835165	0.005553	2.718749	0.958938	16	3	104	13	88	
pkg - canline:can filler	2.265014	0.188186	99	12.03602	4.3E-21	2.265014	0.188186	11	8	15	3	4	
pkg - canline:can filler	4.862459	0.958938	99	5.070669	1.85E-06	4.862459	0.958938	130	20	881	110	751	
pkg - canline:can filler	2.693806	0.184283	99	14.61779	1.82E-26	2.693806	0.184283	16	11	22	5	7	
pkg - canline:can palk	4.541186	0.958938	99	4.735639	7.29E-06	4.541186	0.958938	95	15	639	80	545	
pkg - canline:can seal	3.86779	0.180579	99	21.41881	6.01E-39	3.86779	0.180579	49	34	70	15	21	
pkg - canline:canline l	3.032406	0.958938	99	3.162253	0.002079	3.032406	0.958938	22	4	142	18	120	
pkg - canline:canline l	2.395348	0.958938	99	2.497917	0.01414	2.395348	0.958938	12	3	76	9	64	
pkg - canline:canline l	2.842363	0.958938	99	2.964072	0.003803	2.842363	0.958938	18	4	118	15	100	
pkg - canline:canline/l	5.055171	0.587096	99	8.610467	1.16E-13	5.055171	0.587096	158	49	508	108	351	

Aerobic Seasonal Data.



data	Effect	sublocatic	date	Estimate	StdErr	DF	tValue	Probt	Mu	StdErrMu	anti-log	lower 95ci	upper	sub	add
aerobic	date		a01	3.161271	0.268825	99	11.75961	1.68E-20	3.161271	0.268825	25	15	41	10	17
aerobic	date		a02	5.107231	0.50715	99	10.07045	7.68E-17	5.107231	0.50715	166	61	457	105	290
aerobic	date		a03	4.231393	0.566162	99	7.473816	3.16E-11	4.231393	0.566162	70	23	215	47	145
aerobic	date		a04	4.035464	0.537467	99	7.508305	2.67E-11	4.035464	0.537467	58	20	167	37	109
aerobic	date		a05	3.146889	0.214021	99	14.70362	1.22E-26	3.146889	0.214021	24	16	37	8	12
aerobic	date		a06	2.709376	0.566162	99	4.78657	5.94E-06	2.709376	0.566162	16	6	48	10	32
aerobic	date		a07	4.08745	0.663211	99	6.163126	1.55E-08	4.08745	0.663211	61	17	225	44	165
aerobic	date		a08	3.314376	0.566162	99	5.854109	6.25E-08	3.314376	0.566162	29	10	86	19	58
aerobic	date		a09	3.13057	0.497045	99	6.298359	8.33E-09	3.13057	0.497045	24	9	63	14	39
aerobic	date		a10	4.335522	0.497045	99	8.722589	6.64E-14	4.335522	0.497045	77	29	207	48	130
aerobic	date		a11	0.784092	0.566162	99	1.384924	0.169189	0.784092	0.566162	3	2	8	1	5
aerobic	date		a12	3.403202	0.497045	99	6.846865	6.41E-10	3.403202	0.497045	31	12	82	19	51
aerobic	date		a13	2.656349	0.566162	99	4.69185	8.69E-06	2.656349	0.566162	15	6	45	10	30
aerobic	date		a14	2.808328	0.368005	99	7.631214	1.47E-11	2.808328	0.368005	18	9	36	9	18
aerobic	date		a15	3.460842	0.268825	99	12.87398	7.19E-23	3.460842	0.268825	33	20	56	13	23
aerobic	date		a16	4.840597	0.566162	99	8.549841	1.57E-13	4.840597	0.566162	128	42	394	86	266
aerobic	date		a17	3.792737	0.566162	99	6.699028	1.29E-09	3.792737	0.566162	45	15	139	30	93
aerobic	date		a18	6.078495	0.566162	99	10.73631	2.73E-18	6.078495	0.566162	437	142	1355	296	918
aerobic	date		a19	6.078495	0.566162	99	10.73631	2.73E-18	6.078495	0.566162	437	142	1355	296	918
aerobic	date		a20	3.2454	0.497045	99	6.529386	2.85E-09	3.2454	0.497045	27	11	70	16	44
aerobic	date		a21	2.73194	0.566162	99	4.825365	5.08E-06	2.73194	0.566162	16	6	49	10	32
aerobic	date		a22	2.507586	0.566162	99	4.429094	2.44E-05	2.507586	0.566162	13	5	39	8	26
aerobic	date		a23	3.753476	0.566162	99	6.629682	1.79E-09	3.753476	0.566162	44	15	133	29	90
aerobic	date		a24	2.670775	0.663311	99	4.026433	0.000111	2.670775	0.663311	15	5	55	11	40
aerobic	date		a25	2.555513	0.663311	99	3.852665	0.000208	2.555513	0.663311	14	4	50	9	36
aerobic	date		a26	3.278131	0.566162	99	5.790091	8.31E-08	3.278131	0.566162	28	10	83	18	56
aerobic	date		a27	2.755259	0.566162	99	4.866554	4.29E-06	2.755259	0.566162	17	6	50	11	33
aerobic	date		a28	3.773684	0.566162	99	6.665376	1.51E-09	3.773684	0.566162	45	15	136	30	92
aerobic	date		a29	3.981513	0.566162	99	7.032458	2.65E-10	3.981513	0.566162	55	18	167	36	113
aerobic	date		a30	3.679962	0.566162	99	6.499836	3.28E-09	3.679962	0.566162	41	14	124	27	83
aerobic	date		a31	2.256104	0.409233	99	5.513012	2.81E-07	2.256104	0.409233	11	5	23	5	12

Anaerobic All Locations Data.

