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**Energy and Water Assessment and Plausibility of Reuse of Spent Caustic Solution in a
Midwest Fluid Milk Processing Plant**

By

Carly Rain Adams

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

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Under the Supervision of Professors Curtis Weller and Yulie Meneses

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Energy and Water Assessment and Plausibility of Reuse of Spent Caustic Solution in a Midwest Fluid Milk Processing Plant

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The Food Energy and Water Nexus (FEW Nexus) is the inseparable connection linking these resources. The concept of the FEW Nexus within the food industry addresses the connection of water and energy as key members of food production. The steady increase in population and the increase in food demand are directly related, therefore, the need for water and energy. Immediately taking on this critical challenge will lead to tangible impacts on the water and energy crisis facing the food system. To reduce the distance between process productivity and resource efficiency it must first be determined, within food processing, where water and energy are being consumed. Therefore, this research focused on determining opportunities for water-energy optimization and wastewater reduction in a medium sized dairy. The partnering plant processed pasteurized fluid milk.

To reach the overarching objective, the first task was to develop a baseline of the current consumption of water/energy and wastewater generation. Results from the partnering plant indicate that the production of one gallon of pasteurized fluid milk demands 0.13 kWh and 0.01 Therms of electricity and natural gas, respectively. In addition, every gallon of pasteurized milk produced demands 0.87 gallons of freshwater. On average, 53.08% of the water used to produce milk is consumed during the cleaning stages. This consumption of resources places a large financial strain on producers. Annually, the medium sized fluid milk processing plant spent more than \$47,000 on

energy and water utilities. The second part of this research explored the efficacy of water reuse during cleaning operations. The increase in reuse cycles is directly related in increases in COD, Turbidity, TSS, TP, and TN. The reduction in surface tension with reuse solution is thought to advance the cleaning ability as hydrolysis of milk components have surfactant properties. Before caustic cleaning solution can be reused, the efficacy must be evaluated. To uphold the integrity of plant safety, a 3-log reduction in attached bacteria should be achieved. CIP operations, including a sanitizer rinse, as noticed in treatment 3 and 5 with solutions 0-50N experimental reuse solutions, showed a 3-log reduction in bacterial density of *P.aeruginosa*. Therefore, experimental caustic solutions showed the potential for reuse.

To my mother, Dawn Marie

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Chapter 1: INTRODUCTION

The research foundation, of this thesis, is to simultaneously address Food, Energy, and Water Nexus (FEW Nexus) within the boundary of processing, specifically of fluid milk processing. The current research takes an alternative and progressive look into food production by exploring the interrelationships of process inputs and outputs at a processing facility while excluding the consumption of resources on the farm and in the home. Research phase one takes place within the walls of a medium sized Nebraska fluid milk processing plant. Plant to plant variation in volume and cost of energy and water consumption exists. However, the partner plant served as a model and reference for recommendations and development of overarching strategies that could affect not just one plant, but the entire dairy industry. Research phase two evaluates the potential for reuse of spent cleaning solution using a benchtop Center for Disease Control (CDC) Bioreactor. Here, the efficacy of reusing spent caustic solutions was evaluated against the \log_{10} reduction of *Pseudomonas aeruginosa* biofilm.

The overarching goal is to transform our findings into a set of industry wide guidelines and standards that will promote a culture of water and energy conservation. The expected benefit is to encourage and assist in creating a culture of water and energy optimization within food production. It is the hope that these findings will catalyze producers to reuse process wastewater with confidence in its' safety, while also providing financial benefits to the production. To reach this goal, the project was divided into two phases, each with manageable objectives:

Phase 1: The quantification of Energy and Water Requirements of a Milk Processing Facility and the Cost Assessment of Resource Consumption

Objective 1: To quantify the baseline consumption of water and energy usage and wastewater generation at a fluid milk processing plant

Objective 2: To pinpoint locations in milk processing that have the potential for resource reduction

Objective 3: To provide understanding on the economic tie of resource consumption at a Midwest dairy compared nationally

Part 2: Determination of Plausibility of Reuse of Spent Caustic Solution from a Milk Processing Facility on the Removal of *Pseudomonas aeruginosa*

Objective 1: To determine if milk processing wastewater can be reused through efficacy evaluation of spent cleaning solutions

Objective 2: To evaluate how physico-chemical properties of experimental caustic solution, as conditions under which spent solutions can be reused, relates to efficacy of reuse

Chapter 2: LITERATURE REVIEW

A literary review was conducted to evaluate the current understanding of the Food Energy and Water nexus (FEW Nexus) as it relates to food processing. In literature, the role of energy and water is viewed on a broad spectrum. Minimal literary references to food processing specially were found. This review also explored the roles of cleaning solutions in food processing.

FEW Nexus

The production of food is unbreakably associated with resource consumption. Water and energy are two key resources engrained in food production from the field to the fork. The Food, Energy, and Water Nexus (FEW Nexus) refers to the intricate relationship between these three commodities (Hanlon et al. 2013). Water and energy for food production includes the agricultural, livestock, and processing demands of resources. Food production can be broken into two sectors: primary sector (agricultural growth and livestock raising) and secondary sector (raw to finished commodities). This paper refers to the conversion of raw goods to finished products as it occurs in a food processing facility, therefore the secondary sector here be referred to as food processing.

Food processing, is an essential link in the food chain, and a tangible example of the FEW Nexus. Actions that occur in the food processing plant hold significant influence on the social, environmental, and economic wellbeing of the public. The role of the FEW Nexus should be of great importance to the processors as the demand for food will increase as the environment shifts with climate change, dietary habits fluctuate, and both population and urbanization increase. Therefore, additional stress will build on the complex relationship of resources (energy and water) with food production (Gulati et al.

2013). By 2050, the world will experience increases in demand of energy (80%) and water (50%) to help reach the growing needs of the public (IRENA 2015). While the need for water and energy will increase so does the variability in available high quality water (IRENA 2015). Efforts to optimize water, should directly seek to optimize energy, as with one comes the other. The demand for resources in combination of their limited supply will cause the price of energy and water to increase.

The NEW Nexus studied in developing countries (Gulati, Jacobs et al. 2013) sought to connect environmental impact of these resources with security of food cost and availability. This study concluded the need for further understanding of the direct link of these resources and the potential for government intervention. Additionally previous studies of the NEW Nexus do not successfully distinguish resource consumption at different stages along the food chain. One study indicates food production accounts for 90% of the freshwater use and 30% of the energy use (Scanlon et al. 2017). Further evaluation determined these numbers are referencing different parts of the food chain. Here they defined food production broadly and refer to the water needed for irrigation purposes (Siebert et al. 2010). However, the referenced energy consumption (FAO 2011) refers to the entire food chain, as opposed to the primary or secondary production explicitly.

The specific consumption of energy and water must be known for the development of effective consumption strategies for the food industry. Research must thoroughly define the FEW Nexus in relation to the food chain, as current stepwise consumption is not well defined.

Energy in food processing

The entire food industry is energy intensive manufacturing (EIA 2016). Food processing accounts for 5% of the U.S. industrial sector energy consumption (EIA 2017). In 2007, developed countries reported more energy consumption needed for processing and transport of food, over agricultural growth (Bazilian et al. 2011). Dairy processing accounted for 13% of the overall natural gas consumed for food processing (Masanet et al. 2014) and is considered to be energy intensive (EIA 2016). One study looked at the energy consumed to produce food from farm fuels to resident's home. They found that processing and packaging made up about 19% of the total energy used (Finley and Seiber 2014). Resource consumption varies by sector and commodity. The energy requirements of dairy products, ranked from least to most energy intensive: fluid milk (310 Btu/lb of product), powdered dry milk (421 Btu/lb of product), butter (552 Btu/lb of product), ice cream (814 Btu/lb of product), cheese (1196 Btu/lb of product), and dry whey (5837 Btu/lb of product) (Masanet et al. 2014). While products may vary in energy demands, many dairy commodities require similar processing operations including standardization, pasteurization, homogenization, and cooling. Additional processes that may be incorporated are clarification, sterilization, evaporation, freezing, or fermentation (Brush 2011). Another study compared the total energy consumed amongst countries and found European milk plants experienced a wider range (0.3-12.6 MJ/kg product) in total energy compared to domestic milk plants (0.2-6.0 MJ/kg product) (Xu and Flapper 2009). These values, generalized for all milk plants, fail to correlate consumption with commodity type or processing volume. This is a common trend in previous research. Evaluation of energy for fluid milk showed energy intensities of 1.06mJ/L and 0.17 kWh/L for natural gas and

electricity, respectively, for Canadian milk processors (National Dairy Council of Canada 1997). Natural gas and electricity are the two main forms of energy consumed during dairy processing. To optimize energy consumption, food processors must understand their baseline energy and water utilization to judge process efficiency and identify processes.

Water in food processing

Water is a finite resource used throughout everyday life. The majority of water found on Earth is not available for use. Only 2.5% of Earth's water supply is freshwater and a little over one percent of the freshwater is surface water (USGS 2016).

The third largest industrial consumer of water, the food industry (Ölmez and Kretzschmar 2009) requires high quality, potable water, as water is intimately used throughout processing. The dairy industry accounts for 12% of the freshwater consumed by the food industry (Bustillo-Lecompte and Mehrvar 2015). Water contributes to the conversion of raw food commodities through heat treatment, transportation, cleaning, and as an ingredient. The water quality needed through the different stages in production may require different levels of quality. The Netherlands food industry, in 1997, consumed 247.46 million m³ of freshwater (Casani et al. 2005). Water consumption for dairy processing alone is 1.8 L/kg (Klemes et al. 2008).

The food industry must join in the worldwide quest for clean and safe water, for now and for the future. Without stability in water, the food chain will not stretch to meet the needs of a growing population. A 30% reduction in water consumption could result from both

alterations to plant cultural and processing (Kirby et al. 2003). Understanding wastewater generation throughout processing identifies areas of possible reduction.

Wastewater water reuse

When discussing water conservation a few terms are used and not interchangeable. Water regeneration or reconditioning refers to water that has undergone treatment and intended for reuse (Codex Alimentarius Commission 1999). In this research water, reuse is referring to the continued subsequent use of spent water, without the addition of treatment. This water should not negatively reduce product quality (Codex Alimentarius Commission 1999).

The food industry generates wastewater throughout processing, due to cleaning demands and product residue and loss through spillage. Generated wastewater not only inflicts treatment charges but also contains lost revenue from lost product. Inadequate discharge could result in financial harm to processors due to the risk wastewater possess to the public and the risk of government fines. These wastewaters must be treated or distributed properly. Improper treatment of wastewater can lead to water contamination and therefore a reduction in quality of product and public water. The potential for water reuse can concern food processors due to the organic load and potential of bacterial contamination in used processing water. One study believes that not all processing stages require potable water (Kirby et al. 2003), but the use of alternative water should be verified to avoid contamination concerns.

The Environmental Protection Agency regulate discharge of processing wastewater. Discharge Effluent limitations are based on Biochemical oxygen demand (BOD₅) and

Total Suspended Solids (TSS) (e-CFR 2018). Limitations vary based on receiving milk equivalents for dairy processing. Effluent limitations for milk processing plant changed based on receiving load (**Table 1**). Lower limitations were formatted for processing plants with higher processing loads.

Table 1: Effluent Limitations for CFR Subpart B

Parameter	Large fluid milk plant (>25,900lb/day of BOD ₅ input)	Small fluid milk plant (<25,900lb/day of BOD ₅ input)
BOD ₅ ^a	0.338	0.450
TSS ^a	0.551	0.675
pH	6.0-9.0	6.0-9.0

^a lb/100lb BOD₅ input

(e-CFR 2018)

The National Pollutant Discharge Elimination System (NPDES) develops industry specific permits based on first upholding water quality and second based on accessible and plausibly treatment for the direct discharge of industrially generated wastewater. The EPA sets Effluent Guidelines by industry (**Table 1**). Limitations and types of potential pollutants labeled as conventional, set by the EPA, reflect The Clean Water Act. The main factors contributing to the effluent limitations of conventional pollutants (BOD₅, pH, TSS, fecal coliforms, and oil and grease) consider 1. Cost of treatment (\$/lb. pollutant removal for BOD₅ and TSS), 2. Energy demand, 3. Age of treatment and 4. Engineering controls of best practicable control technologies (BPT) currently available. It is not easy to identify clear definitions of industry wide effluent. However, U.S. EPA discharge limits are reported as 26 mg/L BOD₅, 30 mg/L TSS, and 8mg/L TN (Bustillo-Lecompte and Mehrvar 2015). Effluent limits for Nitrogen and Phosphorus are

implemented in 34% of the major municipal sewage treatment facilities across the United States (NPDES 2017).

Wastewater treatment coupled with distribution of water is considered energy intensive (1,100-4,600 kWh/mg) (Bauer et al. 2014). Current common wastewater treatments include sedimentation, flocculation, coagulation, filtration, chlorination, anaerobic and aerobic lagoons, aeration, activated carbon, and ozone. Food processing wastewater, generated through spent processing water, undergoes treatment or land application, for irrigation needs. Land application as a route for managing wastewater can be beneficial to the soil due to the nutrient rich loads. However, its' distribution can also pose health risks to the public (WHO 2011). Wastewater pollutants in dairy processing wastewater vary greatly as seen in **Table 2**.

Table 2: Literature Comparison of Dairy Wastewater

Processing	pH	BOD ₅ (mg/L)	COD (mg/L)	TN ^a (mg/L)	TP ^b (mg/L)	TSS (mg/L)	Wastewater volume	Reference
Dairy Processing	6-9	50	250	10	2	50	1.7-14 kg/1000L	(IFC 2007)
Dairy processing	6.8-11.3	709-2297	-	-	36-78	405-1082	11-508 10 ³ gal/day	(Danalewich, Papagiannis et al. 1998)
Market milk	-	750 mg/L effluent	2060 mg/L effluent	29-45 mg/L effluent	8.1-11 mg/L effluent	-	0.86-1.03 l/l milk	(Lampi 2001)
Dairy processing	7.3	-	24.7	-	-	-	-	(Sarkar, Chakrabarti et al. 2006)
Fluid milk	5.0-9.5	500-1300	950-2400	-	-	90-450	-	(Demirel, Yenigun et al. 2005)
Dairy	1-13	1-50000	-	-	75-125	220-340	-	(O. Monroy H . F. Vhquez M. n.d.)
Milk	8-11	15-4790	886-18480	160-807	-	6-8500	-	(Klemes, Smith et al. 2008)

a= Total Nitrogen

b= Total Phosphorus

“-“ No data

The composition of milk processing generated wastewater is variable. Reported wastewater from milk dairy processing showed wide ranges in organic load. The largest range in reported BOD₅ and COD was 1-50,000 mg/L and 24.7-18,480 mg/L respectively. Nutrient load and pH also showed large ranges (1-13 pH, 10-807mg/L TN, and 2-125mg/L TP). Similarly, the volume of generated wastewater varied. The parameters chosen in **Table 2** were the most common parameters reported. However, not all studies gave values for wastewater load and volume. Optimizing the FEW Nexus within food processing, requires a common understanding and transparency when it comes to quality and quantity of resources used and wastewater generated. Filling in these gaps with volume and type of outgoing fluid milk will help link the reason for variable wastewater generated. Reporting consumption in relation to the volume of milk processed, will help processors understand the generation of each process. Additionally, defining these parameters would help explain the observed variability in organic load of spent processing wastewater.

Dairy Industry

The U.S. dairy industry is shifting away from family owned small production to multi-farmer corporations. The dairy industry produces various products that require different quantities of resource consumption, and in turn produce different wastewater streams (both composition and volume) The U.S accounts for 10% of the global milk production (IUF n.d.). Successful production of dairy products involves adequate treatment and cleaning processes. Together, these operates help maintain the high integrity of

productions. Dairy plant cleaning accounts for 21% of the energy consumption (Ramirez et al. 2006) and 28% of the water in Australian milk processing (Rad and Lewis 2014).

Cleaning Operations

The goal of cleaning operations is to kill bacteria and eliminate the possibility of product contamination. Inadequate cleaning risks the possibility of product contamination and recalls. Product recalls affect the financial and social aspects of food processors. In addition, inadequate cleaning results in expenses of wasted water and cleaning solutions. Automated cleaning was designed to assist the dairy industry in developing repeatable consistent cleaning. Wastewater from cleaning operation can account for 54% of the total wastewater volume (Dresch et al. 1999).

Cleaning in Place (CIP)

CIP operations, in dairy processing typically involve four steps. The first step in cleaning operations is the freshwater rinse. This step involves flushing heated water throughout the piping and holding tanks. Water used to flush lines exits to the floor containing not only fresh water but also leftover/lost product. The second step is a caustic rinse, which consumes large amounts of caustic soda. A dairy plant processing 260,000 gallons of milk a year will consume 120 tonnes (Marie Furic 2015). The role of caustic solutions is removal of soil left from carbohydrate and protein residues (Chisti 1999). The third step in CIP operations is an acid rinse. The role of this step is twofold; neutralize of residue caustic solution and removal of mineral deposits (Chisti 1999). The final step, sanitization, should have a bactericidal effect. This step is essential to complete cleaning, and ensure the future of product safety.

Caustic Reuse

Some dairy processors may reuse caustic solution, without quantitative data to back up their decision. When the decision to reuse water is based on arbitrary data of color and odor, the repeatability of automated cleaning could be lost. Some processors will choose to use commercial blends of Sodium Hydroxide (NaOH) and surfactants, while others choose to use a low concentration NaOH solution. Research regarding caustic solution reuse have focused on cleaning solutions ability to clean fouled membranes (Dresch et al. 1999, Bremer et al. 2006, Marie Furic 2015). Studies have observed a reduction in surface tension when caustic solutions are reused, increases their efficiency. One study observed that reusing caustic solution resulted in a decrease in surface tension from 74.0mJ/m^2 (fresh solution) to 59.0 mJ/m^2 and in some cases 27.9 mJ/m^2 . This surface tension correlated to a reused solution with a COD of 300mg/L (Alvarez et al. 2007). Other studies have proposed membrane treatment to regenerate caustic solution, for later use (Trägårdh and Johansson 1998, Gésan-Guiziou et al. 2002, Fernández et al. 2010). With or without treatment of spent solution the cleaning efficiency must be evaluated to ensure sanitary standards are held constant, as improper cleaning is the major source of milk contamination in food processing (Kumar and Anand 1998). The reuse of solution without evaluating the microbial impact can lead to inadequate cleaning operations, and therefore the growth of biofilms.

Biofilms and *Pseudomonas aeruginosa*

Overtime, insufficient cleaning can form environments that foster microorganism growth. If cleaning continues to fail, than microorganisms can multiply and eventually entrap surrounding fragments (Kumar and Anand 1998). This collection of cells is known as a

biofilm. First, the surface is conditioned by an increase in nutrients present. Next, microorganism adhere to the conditioned surface and continue to grow. The bacteria locks into the surface after extracellular polysaccharides forms, which acts as a protecting coat for the biofilm. The formation of biofilms in food processing plants can negatively affect heat transfer and flow rates (Kumar and Anand 1998). Biofilms negatively affect processing as well as pose great risk to the public health.

Pseudomonas aeruginosa is an aerobic Gram-negative rod that pose a health risk to immunocompromised members of the public. Two pigments may be produced as a result of *P.aeruginosa* growth: Pyocyanin (non-fluorescing) and pyoverdin (fluorescing). (WHO 2011). *P.aeruginosa* in biofilm formation produces three types of extracellular polysaccharides (Irie et al. 2010). This strong outer layer contributes to the biofilm's resistance to disinfectants. *P.aeruginosa* can show moderate resistance to Chlorine if biofilm formation occurs.

Further evaluation of the FEW Nexus focused of food processing is needed. A holistic approach would include quantifying resource consumption to understand the interrelation of food energy and water within food processing. These values must be supported by plant operations and processing data. Additionally studies that evaluate the impact of resource optimization should explore the microbial significance. As biofilms present more resistance to removal than planktonic cells, they are ideal for studying efficacy of caustic solution reuse. Together these parameters can help processing facilities understand and optimize their practices, and encourage a sustainable food industry.

List of References

- Alvarez, N., et al. (2007). "The role of surface tension of re-used caustic soda on the cleaning efficiency in dairy plants." International Dairy Journal **17**(4): 403-411.
- Bauer, D., et al. (2014). "The water-energy nexus: Challenges and opportunities." Washington DC: US Department of Energy.
- Bremer, P. J., et al. (2006). "Laboratory scale Clean-In-Place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms." International journal of food microbiology **106**(3): 254-262.
- Brush, A. (2011). "Energy Efficiency Improvement and Cost Saving Opportunities for the Dairy Processing Industry: An ENERGY STAR? Guide for Energy and Plant Managers."
- Bustillo-Lecompte, C. F. and M. Mehrvar (2015). "Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances." Journal of Environmental Management **161**: 287-302
- Casani, S., et al. (2005). "A discussion paper on challenges and limitations to water reuse and hygiene in the food industry." Water Research **39**(6): 1134-1146.
- Chisti, Y. (1999). "Modern systems of plant cleaning." Encyclopedia of food microbiology **3**: 1806-1815.
- Codex Alimentarius Commission, (1999). "Codex Alimentarius Commission: Codex Committee on Food Hygiene." Proposed Draft Guidelines for the Hygienic Reuse of Processing Water in Food Plants.
- Danalewich, J., et al. (1998). "Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal." Water Research **32**(12): 3555-3568.
- Demirel, B., et al. (2005). "Anaerobic treatment of dairy wastewaters: a review." Process Biochemistry **40**(8): 2583-2595.
- Dresch, M., et al. (1999). "Membrane processes for the recovery of dairy cleaning-in-place solutions." Le Lait **79**(2): 245-259.
- EIA, (2016). "Chapter 7: Industrial sector energy consumption."
- EIA, (2017). "Industry Uses Many Energy Sources "
- FAO,(2011). "Energy-Smart Food for People and Climate Issue Paper."

- Fernández, P., et al. (2010). "Nanofiltration regeneration of contaminated single-pHase detergents used in the dairy industry." Journal of Food Engineering **97**(3): 319-328.
- Finley, J. W. and J. N. Seiber (2014). "The Nexus of Food, Energy, and Water." Journal of Agricultural and Food Chemistry **62**(27): 6255-6262.
- Gésan-Guiziou, G., et al. (2002). "Nanofiltration for the recovery of caustic cleaning-in-place solutions: robustness towards large variations of composition." Journal of dairy research **69**(4): 633-643.
- Gulati, M., et al. (2013). "The Water–energy–food Security Nexus: Challenges and Opportunities for Food Security in South Africa." Aquatic Procedia **1**(Supplement C): 150-164.
- Hanlon, P., et al. (2013). Food, Water and Energy: KNOW THE NEXUS. G. C. Foundation. New York, NY, USA.
- IUF, (n.d.). "United States of America Dairy Industry."
- IFC,(2007). "Environmental, Health, and Safety Guidelines for Dairy Processing." World Bank Group.
- IRENA (2015). Renewable Energy in the Water,Energy & Food Nexus. . I. R. E. Agency: 12-21.
- Kirby, R. M., et al. (2003). "Water in food production and processing: quantity and quality concerns." Food control **14**(5): 283-299.
- Klemes, J., et al. (2008). Handbook of water and energy management in food processing, Elsevier.
- Lampi, M. (2001). Best available techniques (BAT) for the Nordic dairy industry, Nordic Council of Ministers.
- Marie Furic, A. E., Walid Blel and Olivier Sire (2015). "Regeneration of Lyes for Cleaning-In-Place (CIP) Processes in Dairies: Comparison of Recycled Potash and Soda Solutions Cleaning Properties " American Journal of Applied Sciences.
- Masanet, E., et al. (2014). "Energy efficiency opportunities in the US Dairy processing industry." Energy Engineering **111**(5): 7-34.
- National Dairy Council of Canada, (1997). "Guide to Energy Efficiency Opportunities in the Dairy Processing Industry."
- O. Monroy H . F. Vhquez M., J. C. D. a. J. P. G. (n.d.). "anaerobic-aerobic treatment of dairy wastewater with national technology in mexico: the case of "El Sauz".".

Ölmez, H. and U. Kretzschmar (2009). "Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact." LWT-Food Science and Technology **42**(3): 686-693.

Rad, S. J. and M. J. Lewis (2014). "Water utilisation, energy utilisation and waste water management in the dairy industry: a review." International Journal of Dairy Technology **67**(1): 1-20.

Ramirez, C., et al. (2006). "From fluid milk to milk powder: Energy use and energy efficiency in the European dairy industry." Energy **31**(12): 1984-2004.

Sarkar, B., et al. (2006). "Wastewater treatment in dairy industries — possibility of reuse." Desalination **195**(1): 141-152.

Scanlon, B. R., et al. (2017). "The food-energy-water nexus: Transforming science for society." Water Resources Research **53**(5): 3550-3556.

Siebert, S., et al. (2010). "Groundwater use for irrigation—a global inventory." Hydrology and Earth System Sciences **14**(10): 1863-1880.

Trägårdh, G. and D. Johansson (1998). "Purification of alkaline cleaning solutions from the dairy industry using membrane separation technology." Desalination **119**(1-3): 21-29.

WHO (2011). "Guidelines for drinking-water quality." WHO chronicle **38**(4): 104-108.

Xu, T. and J. Flapper (2009). "Energy use and implications for efficiency strategies in global fluid-milk processing industry." Energy Policy **37**(12): 5334-5341.

Chapter 3: THE QUANTIFICATION OF THE FOOD, ENERGY, AND WATER NEXUS AND THE ECONOMIC EVALUATION ASSOCIATED WITH RESOURCE CONSUMPTION WITHIN A MILK PROCESSING FACILITY.

1. Introduction:

Water, the truly essential element of life, is a luxury for most. However, it cannot be seen as a right for all. Over 844 million people lack access to clean water daily (WHO 2017). As the population and occurrence of urbanization continues to surge, so does the stress placed on resources like water and energy. By 2050, the world's demand for food and water will grow by more than 50%, while the demand for energy will increase by twofold (IRENA 2015). Water is a finite resource; only 1.2% of the Earth's fresh water is considered surface water and available for use (USGS 2016). The food industry must begin to view resource management as the key to unlocking the viable future of the industry. Without taking progressive steps to reduce energy and water consumption, feeding the growing world will become problematic. With that in mind, current research hopes to assist industrial facilities in creating and enforcing a sustainable food system. Innovative research must take a frontline approach by establishing process optimization and resource reduction within the food processing sector. Efforts to ease resource consumption need to simultaneously address reductions in both energy and water. This can be accomplished through addressing the Food, Energy, and Water Nexus (FEW Nexus).

The FEW Nexus is the inseparable connection linking water and energy consumption with the production of food (Finley and Seiber 2014). Both water and energy are engrained in the production of food from farm to fork. Research addressing

the FEW Nexus is conducted on the large-scale basis of the food system. For example, water is required to produce biofuels, which are used in irrigation systems that aid agricultural production of food commodities (UN Water). Energy is consumed to heat water, which helps ensure the safe production of food (Powell 1997). The food industry is faced with a unique opportunity to co-manage resources, as conservation of one is directly related to the conservation of its counterpart (Gulati, Jacobs et al. 2013). The entire food production chain is charged with 90% of the global freshwater and 30% of the energy consumption (Scanlon, et al. 2017). In fact, the food industry is the fourth largest energy user of the U.S. industrial sector (Milmoie n.d.). The FEW Nexus, as it relates to the entire food system, takes considerations into the production of resources, agricultural growth, food production, transportation, and consumer habits of food, energy, and water (Biggs, et al. 2015). However, large scale consideration fails to address how the FEW Nexus relates to food production, within the walls of a food processing facility. The FEW Nexus must be optimized at each stage within the food system.

It is important to address the FEW Nexus when formulating a sustainability focused action plan as consumption of resources cannot just be monitored at one location in the food chain. It is an overarching view of the FEW Nexus, that resources of water and energy are embedded into finished food. Therefore, as food waste occurs, so does the waste of energy and water (Cuéllar and Webber 2010). When addressing resource use, it is imperative to understand that food waste transpires on the field, in the processing facility, during transportation, and in the consumer's kitchen. As a food processor, food waste can be controlled on the processing floor. Therefore, processors should become conscience of the resource demands of their process. The first step in reducing food

waste is to improve process efficiency. Quantifying the resource demands, inputs, and outputs throughout food processing, allows processors to recognize where resource consumption can be improved. In locating these key points of production, wastewater generation points and the recovery potential of outputs will be uncovered. In determining the plausibility of wastewater reuse or input recovery, the composition of wastewater must be determined. Focusing efforts on the recovery of milk processing wastewater is a plausible step to reducing food waste, and thus optimizes resources. As the production of cheese and milk require different amounts of water and energy, so will the volume and composition of wastewater. Variation also exists amongst processors depending on production scale, manufacturing practices, and operational importance placed on sustainability. Therefore, energy and water demands of milk producers will vary with the volume and composition of the dairy product produced. A case study in Mexico determined that the volume of wastewater generated yearly was 1-3 times the volume of milk processed (O. Monroy et al. n.d.). Other studies found the dairy industry effluent streams contained 1-3% loss of milk components (Luo et al. 2012) and produced 0.2-10 L effluent/ L of processed milk (Vourch, Balannec et al. 2008). It is important to associate product lines with their true resource consumption to understand which points in production contribute the most to loss of milk components, and therefore effluent wastewater lines.

In 2016, the United States produced over 212,000 million pounds of milk (USDA 2017). The US dairy industry is the sixth largest milk producer in the world, with the Midwest producing 32% of the total pounds of milk produced nationally (IUF n.d.). Dairy processing, not specific to farm or facility is responsible for 15% of the food

sectors' economic output (Masanet, Brush et al. 2014) and consumes \$1.5 billion on purchased energy (Brush 2011). Water is a large input for all dairy processing facilities and is used throughout multiple stages of milk processing. The Environmental, Health, and Safety (EHS) guidelines identifies four environmental issues surround dairy processing: wastewater, solid waste, emissions, and energy consumption (IFC 2007). Research on dairy processing does not separate process requirements nor specify variation in product lines. The water consumption of milk production includes the amount needed for the processing of milk as well as the cleaning of processing equipment. The primary amount of wastewater, 50-95% of the waste stream's volume, occurs during washing, cleaning, and operations conducted during CIP cycles (Daufin, Escudier et al. (2001), Kushwaha, Srivastava et al. (2011)). Recovery of desirable components in wastewater can lower the cost associated with wastewater disposal as surcharges, for wastewater with Biochemical Oxygen Demand (BOD₅) levels above 250-300 mg/L, increase the cost of wastewater treatment. In the European Union, the cost of treating dairy industry effluents is great, ranging from 0.62- 2.79 \$/m³ (Fernández, Riera et al. 2010).

Due to the large volume of effluent wastewater, determining ways to treat or repurpose may be a good start in addressing the FEW Nexus at the processing level. The reconditioning of food industry wastewater has been explored as an alternative to reduce waste, recover water, and produce energy. Membrane technology has been used for the treatment of food industrial wastewater (Hafez, Khedr et al. 2007), but faces obstacles such as cost effective, energy consumption, and the control of water reuse (Pouliot 2008). Costs are dependent on the scale of production, location of wastewater treatment

plant, energy requirements, and type of treatment, e.g., nanofiltration, reverse osmosis (Owen, Bandi et al. 1995). Wetlands, coagulation, and algae cultivation are also used for the treatment of wastewater to recycle organic materials (Luo, Cao et al. 2012). As wastewater treatment is not one size fits all, other methods should be developed and explored first. While some literature may recommend the reuse of process wastewater, there is no clear line of requirements for effective reuse. The EHS recommends reuse of water in the form of condensation for heating or cooling, as long as they achieve sanitary requirements (IFC 2007). However, this point of reuse needs definition. Therefore, this project wants to define where that line is, allowing for a more comprehensive understanding on how milk processors can optimize resource consumption.

The recovery and treatment of wastewater must be approached in a way that is plausible for all members of the industry. It is thought that a producer's decision to recover wastewater must be validated with stability in product safety and incentivized by the potential benefit to their bottom line. The desire of a producer to become a “good steward” of resources may not be enough to implement beneficial changes to the FEW Nexus within a milk processing facility. Processing inputs of electricity, natural gas, and water have associated financial costs. It is important to explore the utility cost and savings opportunities of reducing and reusing resources.

Determining the role of water and energy in the food industry has proved to be an ideal starting point for reducing the distance between process productivity and resource efficiency (Meneses and Flores 2016). The key to resource conservation is to pinpoint specific production lines and determine what methods of reduction are specific to that product. Together, they will answer the questions, “where can water be conserved?” and

“is it able to be recovered?.” Knowing process specific requirements of energy and water will help determine the potential long-term benefits of conservation strategies and help to create a secure food system that can hold and supply the nutritional needs of today and tomorrows’ world population (Meneses, Stratton et al. 2017).

It is a challenge to find a process for recovery that simultaneously reduces water and energy while also considering the financial implementation and needs of each recommended technology or practice (Bazilian, Rogner et al. 2011). The holistic approach of addressing the relationship of the FEW Nexus within milk production will uncover specific locations, which hold the potential for resource reduction, helping to make proper process specific recommendations. This research unlocks the doors of a small- medium U.S. fluid milk processing plant to understand the roles of the FEW Nexus in the production of fluid milk and looks for opportunities for optimization and reuse. Therefore, the objectives for the project were as followed:

Objective 1: To quantify the baseline consumption of water and energy usage and wastewater generation at a fluid milk processing plant

Objective 2: To pinpoint locations in milk processing that have the potential for resource reduction

Objective 3: To provide understanding on economic tie of resource consumption at a Midwest dairy compared nationally

2. Materials and Methods:

The production of fluid milk occurs at both the milk parlor and the processing facility. For the purpose of this research the collection of water, energy, and wastewater data occurred within the walls of a small-medium United States fluid milk processing facility and does not reflect consumption on the farm or primary production. In total 22 plant visits were made for data collection and process determination.

2.1. Plant Description

A Midwest processing facility was chosen for determining the baseline of resource consumption within milk processing. This facility produced a variety of fluid milk products including unflavored, chocolate, and strawberry varieties of whole, 2%, 1%, skim, half & half, and occasionally ice cream base. This processor daily produced 7,218 gallons of finished fluid milk. The boundaries for FEW Nexus data collection began when raw milk was pumped from the receiving tanker and ends after heat treated milk is filled and packaged. **Figure 1a** shows the pathway of milk as it transitions from raw to finished commodities. Each morning fresh raw milk is transported from the farm-based milk parlor to the processing facility, about 10 miles. Upon arrival, all raw milk is unloaded from the refrigerated receiving tanker and undergoes separation via centrifugation. Through separation, the raw cream is completely separated from the skim milk. Raw milk is stored in glycerol lined storage tanks until ready for standardization and heat treatment. The plant operator manually conducted fluid milk standardization. The location of standardization fluctuated between the balance tank and the liquefier. After standardization, raw milk is pumped to the liquefier if additional flavor additives are needed (i.e. chocolate). If unnecessary, newly standardized raw milk is held in one of

the three raw milk holding tanks (Tank 1, 2, &3). All processed milk will undergo High Temperature Short Time (HTST) pasteurization and homogenization. After milk is heat treated it is sent to one of the five finished milk tanks (Tank 4,5,6,7,&8) as seen in **Figure 1b**. From here, the pasteurized milk goes into either bottles or pouches. After packing milk is ready for transportation. The quantification of energy and water ends after packaging, and excludes resources consumed during transportation and procurement. The processing facility operated Monday-Thursday, producing finished milk four times a week. The remaining days are held for preventative maintenance or backup production in case of increased demand for finished milk. To determine areas of high resource input, production was broken into two shifts; processing and cleaning. Shift 1: Processing, takes place between 00:00 and 13:59. Shift two: Cleaning, begins when processing is complete and will go until 23:00. Due to varying supply demands, processing and cleaning start times experienced fluctuation.