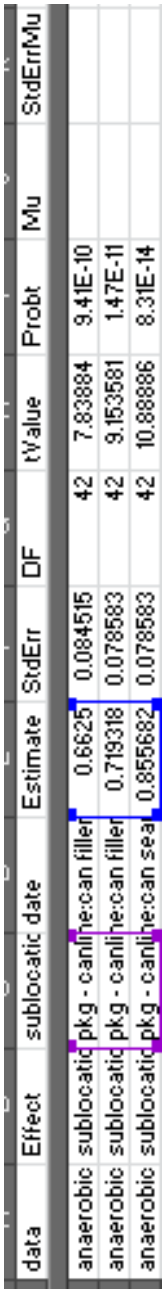


data	Effect	sublocatic	date	Estimate	StdErr	DF	tValue	Probt	Mu	StdErrMu
anaerobic	date		an29	0.548387	0.275978	89	1.987066	0.04999	0.548387	0.275978
anaerobic	date		an30	0.215054	0.275978	89	0.779242	0.437905	0.215054	0.275978
anaerobic	date		an31	-0.11828	0.207562	89	-0.56985	0.570215	-0.11828	0.207562
anaerobic	sublocatic	Brewhouse:BH2 dou		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Brewhouse:BH2 hall		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Exterior (cutside):BH		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Exterior (cutside):acic		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Exterior (cutside):truc		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Exterior (cutside):woo		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Fermentation Cellar:c		0.395422	0.2823	89	1.400719	0.164775		
anaerobic	sublocatic	Fermentation Cellar:c		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Fermentation Cellar:c		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Keg storage:empties		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Lab:micro lab		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Lab:micro lab hood (r		-0.27124	0.2823	89	-0.96084	0.339237		
anaerobic	sublocatic	Warehouse:truck bay		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	wood cellar:climbing		0.062089	0.2823	89	0.219941	0.826421		
anaerobic	sublocatic	Yeast Cellar:prop roc		0.467663	0.227212	89	2.058269	0.042489		
anaerobic	sublocatic	Yeast Cellar:prop roc		0.746072	0.419555	89	1.778244	0.078779		
anaerobic	sublocatic	Yeast Cellar:yeast sto		0.216851	0.250778	89	0.864713	0.389521		
anaerobic	sublocatic	Yeast Cellar:yeast sto		0.216851	0.250778	89	0.864713	0.389521		
anaerobic	sublocatic	Yeast Cellar:yeast sto		0.342663	0.227212	89	1.508121	0.135065		
anaerobic	sublocatic	pkg - canline:can acc		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:can con		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:can filler		0.662738	0.110777	89	5.982643	4.5E-08		
anaerobic	sublocatic	pkg - canline:can filler		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:can filler		0.719557	0.106686	89	6.744647	1.49E-09		
anaerobic	sublocatic	pkg - canline:can palle		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:can sea		0.85592	0.106686	89	8.02283	3.9E-12		
anaerobic	sublocatic	pkg - canline:can stor		-0.25393	0.443174	89	-0.56532	0.573277		
anaerobic	sublocatic	pkg - canline:canline I		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:canline I		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:canline I		0.746072	0.443174	89	1.660987	0.100235		
anaerobic	sublocatic	pkg - canline:canlineN		0.728756	0.2823	89	2.581497	0.011472		

Aerobic Canline Subset Data.

Effect	sublocatic	date	Estimate	StdErr	DF	tValue	Probt	Mu	StdErrMu		antilog	lower 95	upper95	sub	add
sublocatic	pkg - canline	can filler	2.274401	0.180504	51	12.60028	2.77E-17	2.274401	0.180504		10.72209	7.776039	14.94901	2.946052	4.226921
sublocatic	pkg - canline	can filler	2.703193	0.175244	51	15.42534	7.57E-21	2.703193	0.175244		15.92732	11.51398	22.1932	4.413341	6.265882
sublocatic	pkg - canline	can seal	3.877177	0.170238	51	22.77498	2.19E-28	3.877177	0.170238		49.28769	35.35336	68.87403	13.93433	19.58634

Anaerobic Canline Subset Data.



data	Effect	sublocatic	date	Estimate	StdErr	DF	tValue	Probt	Mu	StdErrMu
anaerobic	sublocatic	pkg - canine	can filler	0.6625	0.084515	42	7.83884	9.41E-10		
anaerobic	sublocatic	pkg - canine	can filler	0.719318	0.078583	42	9.153581	1.47E-11		
anaerobic	sublocatic	pkg - canine	can seal	0.855682	0.078583	42	10.88886	8.31E-14		