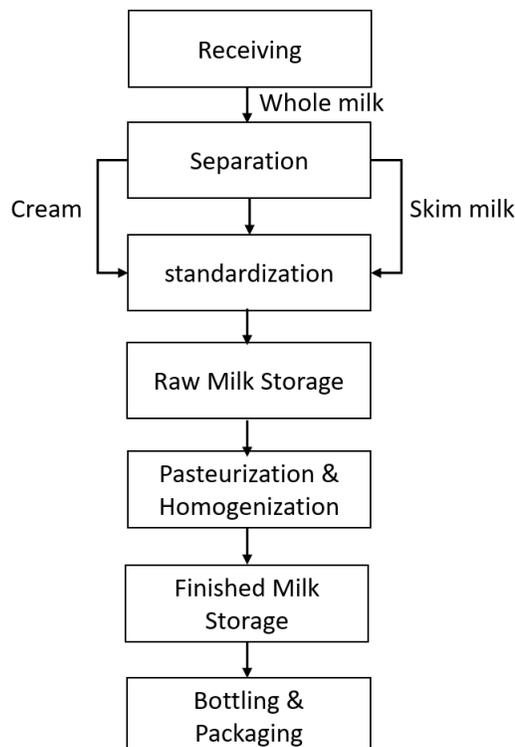


Figure 1a: Process Flow Diagram of Fluid Milk Processing in a Medium Sized Dairy Plant

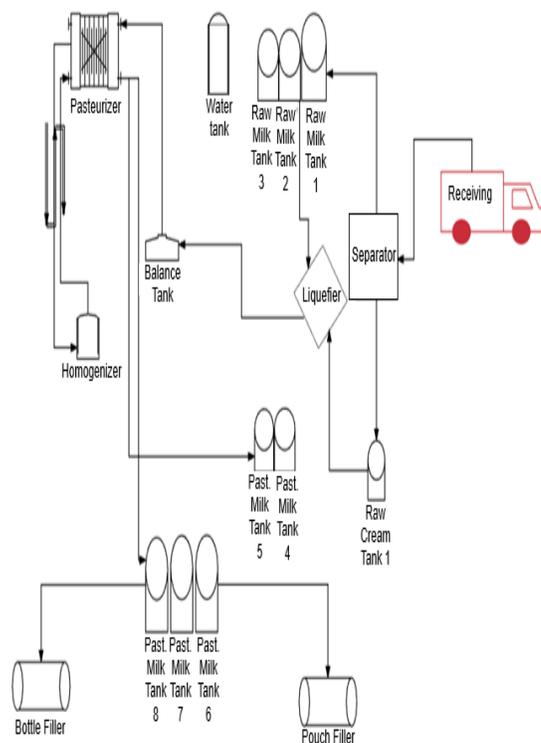


Figure 1b: Plant Diagram of Medium Sized Dairy Processing Plant

Developing a process flow diagram, step 1, allows for determination of the flow of milk through the plant. It also identified the process inputs of milk, water, and energy, and process outputs (finished milk and wastewater). The fluid milk processor consumed two forms of energy, natural gas and electricity. The next step was to quantify these inputs.

2.2. Electricity

Electricity was the primary form of energy used throughout production. This energy source is used to power motors, machines, and pump milk through the flow of processing. To determine the total electrical baseline consumption of milk processing, 24 months of electrical bills were consolidated and analyzed. Electrical data sheets provided

monthly usage (kWh), days of usage, and monthly utility cost. This location was free of demand rate charges, which is not the case for all industrial processors. Therefore, fluctuations in electrical charge was due to seasonal changes and production scale variation. The daily electricity consumed for the entire facility was estimated using Equation (Eq.) 1. While this number reflects the electrical usage of the entire facility, administration/non-processing floor area's electrical consumption was thought minimum in comparison to the processing area. A dairy processing plant, processing undisclosed dairy products, consumes 20% of its energy for non-processing/building operations. (Sun, Reindl et al. 2012) and 9% for fluid milk plants (Ramirez, Patel et al. 2006).

These areas support the processing of fluid milk and therefore were considered. To evaluate which production units had the highest volume of resource consumption additional meters were installed. This data provided justification into areas of focus for optimization efforts of energy and water. The first meter installed was an Automated Metering Infrastructure (AMI) that monitored hourly kWh consumed by the processor. This meter was installed to replace the original meter. Unlike the old meter that was read at the end of the month, this meter took away any operator recording errors of total electrical usage as data was uploaded automatically (Chapman Metering, Iowa). A second meter, commercial three-phase electrical amp meter, was connected onto the electrical control panel (Ted Pro 400, South Carolina). Certified engineers, from a state public power district, installed the second meter. Electrical usage at multiple points within the processing room were monitored. Hourly signals of usage were uploaded to the online record keeping device. Additionally, this meter allowed for independent monitoring of total kWh used each day. Therefore, electrical use for non processing

days could be determined and verified against the AMI meter. This is considered the baseline electrical usage for the facility. The AMI meter allowed for separation of electrical usage for processing vs. cleaning. These numbers were calculated by looking at average total kWh, over a six month, consumed on a processing day. The kWh consumed during processing and cleaning operations were determined by taking the total kWh for each hour during the specified time frame.

$$\textit{Average} \frac{\textit{kWh}}{\textit{day}} = \frac{\textit{Total kWh used}}{\textit{days of use}} \quad (1)$$

2.3.Natural gas

The second form of energy used at the plant, natural gas, was solely consumed by the boiler. Reports from 24 months of billed usage were collected to determine how much natural gas was consumed each month. Natural gas is used to generate hot water utilized throughout cleaning and pasteurization.

2.4.Water

Monthly consumption measured in gallons and cost data were obtained from 24 months of billed data and analyzed using **Eq. 2**. To further evaluate volumes and locations of water consumption two Multi-Jet Water Meter w/ Pulsed Output inline meters (Dwyer, Indiana) were installed. One inline meter was installed into a sliced pipe connected to the boiler feed tank. This meter would measure the volume of water utilized through the boiler for steam production. The second meter was installed near the main city provided water meter. This meter was used to monitor total water consumption of the processing facility. Both meters were connected to iMONNIT (Monnit, Corp, Utah), an online data collection and display system. A pulse monitor allowed for hourly usage volume readings

of both meters. This indicated when the largest volume of fresh water was consumed. Inline meters helped determine the difference in water consumption during processing versus cleaning operations. The water consumed during cleaning is utilized during multistage cleaning in place systems (CIP), cleaning out of place procedures, floor cleaning, and the running of the homogenizer. A material balance of the automated CIP system was conducted. Two portable ultrasonic flowmeters (Fuji Electric Co., Ltd, Japan) were used to measure the flow rate and volume of water in and out during the different stages of cleaning. Each meter was calibrated based on pipe dimensions.

$$\textit{Ave freshwater} \frac{\textit{gal}}{\textit{day}} = \frac{\textit{Total gal used}}{\textit{days of use}} \quad (2)$$

This plant's cleaning process operates between two cleaning in place (CIP) operations and additional cleaning out of place (COP) operations. The first CIP system was designed for automatic cleaning of the processing equipment and the first five tanks (3 raw milk, 2 pasteurized milk). The second system is the automated cleaning of the last three finished milk storage tanks and the pouch filler. CIP stage one involves automated flushing of the pasteurizer and homogenizer with fresh cold water. The water balance for this step was performed using the two flowmeters. One meter installed on the pipe where water flowed into the system. The second meter was installed at the point water was expelled.

Verification of flow meter accuracy was done by comparing the computed totalizer volume with the projected flowrate multiplied by the time. Any variation between the volumes of water in and water out was due to residue milk, leftover from processing. If the output flow volume exceeded the flowmeter capacity the volume of water out was assumed equal to that of water in. This assumption is justified, as operator must have cleared lines before continuing onto the next cleaning stage. Cleaning of the inside of the

tankers are excluded from the first step of the CIP process and thus a part of COP operations. These tankers are cleaned using hot water through an operator controlled hose. The volume was quantified using measured hose flow rate and usage time. This technique was verified by measuring the total volume of water out, once tanks were drained to the floor. As the water out contained any residue amounts of milk left in the tanks, the water consumed was determined using **Eq. 3**. To determine how much milk was left over in the tanks at the start of cleaning **Eq. 4** was used.

$$\text{Water output} = (\text{Ave. Hose flow rate} \times \text{Hose usage time}) \quad (3)$$

$$\text{Milk out} = (\text{Measured volume of water exiting tank}) - (\text{Ave. Hose flow rate} \times \text{Hose usage time}) \quad (4)$$

The second stage of each CIP process is caustic (alkaline) rinse. This step is followed by an acidic rinse, and CIP is completed after sanitization. Fresh water from the water-holding tank is pumped to the balance tank. For the remaining stages water and specific cleaning agents go into the balance tank, flow through the pasteurizer and homogenizer and then move to the fifth and final tank of the first CIP process. Once the water has been cycling in tank 5, for 11 minutes it returns to the balance tank. Additional water is added to the tank and the cycle repeats until all five tanks are cleaned. Each water balance was conducted in triplicate. CIP pathways will vary by processor.

2.5.Wastewater

Process wastewater was composed of lost product, spent water, and used cleaning chemicals. Wastewater was generated for everyday of production. The processing floor contained one main drain in the middle of the facility. All spilled product or spent

solutions were pulled to this drain. Drained liquid was pumped outside off the facility and into a collection tanker. This tanker remained stationary for two day, or until it reached at least half full. From their , the tanker was either sent off to the fields, to be directly land applied or disposed into a designated lagoon. Here the water would stand, and organic components would settle out. This water would be used to meet irrigation demands. When the truck is sent to the lagoon, it passes over a scale; data collected for the analysis of wastewater generation are associated to these recorded weights. However, the days where the wastewater is directly land applied, the truck weight was not recorded. Therefore, detailed records of wastewater generated were sporadically monitored.

2.6.Economic Evaluation

While each dairy processor is unique, high utility processes remain constant for fluid milk production. In order to evaluate the role of economics in dairy processing, comparisons across the industry were made. Industrial energy costs were determined and compared to partnering processor. The U.S. Department of Energy's industrial rates for natural gas and electricity were compared to the Midwest dairy plant (DOE 2017). Determining these cost relationships may help processors when evaluating the plausibility of wastewater recovery and reclamation efforts.

3. Results and Discussion

The annual resource consumption and their affiliate cost to the partnering processor were determined through utility bill data analysis. Each year the processor consumed 230,000 kWh (\$25,805) 25,000 Therms (\$16,338), and 1.4 million gallons (\$5,038) on electricity,

natural gas, and water respectfully. The further analysis of both billed and metered data is seen below.

3.1. Electricity and Natural Gas

In addition to understanding the yearly impact of resource consumption, consolidation of billed data reviewed trends in monthly usage. The average monthly billed energy consumption was 16,791 kWh (electricity) and 1,964 Therms (Natural Gas). **Figure 2** shows the month-to-month variability in energy consumption. This plant saw an increase in electrical consumption during months of fall and spring. The monthly electrical usage peaked at 24,657 kWh in September. After this peak, the electrical usage fell but remained relatively stable until April. In April, the electrical usage spiked again (21,407 kWh) but experienced a lower spike than September. The lowest monthly electrical consumption happened right after the second spike. During the month of May, the electrical usage was only 12,412 kWh. The electrical usage for the month of July was captured for 2015 and 2016. The electrical consumption in this comparable month was 17,151 kWh in 2016 and 21,093 kWh in 2015 (**Figure 2a**). This could be a result of increases in efficiency from 2015 to 2016, prior to this research's plant observations. During the duration of the experiments, while conducting plant research, no improvement measures or changes in processing or cleaning were implemented. Information on July 2015 & 2016 for fluid milk production was not assessable. Therefore, changes in production volume cannot be used to justify the specific difference in relation to July 2015 and July 2016.

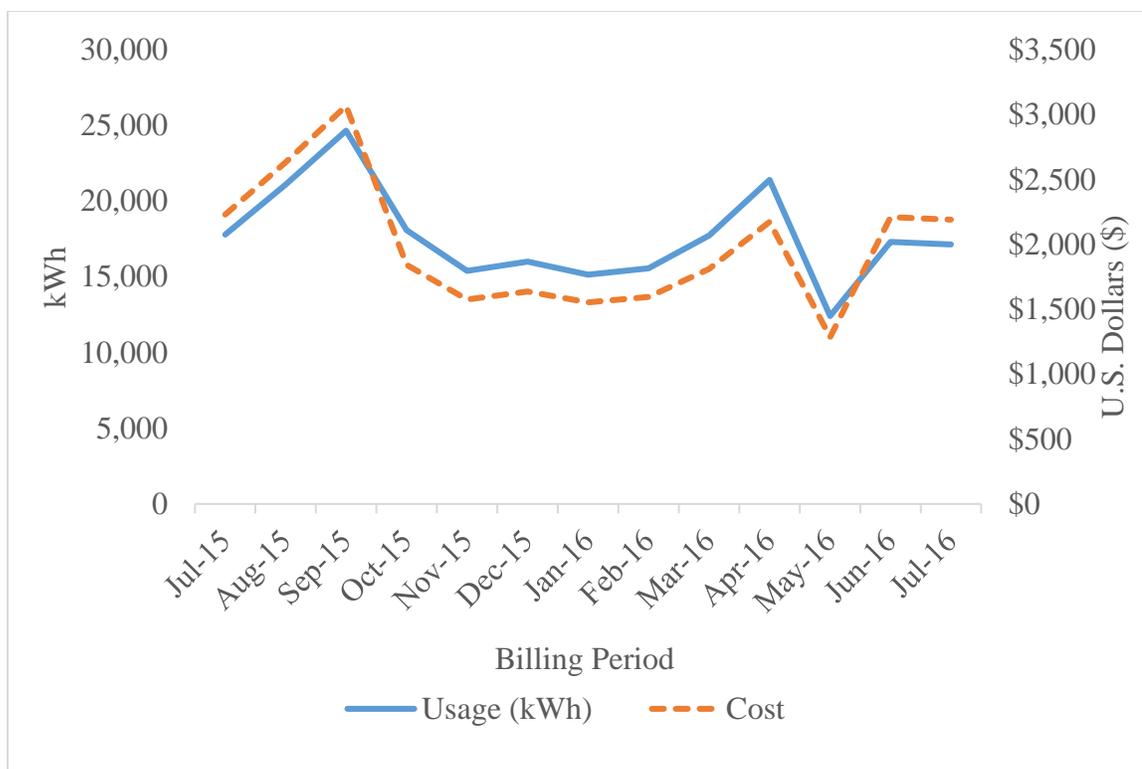


Figure 2a: Monthly Usage and Cost of Electricity

However, **Figure 2b** shows the trend in electrical consumption in relation to fluid milk production. Monthly variation in electrical consumption followed a similar trend alongside milk production. While the milk production values for July 2015 and 2016 is not available, the trend shown in **Figure 2b** indicate the difference is most likely due to a reduction in total finished milk production.

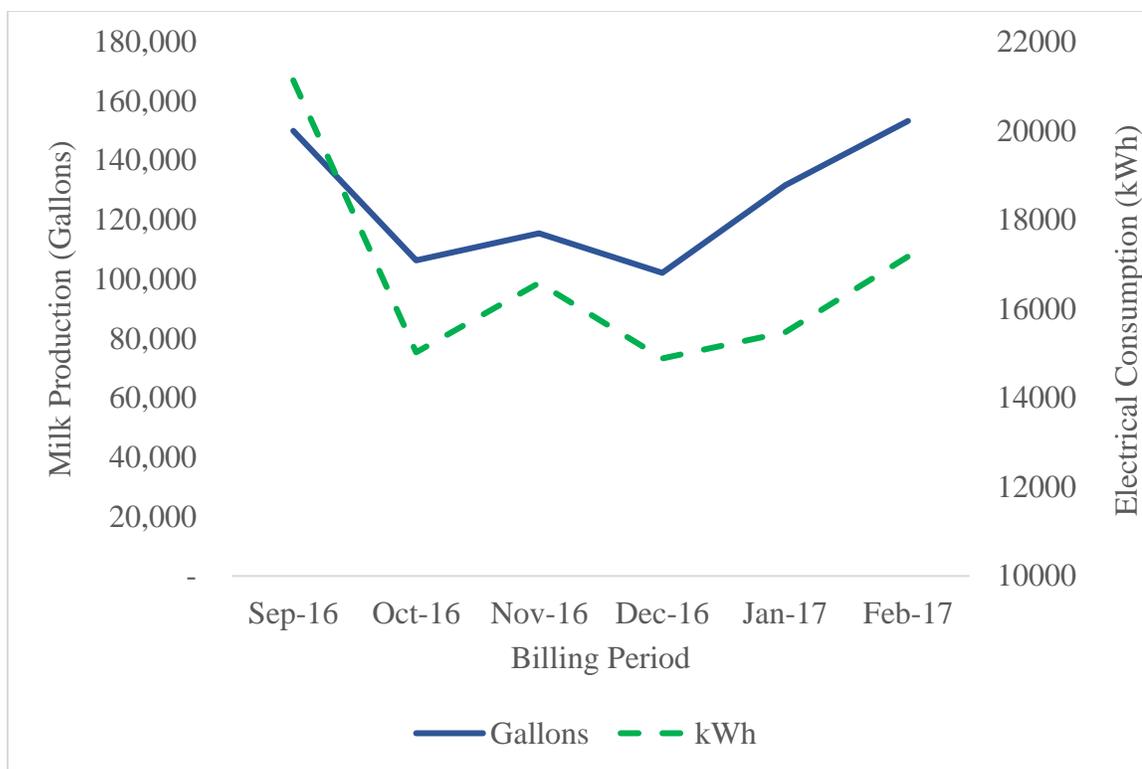


Figure 2b: Monthly Electrical Usage and Milk Production

Over the months, the natural gas consumption ranged slightly from 1,340 -2,240 Therms. The largest months of natural gas consumption were during winter, colder temperature months. The Natural Gas usage for the month of July (**Figure 2c**) was also captured at two points for 2015 (1,340 Therms) and 2016 (1,815 Therms).

An estimate for daily utility consumption can be interpreted from billed data. The average daily energy consumption, found from consolidated bills, was 568.81 kWh /day and 61.28 Therms/day. Interpreting the daily energy consumption in this way does not address variability between processing days and non-processing days. Billed data also fails to detail specific operations energy consumption. Therefore, additional meters to pinpoint specific areas of resource consumption. As the boiler was the sole consumer

of natural gas the location and purpose of natural gas consumption was known. The boiler produced steam used in processing and cleaning.

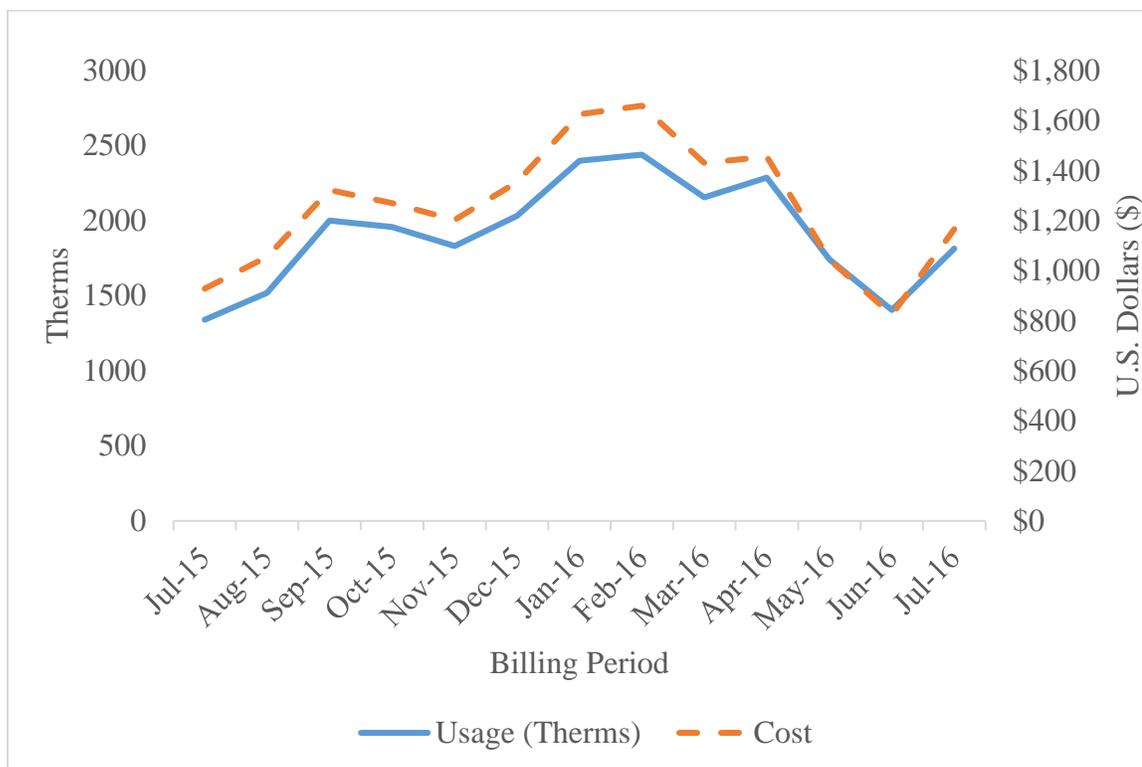


Figure 2c: Monthly Usage and Cost of Natural Gas

Differences in processing day and operations (cleaning vs. processing) were determined by six months of hourly kWh readings by the AMI meter. Evaluating hourly meter data revealed that the processing plant had a kWh consumption on days where no processing was occurring. The total kWh hour consumed on both processing and non-processing days varied. On a processing day, the average electricity for a processing day ranged from 505.55-1221.86 kWh. The average non-processing day electrical consumption ranged from 36.87-544.47 kWh (**Figure 3**). The upper level of electrical consumption range on non- processing days could be a result of nonscheduled make up production or attendance. The overall average amount of electricity consumed on non- processing days

account for 24.80% of the total electrical consumption experienced on processing days. Non-processing days are Friday, Saturday, and Sunday where the milk demands have already been met for the week. However, make up processing runs, along with preventative maintenance can explain the large range in non-processing day electrical consumption.

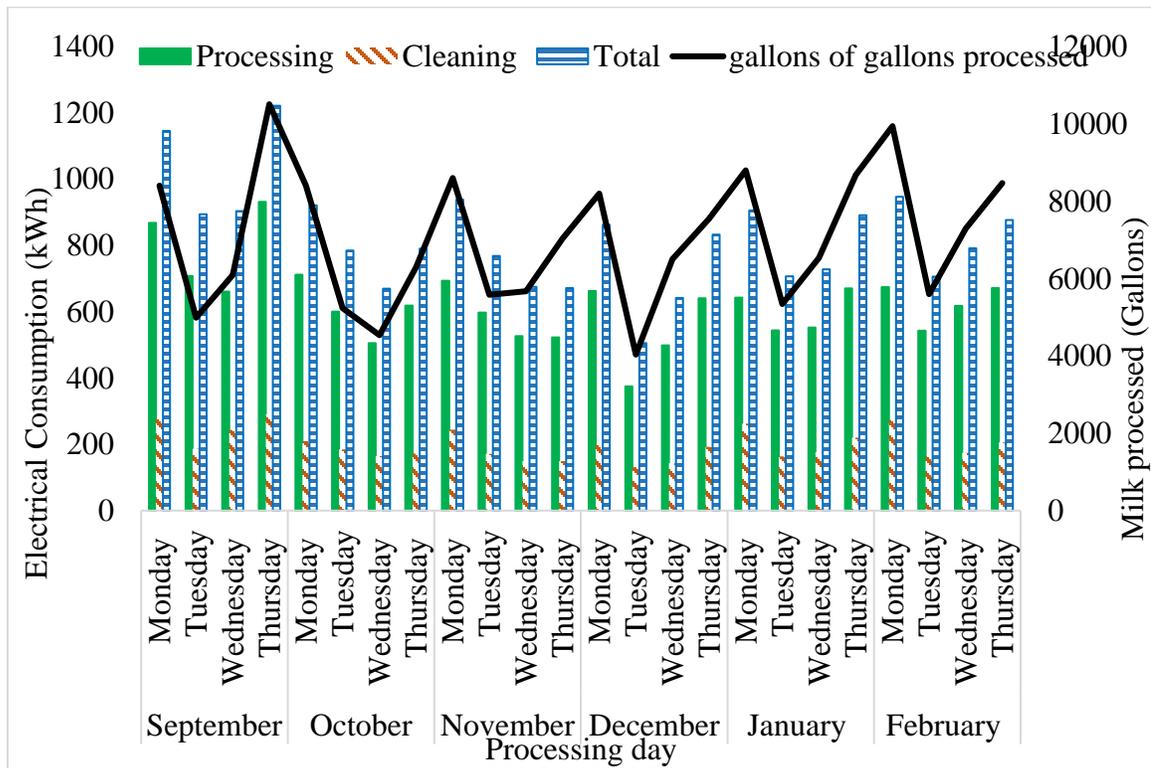


Figure 3: Average kWh Consumption by Processing Day

During processing days, the plant runs on two operation schedules: processing and cleaning. Hours of processing operations are 00:00 and 13:59, while cleaning operations run from 14:00 to 23:59. After standardizing, heat-treating, and packaging of finished milk, cleaning operations were launched. **Figure 3** shows the total kWh consumption broken down by processing and cleaning operations during processing days. The kWh

distribution is shown in relation to the amount of finished milk processed. Here the milk is reported as gallons of gallons processed. The final weight of finished milk was converted from pounds of gallons processed to gallons of gallons processed. Therefore, this is gallons of milk processed. As production volume fluctuates (**Figure 3**), so do the total peaks in electrical usage. The average total amount of electricity consumed on a processing day was directly related to the amount of milk processed. The percentage of electricity consumed during cleaning operations, varied slightly with a minimum of 21.21 % and a maximum of 28.84% of total electrical use. On average the cleaning operations account for 24.12% of the total kWh consumed during a processing day. This is similar to Canadian fluid milk plants where CIP operations accounted for 25% of the total energy (Xu and Flapper 2009). However, CIP operations in Dutch dairies accounted for only 9.5% of the energy demand of fluid milk production in Dutch dairies (Ramirez et al. 2006). Variation could be caused by production scale and level of automated cleaning.

After evaluating the distribution of electrical consumption between operations, circuit specific electricity was evaluated. The 3-phase amp meter allowed for automated data collection from September 2016 through January 2017. Each day the electricity consumption of 55 units was measured and signals were automatically uploaded to an online database. The percentage for each unit was determined by taking the daily kWh data collected divided by the total kWh of the measured points. To sort through data and determine which circuits were essential electrical consumers, 2% electrical consumption was used as a marker of a large consumer.. Over the months, data showed that 24% of the monitored points (55) contributed to 2% or higher.

The average kWh of each measured unit, consumed on a processing day, followed the same pattern throughout the measured months (**Figure 4**).

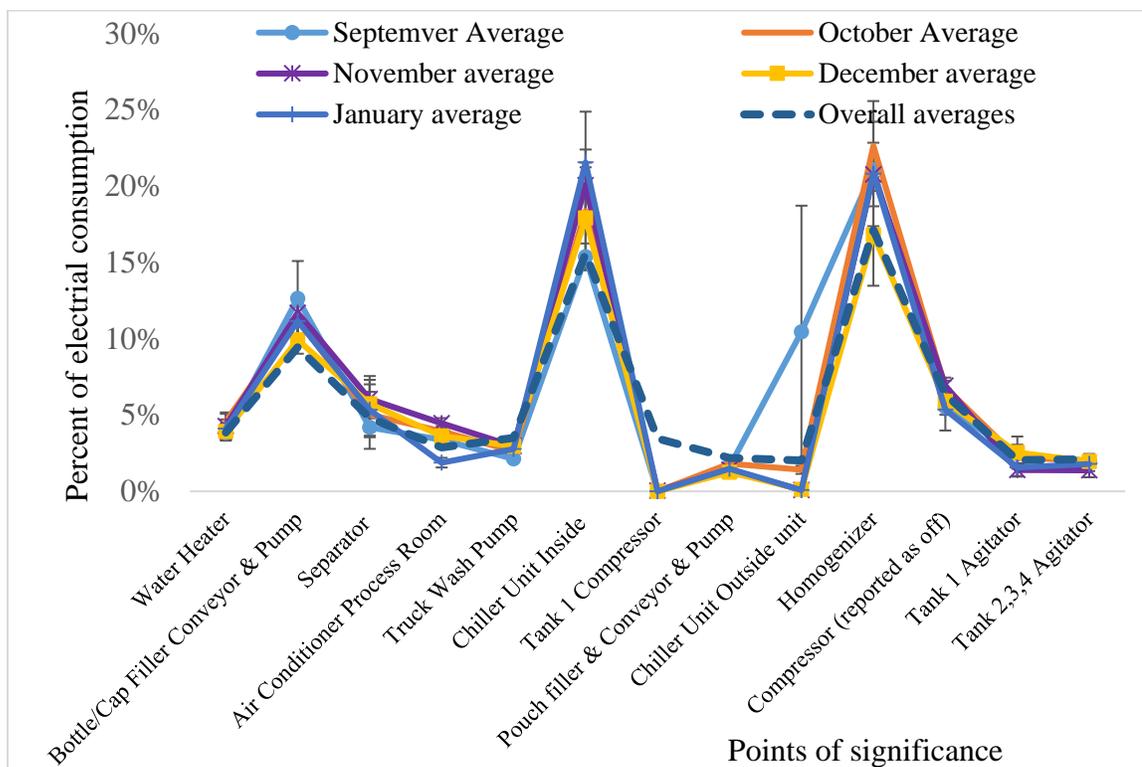


Figure 2: Average Percentage of Energy Consumed Over Six Months

The average of these 13 circuits that reached an average of 2% or higher are seen in **Figure 5**. Together, these 13 circuits accounted for an average of 75.31% of the total kWh monitored over the course of the six months. The remaining electricity monitored was broken into two categories based off percent consumption. Unit operations that showed percent consumption between 1-1.99% accounted for over 15% of the monitored electricity. The remaining electrical consumption was from units consuming less than one percentage.

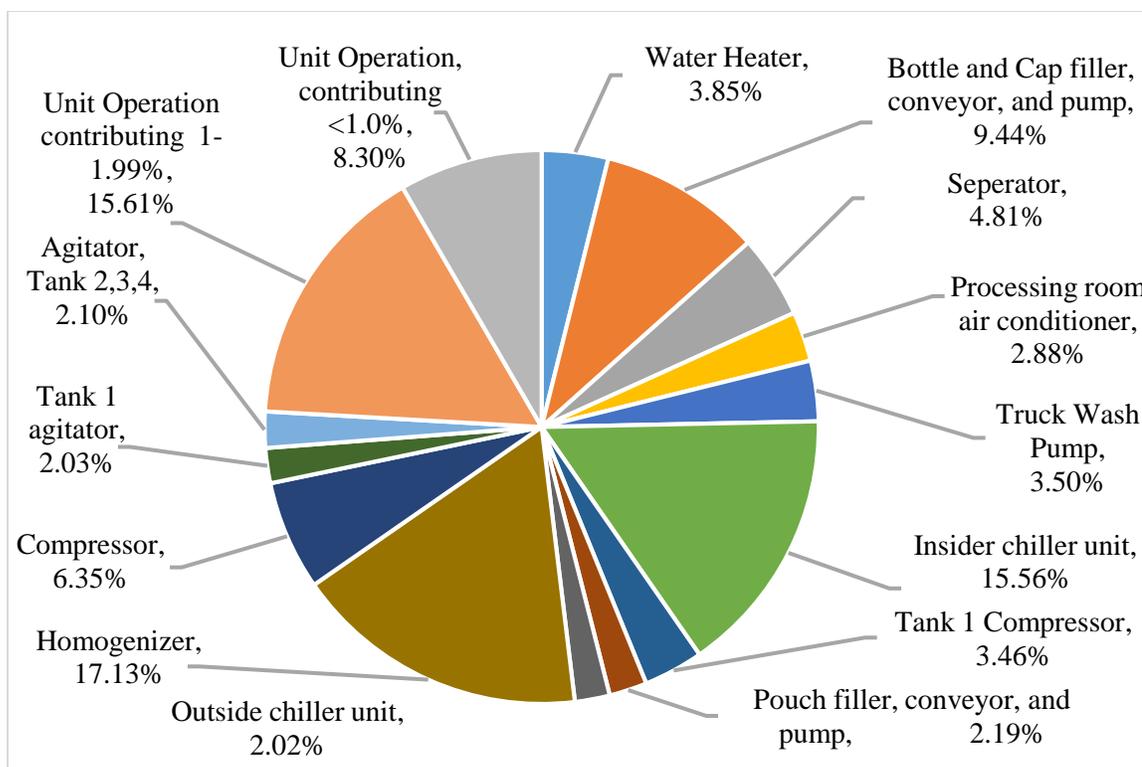


Figure 3: Overall Percentage of Electrical Energy Consumed during a Processing Day

At this plant centrifugation via separator and homogenization account for 21.94% of the electrical consumption, lower than the 38% found for fluid milk in Dutch facilities (Ramirez et al. 2006). These steps together with pasteurization are referred to in this document as milk treatment. The energy requirement on pasteurization is accounted for through natural gas consumption.

The chilling units accounted for 17.58%. This closely compared to the 19% cooling and refrigeration account for in Netherlands milk processing, but higher than 2% consumed for cooling and refrigeration found in Canadian plants (Xu and Flapper 2009). One third of the energy used in the dairy industry is used for heating and cooling purposes (Brush

2011, Sun et al. 2012). However, this does not distinguish which dairy commodities contribute the most to this. This is important, as the energy requirements for cooling are product depended. One study found cooling energy requirements for fluid milk (19%) were less than the energy consumption for butter (66%), yet similar to Cheese (19%) (Ramirez et al. 2006).

A survey of Canadian milk production facilities showed a combined electrical and natural gas consumption by operation. They found that 48% of the total energy was consumed during standardization and heat treatment and 8% was consumed during packaging (Xu and Flapper 2009). Together the packaging, pouch and bottle filler, accounted for 11.63% of the total energy of the processing plant (**Figure 5**). While For fluid milk, the most energy intensive processes have been reported as pasteurization followed by cooling (Brush 2011) homogenization and cooling were the most electrical energy intensive circuits measured here.

3.2. Water

The average monthly consumption of freshwater for the fluid milk processing facility was 113,446 gallons (\$373.86). Similarly to energy analysis an estimate for daily freshwater can be taken from billed data, to be 3,682 gal/day. Similar to energy consumption, that of water varies, as seen in **Figure 6**.

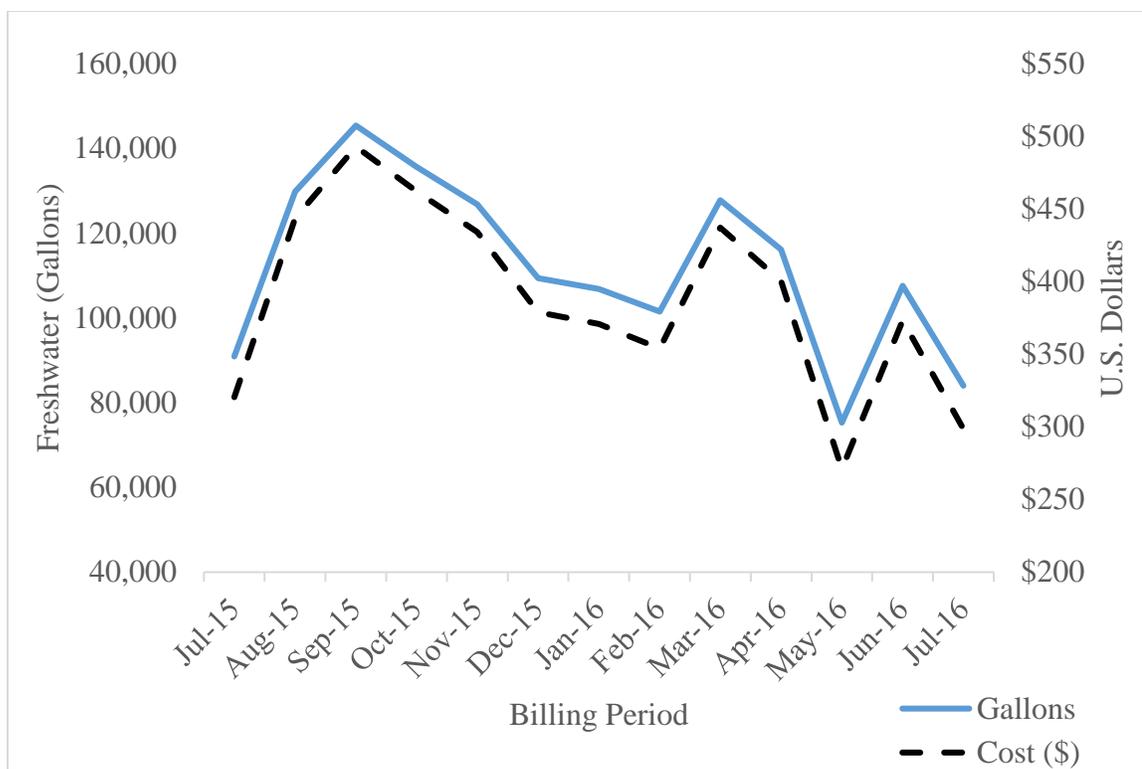


Figure 4: Fresh Water Consumption and Cost

Similar to the trend seen with electrical billed data, July 2015 consumed more freshwater (90,950 gal.) than July 2016 (84,000 gal.). The highest freshwater consumption occurred in September, followed by March, similar to times electrical consumption spiked,

To help understand the trends and areas of freshwater consumption, additional meters were installed. These meters allowed for distinguishing between water consumption on processing vs. non-processing days.

For processing days, the inline water meters revealed that the boiler, the sole consumer of natural gas, consumed an average of 11% of the total fresh water (**Figure 7**). This water was utilized during pasteurization, automated (CIP) and manual (COP) cleaning shifts

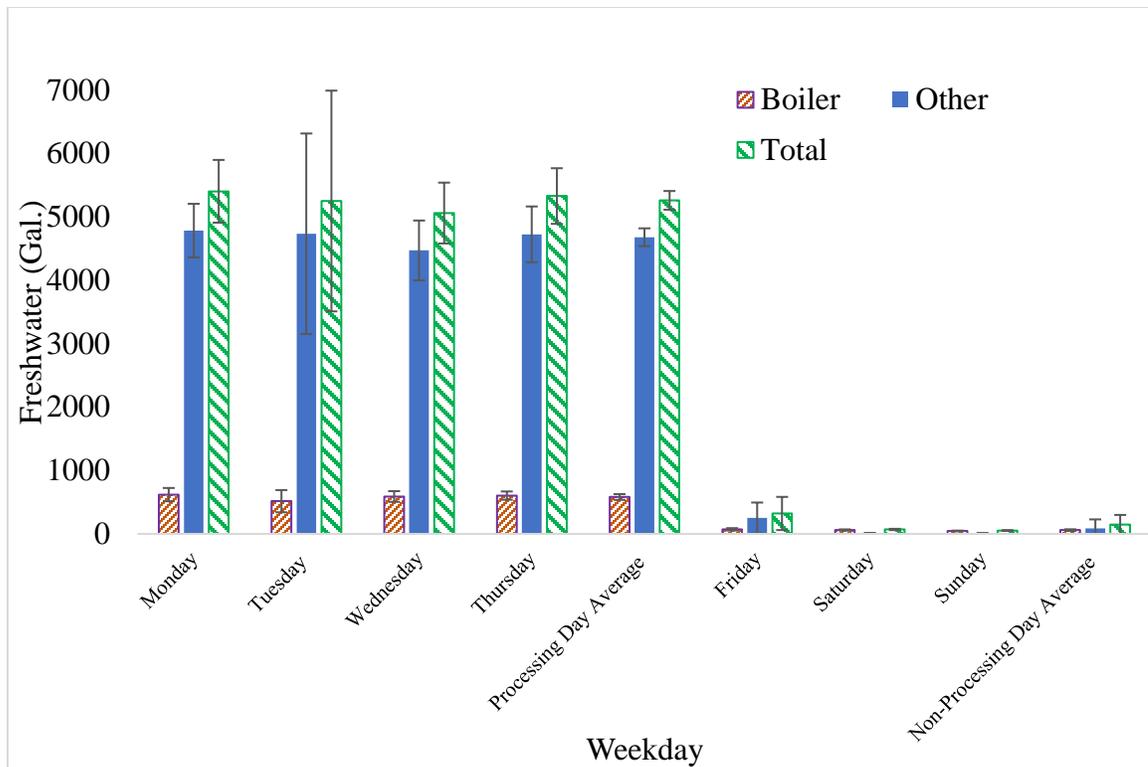


Figure 5: Freshwater Consumption, Determined by Inline Water Meters

The second inline water meter indicated that 53% of the total water consumed during a production day is utilized during cleaning operations. This refers to the water taken up for activities during the hours of cleaning operations. This includes, CIP systems (two cycles), COP systems, washing floors, and to meet the needs of the homogenizer.

To further focus these efforts, a material balance assisted in understanding where the cleaning operations water was used. The material balance measured both CIP and COP operations. The manually (fuji meter data) quantified volume for these cleaning operations is 1470.18 gallons. This value represents 52.6% of the total cleaning operations water (2794.4 gallons) determined by the inline water meters. These results indicate that more gallons of freshwater are consumed than gallons of wastewater

produced. The difference in the water monitored by the inline water meters vs. the material balance using ultrasonic meters can be attributed to evaporation, leaking seals, or operator caused spillage. The loss due to evaporation is a common trend among dairy processors (IFC 2007). The first step in the cleaning operations consisted of using fresh hot water rinse. This first rinse consisted of three parts: 1) The inside of the stainless steel tanks were manually washed to send away any remaining milk out , 2) The automated fresh water used to flush the pasteurization pipe, and 3)The automated fresh water rinse for finished milk tanks 6,7,8. Together, this fresh water rinse accounted for 21.30% of the spent water during cleaning operations (**Figure 8**). The second stage in cleaning uses a highly alkaline solution. The caustic solution used accounts for 22.75% of the total water used during cleaning operations. The third stage, acid rinse, accounted for 4.66%, while the fourth stage, sanitization rinse, accounted for 16.78% of the cleaning operations water. After the automated cleaning, operators proceed to use hot water to rinse the outside tanks and machines. The amount of water used to complete this, as well as water to clean floor, and push spent milk and water to the drain was 22.46%. The homogenizer remains on during the duration of all operations, including cleaning. During cleaning operations alone, the water used to cool the homogenizer's piston pumps accounted for 12.05% of the total water consumed during cleaning operations. This does not reflect its additional water consumption during hours of processing.

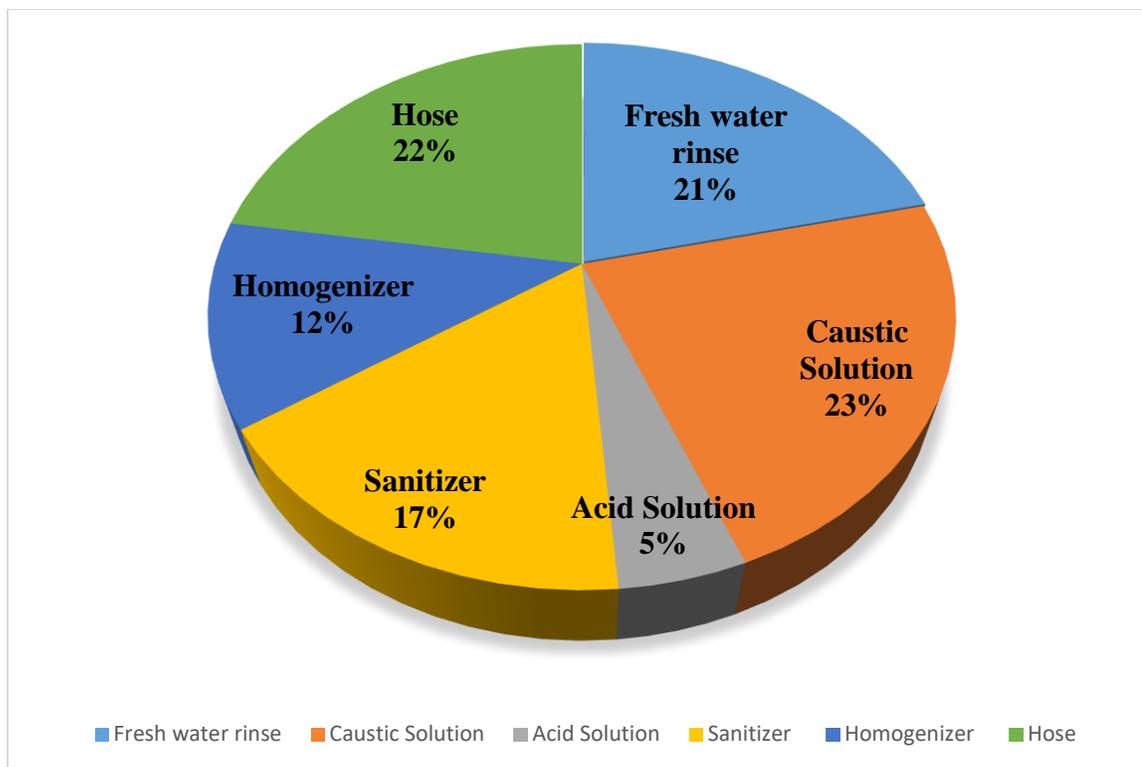


Figure 6. Percentage of Water Consumed during Cleaning Operations

The spent solutions used during cleaning operations are all sent to a central drain for discharge. The wastewater from milk processing is a combination of lost milk, spent water, and cleaning detergents. Due to the milk lost, wastewater has high organic load (20,120mg/L COD and 4,950 mg/L BOD₅). The combined wastewater, taken from the collection tanker, less total suspended solids and lower BOD₅, compared to the Tanker rinse water (25,110 mg/L BOD₅, 11,606 mg/L TSS). The generation of wastewater was directly related to finished product (**Figure 9**).

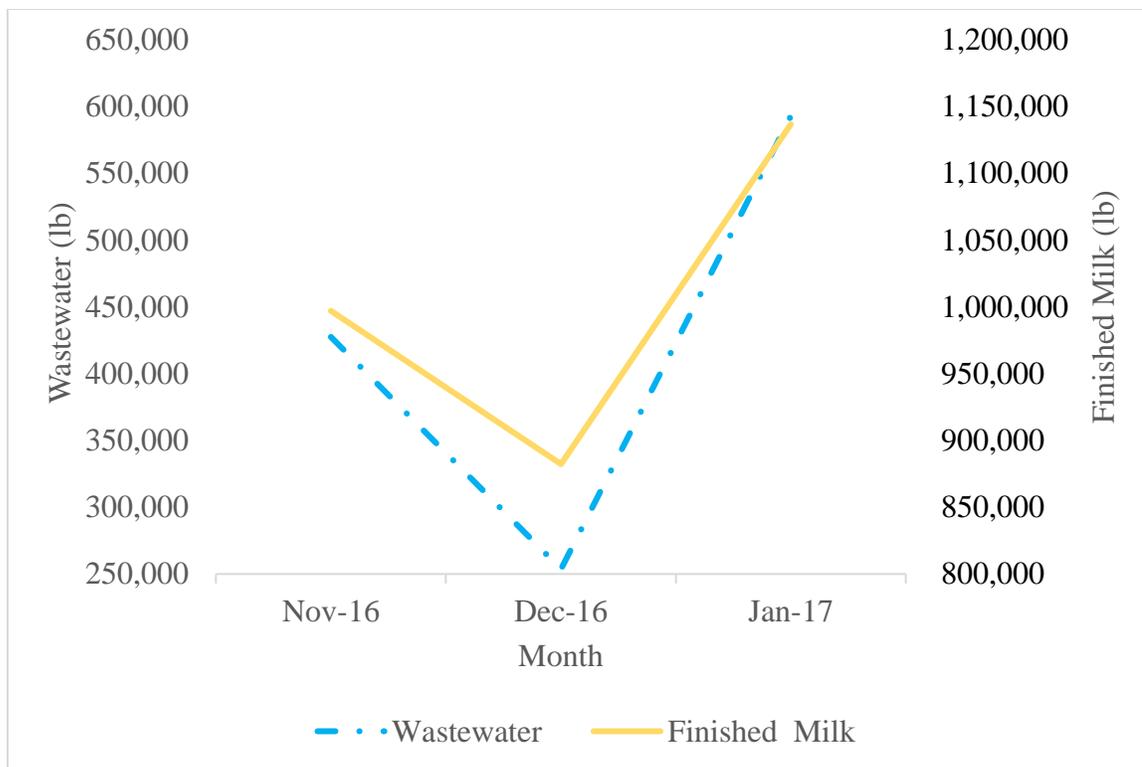


Figure 7: Monthly Wastewater Load Volume

Water quality analyses were independently determined, through a third party laboratory. These parameters were measured to determine how polluted the wastewater was (**Table 3**). The BOD₅ of the partnering plants compiled truck wastewater (4,950 mg/L) was greater than the raw wastewater BOD₅ (1,120 mg/L) of a large processing plant (Andrade et al. 2014). This could be due to more water being used for cleaning, causing their levels to be diluted. Another reason could be difference in lost product due to a more efficient production. Tank rinse water, the first stage in cleaning, had the highest levels for BOD₅, COD, Conductivity of the samples tested.

One case study found that dairy processing wastewater had a Chemical Oxygen Demand (COD) of 760 mg/L, far lower than the material balance samples. That study also

reported TSS of 600 mg/L, quite lower than the results seen in **Table 1** (Buabeng-Baidoo et al. 2017). Another paper saw that raw wastewater from dairy processing had a COD and BOD₅ level of 1500-3000 and 350-600. They reported the total suspended solids (TSS) as 250-600 mg/L (Hafez et al. 2007) all of which are lower than those found in the cleaning operation material balance. Wastewater quality parameters show great variable from plant to plant. Wastewater variability is related to process efficiency, cleaning operations, volume of lost product, age of equipment, improvements in key technologies, processing and facility size, and plant standard operating procedures.

Table 3: Water Quality Characteristics of Spent Cleaning Solutions

Parameters	Tank Rinse water	Tank Caustic Water	Tank Acidic Water	Tank Sanitizer Water	Truck Compiled Wastewater	Pasteurizer Homogenizer Caustic Water
BOD ₅ (mg/L)	25,110	1,230	51.0	183	4,950	1,230
COD (mg/L)	29,610	-	11.0*	829	20,120	4,310
BOD ₅ /COD	0.85	-	-	0.22	0.25	0.29
Conductivity (uS/cm)	1,170	51.2	46.9	1,020	7.29	45.5
pH	7.46	13.3	1.25	4.27	12.3	13.6
TSS (mg/L)	11,606	19.0	*1.00	*1.00	2,088	924
Total Kjeldahl Nitrogen	28.8	0.48	1.09	0.20	36.6	15.5
Total Kjeldahl Phosphorus	15.5	1.05	166	11.4	73.6	94.7
* Below method detection limit						

The compilation of baseline resource consumption resulted in resource consumption rates for the partnering facility. The water, energy, and wastewater requirements of dairy

plants can show great range in consumption by day and should be evaluated as a rate of consumption. For every gallon of finished milk 0.13 kWh, 0.01 Therms, and 0.87 gallons freshwater are needed. According to (Masanet et al. 2014) the natural gas requirement of pasteurization is 0.0079 Therms/ gal. fluid milk. This finding includes products like yogurt and cottage cheese in the same category as fluid milk. This value in combination with these research findings, seen in **Table 4**, of natural gas (0.01 Therms/gal. finished milk) projects the energy demands of heat treatment. Therefore, pasteurization accounts for 79% of the natural gas requirement. The remaining natural gas is used to heat water for cleaning operations.

Table 4: Fluid milk processing utility consumption for partner facility

Utility	Unit	Amount used
Electricity	kWh/ gal. finished milk	0.13
Natural Gas	Therms/gal. finished milk	0.01
Fresh Water	gal. water /gal. finished milk	0.87
Wastewater	Gal. wastewater/gal. finished milk	0.59

Surveyed data of Nordic dairy processors determined that market milk and cultured products required higher volumes of water 1.0-1.5 gal. water/gal. milk and electricity 0.38-0.76 kwh/gal milk for production compared to the processing of fluid milk alone (**Table 4**). The wastewater generated from market milk and cultured (0.9-1.4 gal. wastewater/gal. milk) exceeded the average wastewater generated through the production of fluid milk (0.59 gal. wastewater/ gal. finished milk) (Lampi 2001).

The partnering plants electric demand (0.13 kWh/ gal. finished milk) fell below the reported U.S. national average for dairy processing (1.08-1.62kWh/gal.) (Xu and Flapper 2009). However it fell within the electrical range, surveyed from 17 U.S. fluid milk-processing plants, of 0.10 kwh/gal. to 3.46 kwh/gal. (Xu and Flapper 2009). The energy consumption at the partnering plant was similar in natural gas consumption but below the reported electrical consumption for fluid segment of the Canadian dairy industry (0.6435 kWh/ gal. and 0.036 Therms/ gal. (National Dairy Council of Canada 1997).

The production of market milk and cultured products in Swedish dairy plants showed higher rates of wastewater production and electrical usage (0.60-4.1 gal. water/ gal. processed milk, 0.26-1.28 kWh/ gal. processed milk, and 0.8-2.5 gal. wastewater /gal. processed milk) (Lampi 2001). The organic load of the Swedish fluid milk wastewater was 600-2200 mg/L wastewater BOD₅ and 1600-3200 mg/L wastewater COD. (Lampi 2001). This range of BOD₅ and COD were below the reported 4,950mg/L BOD₅ and 20,120mg/L COD for the partnering facility.

The findings of the requirements of a fluid milk processing facility fall within the range of freshwater. This is expected, as pasteurization water requirements are high for all milk products. The difference could be associated with variation in quantity and variety of fluid milk products produced.

3.3.Economic Evaluation

The cost assessment of resource consumption is of great interest to producers. It is important to understand how much financial resources are being used on inputs of energy and water. Second, a producer should know plant's standing in comparison to other

producers. Therefore, energy charges were compared across the nation, **Figure 10**. National evaluation can benefit producers, by understanding where they fall in relation to industry wide variation in charges. The U.S. Energy Information Administration (EIA) publishes data on natural gas (EIA 2017) and electrical (EIA 2018) charges for industrial sectors among others. This is useful in understanding where a plant's charges fall in proximity to other industrial users. After looking at the range of charges across the country, states that represented areas of high cost and low cost of energy were chosen to compare to the partnering facility. Unlike other processors, the Midwest dairy plant was charged a rate for energy free of demand charges. The occurrence of demand charges are correlated with the use of electrical current during processing times associated during peak usage. This may be implemented for larger processing facilities, as their production is continuous due to demand for finished product. Additionally, as production of milk increases, so will the need for electricity (Xu and Flapper 2009) and the potential for demand charges and increased electrical rates. While a medium to small dairy plant may not experience demand charges, due to production time and energy needs of processing, it may experience seasonal changes in electrical rates. This was observed in the partnering plant during winter months (October 16th-June 14th) where they experienced a reduction in electrical cost by 2.2 cents/kWh.

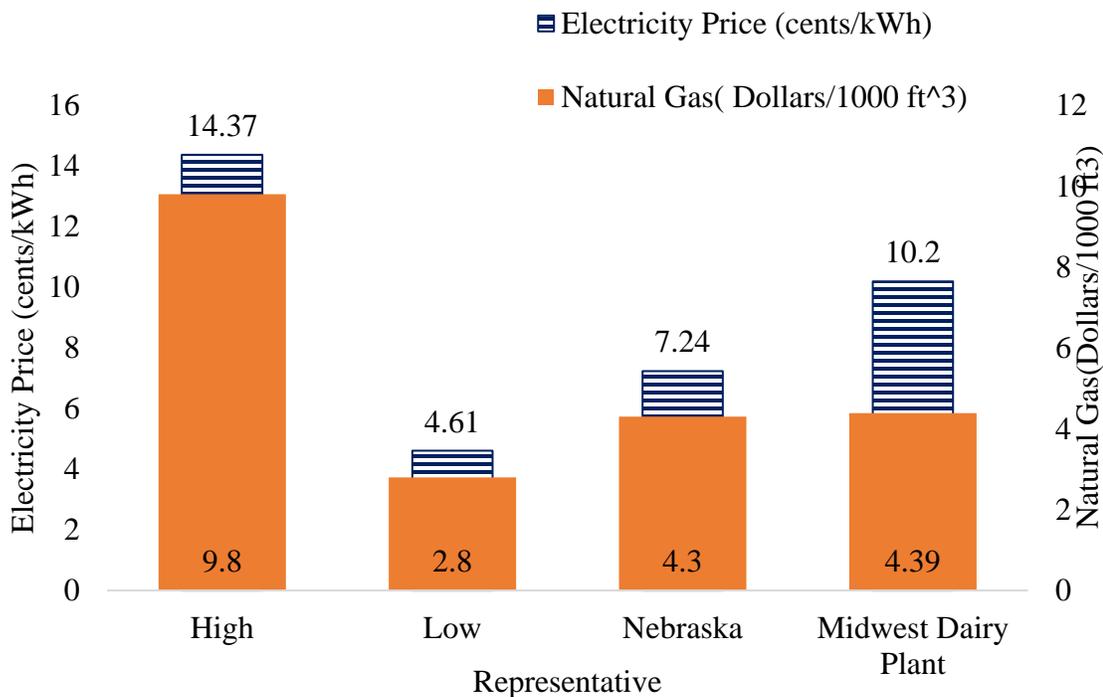


Figure 8: Cost of Energy for Partner Plant vs. Industrial Facilities across the USA

Three states, a high rate, low rate, and a Nebraska rate, were chosen to compare electrical and natural gas rates (**Figure 10**). The Midwest dairy plant, experienced natural gas (\$4.39/1000 ft³) and electrical (10.2¢/ kWh) charges that fell within the high and low representative states. While energy costs may only account for an average of 1-2% of the total cost of operation for overall food production, the economic cost of energy showed continued increases from 1997-2009 (Masanet et al. 2014).

Evaluating the cost of water is twofold: freshwater cost and wastewater charges. As for freshwater, the partnering plant was charged 0.003 \$/gallon freshwater. The partnering milk processor did not send their wastewater to a treatment facility. Instead, the processor independently held and land applied the generated wastewater. To determine which wastewater disposal method is right for a particular food processing plant, the wastewater

quality should be assessed and compared with the local regulations of discharge. The National Pollutant Discharge Elimination System (NPDES) is a permit program created by the EPA for state-to-state discharge regulations into bodies of water (EPA 2018). Based on the electronic Code of Federal Regulations for a milk processing plant receiving 250,000lb/day or less of milk equivalents, maximum daily effluent limitations are set. The pH must be between 6-9 and the BOD₅ and TSS must not exceed 0.450 and 0.675 lb. per 100lbs of BOD₅ input (ElectronicCodeofFederalRegulations(e-CFR) 2018). As dairy industry wastewater is highly loaded due to discharge of lost milk and spent cleaning solutions, pretreatment of wastewater may be necessary before disposal. Dairy processors that have farm land may choose to use their wastewater for irrigation, by lagoon storage, or choose to compost a portion of the wastewater.

An alternative to land application or disposal into bodies of water is sending the effluent to a wastewater treatment facility. A survey conducted across a variety of industrial facilities, found that larger facilities received cheaper rates on wastewater treatment. Additionally surcharges will be applied for high strength wastewater. This survey reported overall wastewater charges ranging from \$0.00123 to \$0.0034 per gallon of discharged wastewater (Industrial Water World 2011). Another survey conducted in the upper Midwest found that the correlating dairy (cheese) processors had a mean wastewater of 45,000-550,000 gallons/ day (Danalewich et al. 1998). Based on the average wastewater charge for industrial companies, \$2.06 (Industrial Water World 2011) a cheese processor would pay \$92.7-\$1,133/day on wastewater treatment charges, depending on processing size. Another case study found that the treatment of dairy effluent would cost French processors \$0.57 - \$2.57/m³ (Fernández et al. 2010). The

range in charges will fluctuate base on product type and production volume. The partnering dairy plant produced on average 77,590 gallons of wastewater each month. If the processor sent wastewater through a treatment plant, they would pay \$167-\$755/month, depending on volume of wastewater and organic load. This number is reflective of milk processing wastewater with an overall range of dairy processing wastewater costs stated above. These numbers does not reflect the cost of transportation. To evaluate what treatment practices is best for specific dairy plants first determine the organic load of the waste stream and the generated volume. Together, these can be used to estimate expected charges for treatment wastewater. The reduction of wastewater could correlate with high savings for producers. If the partnering plant reduced their wastewater volume by 5%, monthly savings could range from \$7.95-\$37.9/month.

The United States produced 23 billion gallons of milk each year (United Dairymen of Idaho 2014). Based on the data for resource consumption per unit of finished milk, found in this research **Table 2**, industry wide consumption rates were estimated. The 2011 milk production of 23 billion gallons, had energy demands of 3 billion kWh and 230 million Therms. Additionally, it used 20 billion gallons of freshwater and produced 13.5 billion gallons of wastewater. These consumption rates can serve as a benchmark for medium sized fluid milk processors. Understanding a milk processor's resource consumption can help to improve the efficiency of production. The role of cost assessment will help processors make process specific changes that will help plants become better stewards to the environment, and may increase the bottom line.

Recommendations for conservation include replacing insulation on water lines and trap steam leaks (National Dairy Council of Canada 1997). Also proper boiler maintenance

can save up to 10% in energy (Brush 2011). A processor should consider installing high efficiency lighting as it could save up to 33% in energy (Anantheswaran et al. 2014). Continued monitoring of resource consumption could help optimize process, through installing permanent monitoring systems for water and energy. Reduce wastewater load through redirecting product leftover away from the drain could also save money from lost product. Install recycling pump on homogenizer could reclaim lost water, as recycling process water can reduce both energy and water consumption. (IFC 2007).

1. Conclusion

To optimize resource consumption, fluid milk processors must first understand the demands of processing, by assessing the baseline consumption of resources. The usage of energy and water should be compared to the utility consumption of 0.13 kWh/ gal. finished milk, 0.01 Therms/ gal. finished milk and 0.87 gal. freshwater/ gal. finished milk. The findings in **Table 2**, to help processors understand where their current consumption falls. However, before any optimization can be done a culture of conservation and awareness must be established. This culture can be implemented through transparency between leadership and operational staff about the social, environmental, and financial benefits of conscious resource consumption. Additionally ongoing training may help with ongoing efforts of operator efficiency and diligence with resource consumption.

Resource consumption of milk production was evaluated based on processing (standardization, pasteurization etc.) and for cleaning (CIP and COP) operations. The cleaning operations attributed to the majority of water consumption and over 20% electricity and. Therefore, the concentration of efforts to optimize resources fell during

the cleaning operation. Future research should be conducted on the plausibility of resource reduction within cleaning operations, as this is a potential operation for water reuse.

List of References

- Alvarez, N., et al. (2007). "The role of surface tension of re-used caustic soda on the cleaning efficiency in dairy plants." International Dairy Journal **17**(4): 403-411.
- Andrade, L., et al. (2014). "Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor." Separation and Purification Technology **126**: 21-29.
- ASTM, E. (2004). "1054-08. Standard test method for evaluation of inactivators of antimicrobial agents." Designation. West Conshohocken, PA: ASTM International.
- ASTM International, (2007). Standard Test Method for Quantification of Pseudomonas Aeruginosa Biofilm Grown with High Shear and Continuous Flow Using CDC Biofilm Reactor, ASTM International.
- ASTM International, (2013). ASTM E2871-13 Standard Test Method for Evaluating Disinfectant Efficacy against Pseudomonas Aeruginosa Biofilm Grown in CDC Biofilm Reactor using Single Tube Method, ASTM International West Conshohocken, PA.
- Bazilian, M., et al. (2011). "Considering the energy, water and food Nexus: Towards an integrated modelling approach." Energy Policy **39**(12): 7896-7906.
- Biggs, E. M., et al. (2015). "Sustainable development and the water–energy–food Nexus: A perspective on livelihoods." Environmental Science & Policy **54**(Supplement C): 389-397.
- Bremer, P. J., et al. (2006). "Laboratory scale Clean-In-Place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms." International journal of food microbiology **106**(3): 254-262.
- Bridier, A., et al. (2011). "Resistance of bacterial biofilms to disinfectants: a review." Biofouling **27**(9): 1017-1032.
- Bridier, A., et al. (2015). "Biofilm-associated persistence of food-borne pathogens." Food microbiology **45**: 167-178.
- Brush, A. (2011). "Energy Efficiency Improvement and Cost Saving Opportunities for the Dairy Processing Industry: An ENERGY STAR? Guide for Energy and Plant Managers."
- Buabeng-Baidoo, E., et al. (2017). "Study of water reuse opportunities in a large-scale milk processing plant through process integration." Chemical Engineering Research and Design **121**: 81-91.
- Bylund, G. (2003). Dairy processing handbook, Tetra Pak Processing Systems AB.
- National Dairy Council of Canada. (1997). "Guide to Energy Efficiency Opportunities in the Dairy Processing Industry."
- Carpentier, B. and O. Cerf (1993). "Biofilms and their consequences, with particular reference to hygiene in the food industry." Journal of applied bacteriology **75**(6): 499-511.

Casani, S., et al. (2005). "A discussion paper on challenges and limitations to water reuse and hygiene in the food industry." Water Research **39**(6): 1134-1146.

CDC, (2016). "Estimates of Foodborne Illness in the United States."

CDC, (2008). "Guideline for Disinfection and Sterilization in Healthcare Facilities".

Chisti, Y. (1999). "Modern systems of plant cleaning." Encyclopedia of food microbiology **3**: 1806-1815.

Cuéllar, A. D. and M. E. Webber (2010). "Wasted Food, Wasted Energy: The Embedded Energy in Food Waste in the United States." Environmental Science & Technology **44**(16): 6464-6469.

Danalewich, J., et al. (1998). "Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal." Water Research **32**(12): 3555-3568.

Daufin, G., et al. (2001). "Recent and emerging applications of membrane processes in the food and dairy industry." Food and Bioproducts Processing **79**(2): 89-102.

Demirel, B., et al. (2005). "Anaerobic treatment of dairy wastewaters: a review." Process Biochemistry **40**(8): 2583-2595.

Dresch, M., et al. (1999). "Membrane processes for the recovery of dairy cleaning-in-place solutions." Le Lait **79**(2): 245-259.

Danalewich, J., et al. (1998). "Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal." Water Research **32**(12): 3555-3568.

e-CFR, (2018). "PART 405—DAIRY PRODUCTS PROCESSING POINT SOURCE CATEGORY."

EPA, (2018). "National Pollutant Discharge Elimination System (NPDES)."

EPA, (2016). "Standard Operating Procedure for Germicidal and Detergent Sanitizing Action of Disinfectants Test SOP Number: MB-27-02."

EPA. (2017). "Nutrient Pollution, The Effects: Environment".

EIA, (2017). "Electric Power Monthly." Retrieved September, 2017, from https://www.eia.gov/electricity/monthly/epm_table_grapher.php?t=epmt_5_6_a.

EIA, (2017). "Natural Gas Prices."

Fernández, P., et al. (2010). "Nanofiltration regeneration of contaminated single-phase detergents used in the dairy industry." Journal of Food Engineering **97**(3): 319-328.

Finley, J. W. and J. N. Seiber (2014). "The Nexus of Food, Energy, and Water." Journal of Agricultural and Food Chemistry **62**(27): 6255-6262.

- Gésan-Guiziou, G., et al. (2002). "Nanofiltration for the recovery of caustic cleaning-in-place solutions: robustness towards large variations of composition." Journal of dairy research **69**(4): 633-643.
- Gibson, H., et al. (1999). "Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms." Journal of applied microbiology **87**(1): 41-48.
- Gulati, M., et al. (2013). "The Water–energy–food Security Nexus: Challenges and Opportunities for Food Security in South Africa." Aquatic Procedia **1**(Supplement C): 150-164.
- HachCompany (2014). "Photometric Method1 Method 8006."
- HACHCompany (2016). "Ascorbic Acid Method Method 10209/10210."
- HachCompany (2018). "Persulfate Digestion Method Method 10208."
- Hafez, A., et al. (2007). "Wastewater treatment and water reuse of food processing industries. Part II: Techno-economic study of a membrane separation technique." Desalination **214**(1-3): 261-272.
- Hamilton, M. (2011). The log reduction (LR) measure of disinfectant efficacy, KSA-SM-07.
- Hoa, B., et al. (2015). "Efficacy of sanitizers on *Listeria*, *Salmonella*, and *Pseudomonas* single and mixed biofilms in a seafood processing environment." Agriculture, Food & Analytical Bacteriology **5**(1): 15-28.
- International Water World, (2011). "SURVEY EXAMINES WASTEWATER TREATMENT COSTS."
- IFC (2007). "Environmental, Health, and Safety Guidelines for Dairy Processing." World Bank Group.
- Koyuncu, I., et al. (2000). "Application of low pressure nanofiltration membranes for the recovery and reuse of dairy industry effluents." Water Science and Technology **41**(1): 213-221.
- Kushwaha, J. P., et al. (2011). "An overview of various technologies for the treatment of dairy wastewaters." Crit Rev Food Sci Nutr **51**(5): 442-452.
- Lampi, M. (2001). Best available techniques (BAT) for the Nordic dairy industry, Nordic Council of Ministers.
- Luo, J., et al. (2011). "A two-stage ultrafiltration and nanofiltration process for recycling dairy wastewater." Bioresour Technol **102**(16): 7437-7442.
- Luo, J., et al. (2012). "Treatment of dairy effluent by shear-enhanced membrane filtration: The role of foulants." Separation and Purification Technology **96**(Supplement C): 194-203.

Marie Furic, A. E., Walid Blel and Olivier Sire (2015). "Regeneration of Lyes for Cleaning-In-Place (CIP) Processes in Dairies: Comparison of Recycled Potash and Soda Solutions Cleaning Properties " American Journal of Applied Sciences.

Masanet, E., et al. (2014). "Energy efficiency opportunities in the US Dairy processing industry." Energy Engineering **111**(5): 7-34.

Meneses, Y. E. and R. A. Flores (2016). "Feasibility, safety, and economic implications of whey-recovered water in cleaning-in-place systems: A case study on water conservation for the dairy industry." J Dairy Sci **99**(5): 3396-3407.

Meneses, Y. E., et al. (2017). "Water reconditioning and reuse in the food processing industry: Current situation and challenges." Trends in Food Science & Technology.

Milmoe, N. A. P. H. "The Food Processing Industry at a Glance."

Mosteller, T. M. and J. R. BISHOP (1993). "Sanitizer Efficacy Against Attached Bacteria in a Milk Biofilm." Journal of food protection **56**(1): 34-41.

Munavalli, G. R. and P. S. Saler (2009). "Treatment of dairy wastewater by water hyacinth." Water Sci Technol **59**(4): 713-722.

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Owen, G., et al. (1995). "Economic assessment of membrane processes for water and waste water treatment." Journal of membrane science **102**: 77-91.

Özbay, A. and G. N. Demirer (2007). "Cleaner production opportunity assessment for a milk processing facility." Journal of Environmental Management **84**(4): 484-493.

Painter, J. A., et al. (2013). "Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008." Emerging infectious diseases **19**(3): 407.

Pouliot, Y. (2008). "Membrane processes in dairy technology—From a simple idea to worldwide panacea." International Dairy Journal **18**(7): 735-740.

Powell, K. D. R. G. M. (1997). "Dairy Processing Methods to Reduce Water Use and Liquid Waste Load." K-State Research and Extension(MF-2071).

Ramirez, C., et al. (2006). "From fluid milk to milk powder: Energy use and energy efficiency in the European dairy industry." Energy **31**(12): 1984-2004.

Ryther, R. (2014). Chapter 27 - Development of a Comprehensive Cleaning and Sanitizing Program for Food Production Facilities. Food Safety Management. Y. Motarjemi and H. Lelieveld. San Diego, Academic Press: 741-768.

Sarkar, B., et al. (2006). "Wastewater treatment in dairy industries — possibility of reuse." Desalination **195**(1): 141-152.

Scanlon, B. R., et al. (2017). "The food-energy-water Nexus: Transforming science for society." Water Resources Research **53**(5): 3550-3556.

Sharma, M. and S. K. Anand (2002). "Biofilms evaluation as an essential component of HACCP for food/dairy processing industry – a case." Food control **13**(6): 469-477.

Sun, A., et al. (2012). Energy Use in Wisconsin's Dairy Industry and Options for Improved Energy Efficiency.

Trägårdh, G. and D. Johansson (1998). "Purification of alkaline cleaning solutions from the dairy industry using membrane separation technology." Desalination **119**(1-3): 21-29.

USDA (2017). Milk Production. USDA.

USGS (2016). "The World's Water."

UW Soil and Forage Lab, (2009). "Total Solids (Dry Matter Content) , Standard Method 2540c."

United Dairymen of Idaho, (2014). "U.S. Dairy Industry." Where Good Comes From.

Uzi MERINA, G. G.-G., Evelyne BOYAVALb, Georges DAUFINb (2002). "Cleaning-in-place in the dairy industry: criteria for reuse of caustic (NaOH) solutions." INRA, EDP Sciences.

Vourch, M., et al. (2008). "Treatment of dairy industry wastewater by reverse osmosis for water reuse." Desalination **219**(1): 190-202.

WHO (2017). Drinking - water, World Health Organization.

Wirtanen, G., et al. (2001). "Microbiological methods for testing disinfectant efficiency on Pseudomonas biofilm." Colloids and Surfaces B: Biointerfaces **20**(1): 37-50.

Xu, T. and J. Flapper (2009). "Energy use and implications for efficiency strategies in global fluid-milk processing industry." Energy Policy **37**(12): 5334-5341.

Chapter 4: DETERMINATION OF PLAUSIBILITY OF SPENT CAUSTIC SOLUTION REUSE GENERATED FROM A MILK PROCESSING FACILITY

Introduction

Demands for high quality and safe water seen throughout the entire path of food production, from farm, through the processing line, and to the consumer. In the food processing facility, water is used as an agent for processing and cleaning, and in some cases is incorporated into finished products. The food processing industry is a large consumer of water, the Netherlands food industry accounted for 247.46 million m³ total water consumption (Casani et al. 2005). The versatility of water in the food processing industry makes this industry a great consumer of water. Water utilization within the food industry varies greatly by more than just sector. Even within the dairy industry, the consumption of water showed a wide range of water consumption from 0.2-11.0 L/ L milk (Gésan-Guiziou et al. 2002). The reuse of spent water may reduce the amount of freshwater consumed and volume of wastewater generated each year.

Off farm (secondary) processing of fluid milk is broken into two operations: processing and cleaning. Processing includes the separation, standardization, homogenization, heat treatment, and bottling of milk. Together, these operations demand resource inputs of energy and water to ensure product quality and safety. The connection of resources with the processing of food commodities is often referred to as the Food Energy and Water Nexus. The first phase of this project, Chapter 2, conducted at a medium sized fluid milk processing facility, revealed that cleaning operations accounted for 53% of the total water consumed during a production day. Percentage of water used for cleaning can reach as high as 95% (Gésan-Guiziou et al. 2002). The authors determined that an operation

(cleaning vs. processing) consuming the majority of water would prove to be worth exploring water reuse. The operation consuming the majority of water was the cleaning operation. Literature shows that cleaning not only is a large consumer of water but also generates large amount of pollution (Fernández et al. 2010). During these operations, plants operate both Cleaning in Place (CIP) and Cleaning out of Place (COP) practices. CIP operations, created for dairy processing, is an automated closed system operation. These operations eliminates the requirement for disassembly of processing machines and reduces the risk of error and cross contamination.

Throughout the processing of milk, milk deposits are left behind on the surface of the holding tanks and production lines. The first step in the CIP process is a preliminary rinse. This step removes any loose product residue and rejected milk. Through a water mass balance, conducted during preliminary phase of this project, it was determined that caustic cleaning, the second step in the CIP process, accounted for 22.75% of the total water used during overall cleaning operations. Caustic cleaning is responsible for removal of both fat and carbohydrate deposits (Chisti 1999). This alkaline solution is composed of Sodium Hydroxide (NaOH) or a propriety commercial cleaning solution. Commercial caustic solutions contain added surfactants to assist in the removal of fat deposits. The third step in a CIP process is the acid rinse. This step helps remove milk stone and mineral deposits that remain after the caustic rinse. The acid rinse can assist in removing any remaining residue from the previous alkaline stage (Chisti 1999). The final step in a CIP system calls for a sanitizer to be flushed into the system. This solution should have a bactericidal effect, meaning its role in cleaning is to avoid contamination by killing bacteria (CDC 2008).

As the caustic solution made up the largest portion of cleaning water, it became the focus for potential reuse. Unlike the partnering milk processor, some facilities may already choose to reuse caustic solution. However, it is without confidence that a static number of reuse cycles have been set. Currently, the level of reuse is dependent, not on quantitative standards but, on qualitative observations. The continued use of caustic solution is determined by the operator, who base their decision to reuse or discard cleaning solution based on color and odor (Alvarez et al. 2007) or until considered too polluted (Gésan-Guiziu et al. 2002).

The effluent generated in dairy processing contains high levels of organic material, 1500-3000 mg/L COD, 250-600 mg/L TSS (Sarkar et al. 2006). These high pollution levels, which showed great variability: 405-1082 mg/L SS, 709-2297 mg/L BOD₅, and 36-78 mg/L total phosphorus (Danalewich, Papagiannis et al. 1998), are due to loss product as well as spent chemicals used in cleaning operations (Özbay and Demirer 2007). These pollutant levels categorize this sector as one of the heaviest polluting sectors of the food industry (Andrade et al. 2014). One study (Alvarez et al. 2007) evaluated reuse of laboratory composed contaminated caustic solution with or without suspended solids against fresh NaOH solution. Fouled membranes were used to evaluate efficacy and cleanliness, regarding hydraulic resistance, of the solutions. They found that both contaminated and new NaOH solution resulted in similar efficiency of cleanliness, and that the presence of suspended solids did not reduce the efficiency. Research evaluating the use of reuse caustic solutions look at their mechanical action, but fail to address their microbial effectiveness. Each year 9.4 million illnesses related to major food borne pathogenic agents occur (CDC 2016). Dairy commodities account for 18% of the

estimated annual bacterial foodborne illnesses and 14% of the deaths associated with bacterial foodborne illnesses in the USA (Painter et al. 2013). Biofilms are the major cause for dairy product contamination (Bremer et al. 2006). If cleaning is not proficient, microorganisms in the form of biofilms may grow, some of which become permissive to sanitizers (Hoa et al. 2015). Biofilms develop a strong matrix of cells that can protect the organism from stress (Bridier et al. 2015). This strengthening of microorganisms makes it more difficult to detach from processing equipment. These biofilms tend to be resistant to the action of the disinfection (Bridier et al. 2011), one of the stages in automated CIP cleaning. The increase in tolerance to sanitizers of biofilms is accredited to the formation of a matrix of extracellular polymeric substances (Bridier et al. 2011). Processors must find validity in the successful cleaning with the use of reused solutions before they can be implemented in a processing facility. Product integrity and safety must be upheld.

Dresch et al. (1999) stated that benefits of recovering cleaning solution are resource savings and maintained cleaning efficiency. Water reuse has the potential to offset some of the pressure that a growing population places on the world's food and water supply. Efforts on behalf of the food industry to reuse water may lead to a 20-50% reduction in the volume of water consumed (Casani et al. 2005). However, this broad range is not specific to one sector of the food industry. The recovery of spent water through treatment, either by physical or chemical methods, has been explored (Trägårdh and Johansson 1998, Gésan-Guiziou et al. 2002, Uzi Merina 2002, Fernández et al. 2010, Marie Furic 2015). Depending on the level of contamination in process effluent, dairy wastewater treatment can be performed by both aerobic and anaerobic methods (Demirel et al. 2005). The combined effect of anaerobic lagoons and aeration in lagoons reduced the COD from

3436-112 mg/L (O. Monroy H . F. Vhquez M. n.d.). Membrane filtration has also been used to treat waste effluent from dairy processing. Nanofiltration membranes were over 98% efficient at removing COD (Koyuncu et al. 2000). Similar removal efficiencies were seen when membrane bioreactors followed by nanofiltration were implemented (Andrade et al. 2014). Additional studies used a combination of membranes, including ultrafiltration and nanofiltration (Luo et al. 2011), to separate nutrients for bioenergy processing. Treatment of dairy wastewater by reverse osmosis (Vourch et al. 2008) could be implemented for water purification and recycle. Some researchers even looked at the use of aquatic treatment systems to remove organic loads from dairy wastewater (Munavalli and Saler 2009). However, this treatment option is not suitable for large scale processing. Mechanical mechanisms, filtration have been explored for the specific reuse of cleaning solution (Dresch et al. 1999) (Trägårdh and Johansson 1998, Koyuncu et al. 2000, Gésan-Guiziu et al. 2002). Coagulation and flocculation, chemical treatment methods, have been employed as pretreatments for dairy wastewater (Luo, Cao et al. 2012). The regeneration or treatment of cleaning solutions involve both capital and operational costs. These economic and potential hazardous impact of these treatments are not incentivizing for producers to implement reuse strategies. While guidelines surround water reuse, this topic has been a mostly unexplored area in food production. This could be due to concerns with potential consequences on hygiene standards (Casani et al. 2005). The safe processing of food requires high-volume high-quality water. It is important that the reuse of spent water does not pose a health risk to consumers by compromising product safety. This paper looks to see if the microbial impact of water reuse can be used as an indicator of reuse efficiency against *P.aeruginosa* biofilm.

Without exploring the effectiveness of reused caustic solution, the reuse of spent cleaning solution may be met with confrontation and fear surrounding the safety of its reuse. The effectiveness of reused caustic solution and their potential to maintain cleaning efficiency without a recovery/treatment step still needs exploration. Therefore, the goal of this research is to assess the impact in the microbial reduction of reusing spent caustic solutions as part of a CIP operation. The first objective of this research is to explore the potential for reusing isolated spent caustic solution at removing *P. aeruginosa* biofilm. The second objective of this project is to evaluate physical-chemical characteristics of reuse solutions, to be used as an additional indicator to their effectiveness. It is the hope that microbial and physical- chemical analyses of samples can assist plant operators in making informed decisions and conclusions to the plausibility and limit of caustic solution reuse.

1. Materials and Methods

2.1 Biofilm formation in bioreactor

A standard Center for Disease Control (CDC) bioreactor chosen to grow a reproducible *Pseudomonas aeruginosa* biofilm. The CBR 90 Biofilm Reactor (BioSurface Technologies Corp., USA) was designed to hold eight polypropylene rods, each with three coupons, totaling 24 coupon samples. This biofilm, a community of cells, mimics one that would develop as a result of high shear stress (International 2007). ASTM standard method E2562-17 was followed for culture preparation, reactor assembly, and operational procedure (steps 10.1- 10.2.4). An overnight culture was prepared by growing *P. aeruginosa* in 100 mL of Tryptic Soy Broth (TSB) (300 mg/L) for 24 hours at 37 °C. Batch mode time started when the bioreactor, containing 350 mL TSB (300mg/L), was

inoculated with 1 mL of the overnight culture. After the first 24-hour cycle, the reactor was transitioned to continuous mode by connecting tubing to a 20 L carboy containing a continuous stream of nutrients, 100 mg/ L TSB. A continuous flow rate of 11.7 mL/min was held for a 24 hour run time. To represent shear stress, the reactor stir bar was held at 120 r/min for the 48-hour growth cycle. The bioreactor was held at 20 °C for the duration of the growth cycle. After inoculation of the bioreactor, the viable bacterial density of the inoculum was confirmed by serial dilution and spread plating.

2.2 Experimental Cleaning Solutions

Caustic cleaning solutions were composed to assess the effect that reuse has on the cleaning efficiency of the spent solution. These solutions were created to replicate the solutions used in CIP operations that occur at a medium sized fluid milk processing facility. The general steps for CIP operation are; initial water rinse, caustic solution rinse, acid solution rinse, and sanitization rinse. Some plants will include an additional water rinse before the acid solution rinse. However, this step is not always implemented, as was the case at the partner plant. Therefore, rinse step was not included in the standard CIP system. Spent caustic solution from the partnering plant had three components; concentrated caustic solution, water, and loss milk. The concentration of caustic solution was determined by plant operations and by supplier recommended dilution. Each experimental solution was composed of one portion of 2% caustic solution and one portion of pasteurized 2% reduced fat milk. The next step was to determine how much milk was present at varying levels of spent caustic solutions. To do this, a water balance of the cleaning operations was conducted on the processing floor. The procedure for the cleaning operations water balance can be found in Chapter 2. The milk component of

these samples was determined based off the biochemical oxygen demand (BOD₅) of spent CIP solutions taken during the material balance. Each pound of BOD₅ in spent milk processing wastewater correlates to nine pounds of milk lost (Powell 1997).

Equation 1 was used to quantify product loss in cleaning in place systems.

$$CIP\ Milk\ Loss\ (lbs.) = \left(\left(BOD5 \frac{lb}{L} \right) * (9) \right) x\ volume\ spent\ CIP\ water\ (L) \quad (1)$$

Calculations revealed that the partnering milk processor had 1.07% of the spent caustic solution was milk loss. Additionally, oral communication with chemical supplier and literature reference to 1-2% milk loss (Munavalli and Saler 2009, Marie Furic 2015) helped to validate the finding. Therefore, replicated spent caustic solutions would contain 1% milk to denote the component of loss product in the caustic solution rinse and to represent the COD that would be found after each CIP operation was conducted. Email correspondents with an industrial chemical supplier (Ecolab, personal communication October 5, 2017) noted that the reuse of CIP caustic solution experienced a 5% loss in spent solution volume per cycle. This percentage of volume lost may be due to evaporation, leaks in connection pipes, or operator awareness. Assuming a 5% loss in volume, after 20 cycles of recovery and reuse the original spent caustic solution would be gone. The samples used to evaluate the plausibility of water reuse were chosen in increments of five reuse cycles from zero reuse cycles (fresh caustic solution) to 20 reuse cycles. To create the reused caustic samples, newly prepared 2% NaOH solution was heated to 60 °C. Fluid milk component was aseptically pipetted into solution and held at 60 °C for a contact time of five minutes. Caustic samples were characterized after this contact time. **Table 5** summarizes the composition of the different caustic solutions used

in this experiment. From here out “N” denotes the number of reuse cycles associated with the caustic solution. Sample volume was 100 mL.

Table 5: Composition of Replicate Reused Caustic Solutions

N° of cycles	Fluid milk :2% NaOH	Dry milk :2% NaOH
	Solution (v/v)	Solution (w/w)
0	0:100	-
5	5:95	-
10	10:90	-
15	15:85	-
20	20:80	-
50	-	19:81

To evaluate an extreme level of potential caustic solution reuse, a 50 N solution was developed. This sample was created by extrapolating percentage of dry matter from the 10 N solution. Standard Method 2540C (Lab 2009) was followed. After drying samples at 105 °C for 16 hours, the percentage dry matter was determined and multiplied five times.

2.3 Physio- chemical analyses

Samples of the experimental caustic solutions were analyzed for different water quality parameters including Chemical Oxygen Demand (COD), Total Phosphate (TP P_04^{3-}), Total Nitrogen (TN), pH, and turbidity. Representative samples were taken at room temperature (20°C). TNTplus vials (HACH, USA) were used with a DRB200 Digital reactor (HACH, USA). U.S. EPA Reactor Digestion Method 8000 was followed for

COD analysis. Each sample was added to TNT 822 plus COD vials and heated for 2 hours at 150 °C. Persulfate Digestion Method 10208 (Hach Company 2018) with TNT826 nitrogen vials were used to determine TN. Total Phosphate was determined following Ascorbic Acid method 10209/10210 (HACH Company 2016) using TNT 845 vials. The HACH DR 3900 Spectrophotometer was used to measure Total Suspended Solids (TSS) followed the Photometric Method 8006 (Hach Company 2014). Sample pH was measured using a Fisher Science Education pH meter (Thermo Fisher Scientific, USA). The turbidity was measured at an absorbance of 600 using a DU 730 UV/Vis Scanning Spectrophotometer (Beckman Coulter Inc., USA). The surface tension of each sample was determined using a pendant drop method. This method used a drop shape analyzer (dsa25e, KRUSS, Germany) to measure the surface tension between each solution and the surrounding air over 5 minutes. This time was chosen as to represent the experimental contact time. In addition to the experimental caustic solutions 0, 5, 10, 15, 20, and 50N solutions, two more samples were analyzed. First, an alternative 2% fresh caustic solution (0_{alt}) was made from a commercial caustic solution (Ac-101, Ecolab, USA). The second solution was made to compare the potential impacts of treatment on physio-chemical characteristics. Therefore, 20 N solution was filtered using Amicon Ultra-15, a centrifugal ultrafiltration regenerated cellulose membrane (Milipore Sigma, USA). This solution is referred to as 20_{filt} .

2.4 Cleaning efficacy of reused caustic solutions

The efficacy of reusing caustic solutions was tested against the removal and reduction of *Pseudomonas aeruginosa* biofilms formed in the stainless steel coupons in the bioreactor. At the end of the 48 hours the bioreactor stopped as the biofilm was ready for analysis.

The bioreactor was disassembled and each rod was removed and dipped into buffered water to remove any planktonic cells. Each coupon was unscrewed and released into a sterile 50mL conical tube. Once all coupons were removed three samples were selected at random for each treatment. Standard method ASTM E2871-13 steps 9.1-9.7.4 (International 2013) was followed for sample removal and efficacy evaluation. This method is referred to as a single tube method, used to evaluate efficacy in a closed system and to eliminate potential for lost cells. For the evaluation of effect of reused caustic solutions in the cleaning efficiency, the CIP cycle treatment procedures below were followed.

Treatment Control: 4 mL dilution water

Treatment 1: 4 mL caustic, neutralize

Treatment 2: 4 mL caustic, drain, 4 mL acid, neutralize

Treatment 3: 4 mL caustic, drain, 4 mL acid, drain, 4 mL sanitizer, neutralize

Treatment 4: 4 mL caustic, drain, 4 mL H₂O, drain, 4 mL acid, neutralize

Treatment 5: 4 mL caustic, drain, 4 mL H₂O, drain, 4 mL acid, drain, 4 mL H₂O, drain, 4 mL sanitizer, neutralize

Each CIP replicated solution came into contact with the exposed biofilm covered coupon for a specific contact time (T=5 min.). After the contact time solutions were neutralized or decanted out of the conical tube and the next solution was added. Control samples, Treatment 0, were treated with 60 °C buffered water, to mimic hose cleaning inside holding tanks. Treatment 4 & 5 used nanopure water for the intermediate rinsing steps.

All the treatments (Trt control -Trt5) were applied to the contaminated coupons using reuse caustic solution at the different reuse cycles described in **Table 5**. The caustic solution was the only reused solution, the rest of the cleaning solutions used in the CIP were prepared fresh for each run. A commercial acid solution (ACID 300, Ecolab, USA) as well as a commercial sanitizer (Vortexx, Ecolab, USA) were used. The biofilm was removed from the coupon through a process of alternating vortex and sonication, as indicated in the standard method (International 2013). Each sample was quantified using serial dilution and spread plating on BD Difco R2A Agar (Thermo Fisher Scientific, USA). Samples were incubated at 37° C for 24 hours.

2.4.1 Neutralization of cleaning solutions

The appropriate volume of neutralizer was determined through preliminary studies. The evaluation of Dey Engley Neutralizing Broth as an effective inactivator of antimicrobial agents (CIP treatment solutions) was conducted. To determine if this broth neutralized the effect of the cleaning solution and to see its' toxicity 8.1-8.4.7 Test A and B (ASTM 2004) were evaluated. There was a 5 minute contact time. To confirm proper neutralization Test A and B, modified for efficacy volume, were repeated. The product (CIP treatment solution) volume was increased to 4 mL and the neutralizer 36mL L Dey Engley Neutralizing broth. Each sample was inoculated with 0.1mL 10^6 *P. aeruginosa*. Serial dilution and spread plates, BD Difco R2A Agar (Thermo Fisher Scientific, USA), was used to see the effect of the neutralization on the growth of *P. aeruginosa*. The neutralizing solution was considered successful if there was colony growth observed in neutralized samples and no growth when Test B was done. Dey Engley Neutralizing Broth did not successfully stop the caustic solution. Therefore, 4 mL reuse caustic

solution were neutralized by reverse titration and inoculated with 0.1 mL 10^6 *P. aeruginosa*, serial diluted and grown for 24 hours at 37 °C to confirm growth. Each sample used to evaluate the reused caustic solution (treatment 1) was neutralized by titration with 0.1N HCL, 10 uL phenolphthalein indicator. Samples subject to both 0.2% acid solution and 0.6% sanitizer (treatments 2-5) were neutralized using a consistent 36mL Dey- Engley Neutralizing Broth (Sigma-Aldrich, USA). The pH of each sample was measured to confirm proper neutralization of treatment solution.

2.5 Statistical Analysis

To evaluate the efficacy of reused caustic solutions against *P. aeruginosa* biofilms samples were tested in triplicate and the entire experiment was repeated three times. Plate counts obtained from spread plating were used to calculate the \log_{10} (cfu/coupon). This \log_{10} density is done to indicate the reduction of viable cells (International 2013) by each treatment. The experimental design included a split plot analysis with reuse solution as whole plot and treatment as split plot. A completely randomized ANOVA was conducted to statistically determine significant differences among treatments. Coupons resulting in $<10^0$ cfu/mL (lower than detection level) were assigned 0.5 \log_{10} cfu/coupon as recommended in. Zero log is undefined making it inadequate for efficacy evaluation, and should be replaced by 0.5 for log reduction evaluation (Hamilton 2011). This method of replacing the lower than detection limit results has been show before (Hoa et al. 2015). Significance level was $P < 0.05$.

3. Results and Discussion

3.1 Physio-Chemical Analyses

The characterization of experimental caustic solution with increased reuse cycles is represented in **Table 6**. Visual observations noted that increasing the number of reuse cycles of experimental caustic solution resulted in darkening of solutions, caused by mailard browning (Marie Furic 2015). The pH remained high for all experimental caustic solutions ranging from 12.48-12.96. It is important to maintain alkalinity, as its role in cleaning is saponification, or the conversion of fats into soaps (Ryther 2014). An increase in reuse cycles was directly related to the increase in Total Nitrogen (301-6490mg/L TN), Total Phosphate (142-11727 mg/L PO₄³⁻), Chemical Oxygen Demand (33-208600 mg/L COD), and Turbidity (0.001-3.135). The amount of pollutants that can be oxidized increased with increase in reuse cycles as seen here. The increase in turbidity, correlated with increase in organic matter and TSS, was also noted in literature (Marie Furic 2015). This increase in wastewater load is important as treatment ability, discharge regulations depend on it, and can result in excess surcharges if too polluted. Wastewater heavily polluted with nitrogen and phosphorus can result in negative impacts on the environment. Adverse impacts of nutrient heavy wastewater are hypoxia, acid rain, and air pollution. (EPA 2017). According to the Code of Federal Regulations (CFR) subpart B, the maximum effluent limitation for a plant receiving 250,000 lb/day of milk equivalent for a given day are pH =6.0 -9.0 , TSS of 0.675 lbs/100 lbs of BOD₅ input (e-CFR 2018). Each plants effluent limits can be calculated based on product volume and the knowledge of whole fluid milk BOD₅ of 120,000 mg/L (Bylund 2003). All experimental caustic

reuse solutions exceed the discharge limits for Nitrogen (< 25 mg/L TN) and phosphorus (0.3-0.5 mg/L TP) (Bylund 2003).

The increase in reuse cycles showed to be inversely related to surface tension (**Table 6**). This trend is also seen in caustic solutions reused over the course of one week at a processing plant (Uzi Merina 2002), as well as when caustic solutions were regenerated through filtration (Marie Furic 2015). The results of physico chemical analysis showed the surface tension decreased with reuse cycle from fresh experimental caustic solution, 0 N (40.37mN/m) to 50 N (30.15mN/m). The result of TSS and COD caused a reduction in surface tension as a result of fat and protein hydrolysis by NaOH (Alvarez et al. 2007). Other studies showed a stabilizing surface tension around 30mN/m (Gésan-Guiziou et al. 2002, Uzi Merina 2002). The stabilization trend was seen when evaluating spent caustic solutions from a plant that reused solution up to 400 cycles. They reported the caustic solutions used 10 times to have a higher surface tension (47 mN/m), and lower COD (900 mg/L) and TN (20mg/L) (Gésan-Guiziou et al. 2002) compared to the characteristics of 10 N solution that had a surface tension of 33.04 mN/m, 18125 mg/L COD, and 557 mg/L TN.

Unlike the COD of the experimental caustic solution, which continuously increased 33-208600mg/L), the cleaning solutions found in literature reused for 280 and 410 cycles over one week of reuse had the same COD and did not observe a clear trend. Variation between physico chemical analysis of 10N solution used here and the one found in that study could be due to the definition of a cleaning cycle and the composition of the caustic solution. Solutions reused over 20 times would ignore the 5% volume loss after each run. Additionally, the partnering dairy plant ran cleaning at the end of each processing day (4

production days/week) whereas ,that study found that one week correlated to over 400 cycles of cleaning (Gésan-Guiziou et al. 2002). Another study prepared a model caustic solution with a COD of 1650mg/L to mimic a used caustic solution from the dairy industry (Trägårdh and Johansson 1998). This value, if extrapolated for 5 reuse solutions, 8100 mg/l COD was similar to the 5N caustic solution (7538 mg/L COD). The experimental caustic solution, fresh 2% NaOH solution had the greatest surface tension of the caustic solutions. Fernandez et al. also found that the surface tension of a 2% fresh single-phase detergent was 40mN/m compared to 40.37mN/m for the fresh experimental caustic solution (Fernández et al. 2010). The degradation of milk components act in place of surfactants, which explains the reduction in observed surface tension (**Table 2**), correlating to more degradation and more surfactant properties of the solution.

Table 6 : Composition and Physio-Chemical Properties of Experimental Caustic Solutions

N° of cycles	pH	COD (mg/L)	TSS (mg/L)	Turbidity @ A 600	Surface Tension (mN/m)	TN (mg/L)	TP (mg/L PO ₄ ³⁻)
0 _{Alt}	12.48	305	4	0.002	32.07	0 ^B	0 ^C
0	12.80	33	0	0.001	40.37	0 ^B	0 ^C
5	12.95	7538	2617	1.761	34.42	301	142
10	12.96	18125	4977	2.168	33.04	557	292
15	12.96	24381	8917	2.399	31.36	852	978
20	12.95	31894	11117	2.532	31.21	1290	1115
20 _{filt}	-	13467	20	0.005	65.59	138	145
50	12.55	208600	3960	3.135	30.15	6490	11727

Alt commercial caustic solution

Filt Filtered 20N solution

^B under range (1-16 mg/L N)

^C under range (6-60 mg/L PO₄³⁻)

*COD= Chemical Oxygen demand, TSS: Total Suspended Solids, TN: Total Nitrogen, TP: Total reactive phosphorus (phosphate)

After filtration all the values of physio-chemical parameters decreased, except surface tension, due to the removal of TSS in the permeate. Treatment of the 20N solution (20_{filt}) caused a 57.77% reduction in COD and increased the surface tension by 52.41% (**Figure 11**). Lower surface tension is associated with better cleaning action. Alvarez et al. compared contaminated caustic solutions with and without suspended solids. The clarification of the contaminated caustic solution showed a 14.33% increase in surface tension (Alvarez et al. 2007). They found that NaOH solutions would result in similar

efficiency compared to commercial solutions, if the NaOH solution had both low TSS and surface tension. However, efficiency testing was based on membrane fouling, as opposed to testing on stainless steel surfaces, as would be found on the processing floor.

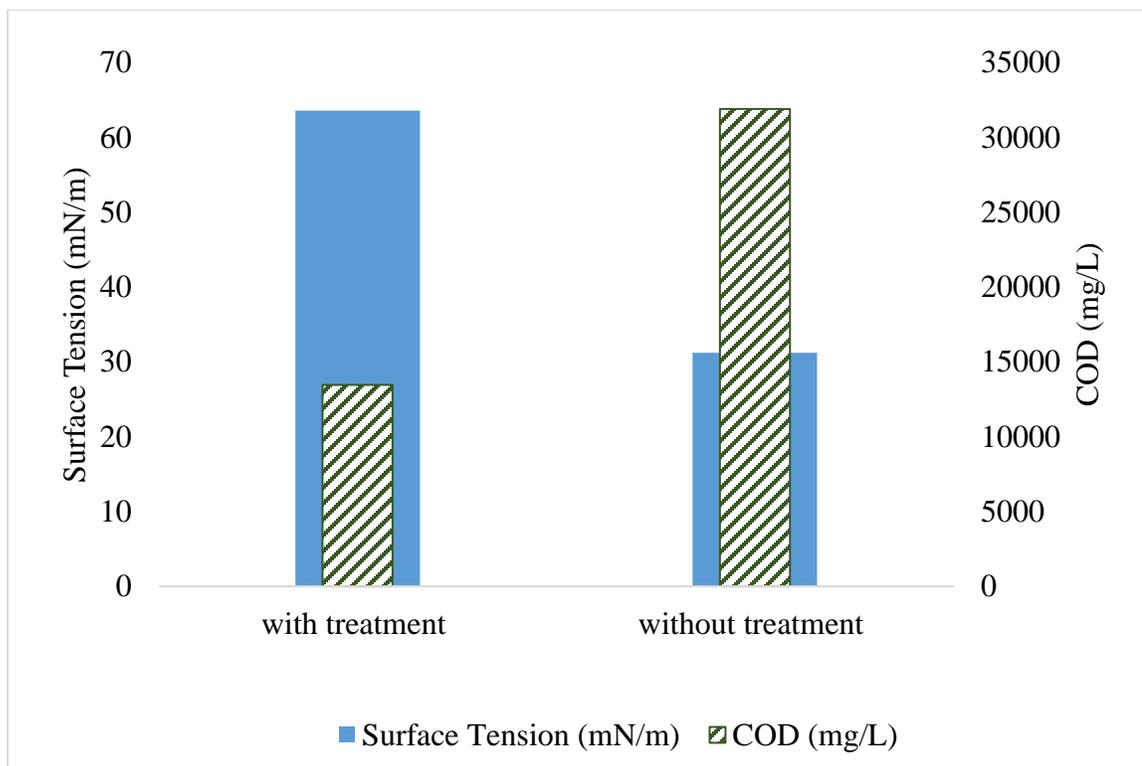


Figure 9: Comparison of Potential Filtration on Characterization of 20N Caustic Reuse Sample

A survey characterizing the waste stream among eight plants showed great variability not only between plants but also at each location (650-2297 mg/L BOD₅, 405-1082 mg/l TSS) (Danalewich et al. 1998). Therefore, it is important to understand the parameters of the waste streams, specifically CIP streams in order to determine their ability for reuse. The next step was to evaluate how these parameters related to their role in CIP operations.

3.2 Cleaning Efficacy of caustic solution

The efficacy of reusing caustic solution for microbial removal of *P.aeruginosa* biofilm was evaluated both individually and in combination of complete CIP operations (**Figure 12**). Efficacy was evaluated based on the change in bacterial density of *P.aeruginosa*. When Treatment 1 was evaluated, experimental caustic reuse solutions representing 10 and 50 cycles were significantly different from 5N (**Figure 12**). The remaining solutions 0, 15, and 20 were not significantly different from the other caustic solutions used in Treatment 1. Treatment 2 evaluated the combined impact of the reuse solution followed by the commercial acid solution. In this treatment the significant difference in efficacy was between both fresh (0 N) solution and 5N or 10N solution. Treatment 3, intended to evaluate the role of a reused caustic solution as part of a complete CIP system, and showed no significant difference between reuse solutions 0-50N. In addition to the CIP operations observed at a processing facility, the impact of additional wash steps was evaluated. In the samples treated under treatment 4, none of the experimental caustic reuse solutions were significantly different from the other. When coupons were exposed to treatment 5, with the addition of 2 rinse cycles, 50N was significantly different from 10N.

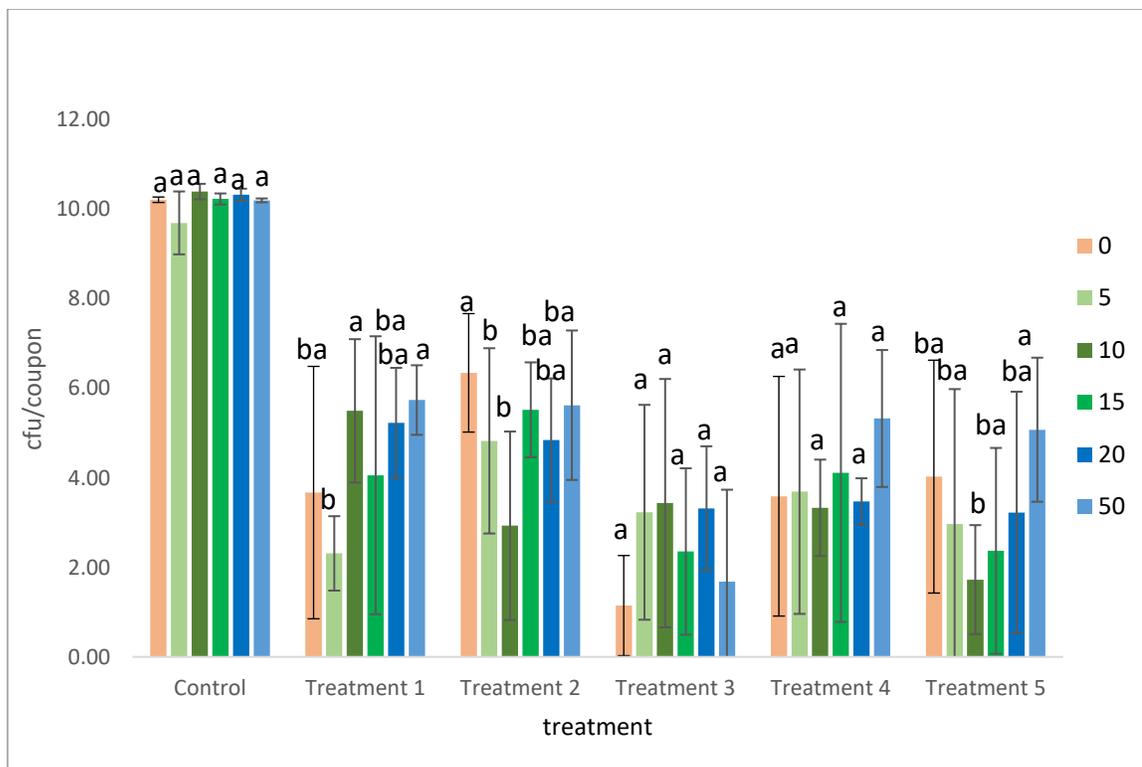


Figure 10: Biofilm Density of *P.aeruginosa* Biofilm after Efficacy Treatment

*Letters denote significance. Shared letters indicate results were not significantly different. Significance level was $P < 0.05$

In order to see the impact of incorporating a rinsing step into the CIP operations, the log reduction of treatment 2 was compared against 4, as well as treatments 3 against treatment 5. No reuse caustic solution showed significant difference when an additional rinsing step was incorporated after the caustic rinse (Treatment 4). This trend was also observed when a rinsing step was added after both the caustic and acid rinsing steps for 0, 5, 10, 15, and 20 cycles of reuse solutions (**Figure 12**). However, with the 50 N solution, the current CIP operations (Treatment 3) there was significant difference, 8.52 log reductions, compared to the addition of two rinse (Treatment 5) which achieved a log reduction of 5.12. For this solution, the water inhibited further reduction in *P.aeruginosa*.

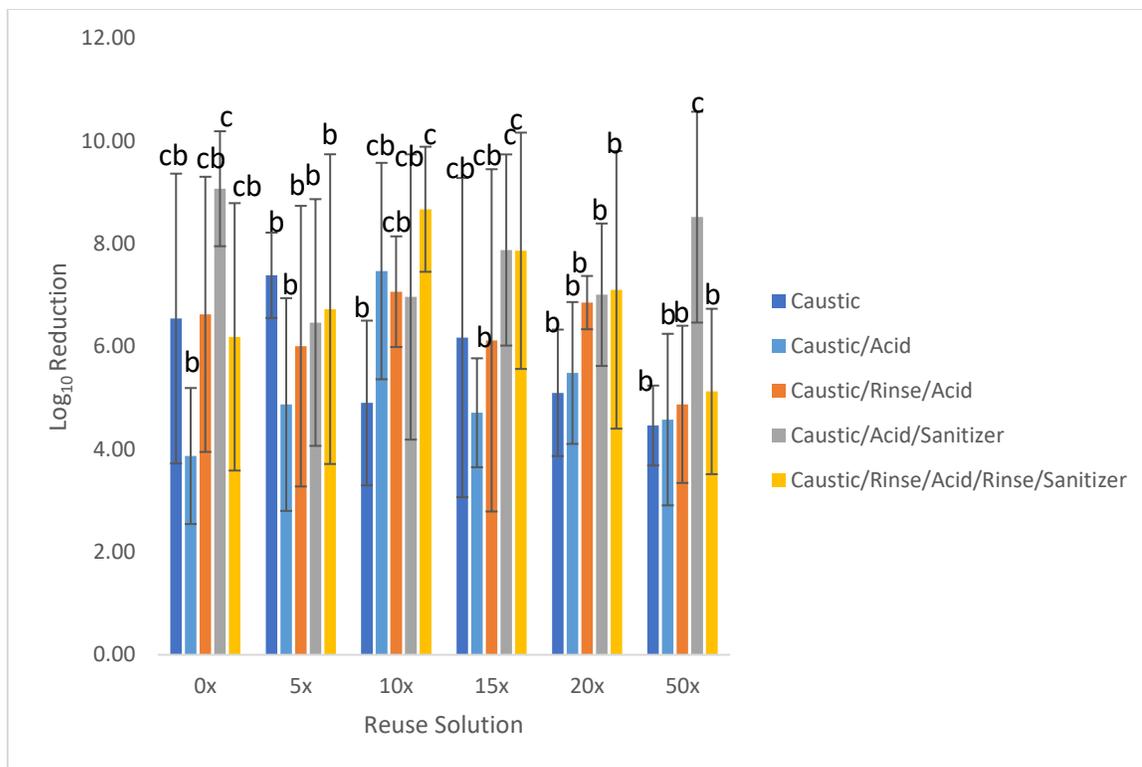


Figure 11: Log₁₀ Reduction of *P.aeruginosa* Biofilm Density with the Incorporation of Rinsing Stages

*Letters denote significance. Shared letters indicate results were not significantly different. Significance level was $P < 0.05$

While the effectiveness of caustic and acid steps on the removal of biofilms has been evaluated previously (Bremer et al. 2006), their original cleaning procedure included two rinse stages and no final sanitation (caustic rinse, water rinse, acid rinse, water rinse). Efficacy evaluation differed from this study, as tubes used for evaluation of different regimens were not randomized, a standard method for biofilm growth was not followed, controlling for microorganism required 18 vs. 48 hours, and effectiveness was testing through swabbing as opposed to the single tube (closed system) method followed. **Figure 12** shows the fresh (0N) 2% NaOH solution experimentally achieved a greater log₁₀ reduction (6.54 +/- 2.82) compared to the log reduction of 1.8 for the 1% NaOH (Bremer

et al. 2006). Additionally, they evaluated the effectiveness of nitric acid rinse and sanitizer. They found no significant difference in bacterial numbers when caustic was followed by an acid rinse nor when a sanitizer was added. For all solutions, (0-50N) the bacterial density log reduction of *P.aeruginosa* were not significantly different between Treatment 1 (caustic) and Treatment 2 (caustic /acid). Treatment 3, complete CIP, was significantly different from Treatment 2 when 0N, 15N, and 50N experimental reuse solutions were used. Interestingly, Treatment 3 was only significantly different from Treatment 1, caustic alone, when 50N solution was evaluated (**Figure 13**). Therefore, the addition of a sanitizer solution did not significantly affected the log reduction of the biofilm compared to caustic alone (treatment 1) except when the caustic solution mimicked 50 cycles of reuse. It is important that a food industry CIP operation involve removal of biofilm throughout the steps, as they are more susceptible to the bactericidal action of sanitizers if they are dislodged from the surface (Carpentier and Cerf 1993).

One study evaluated natural occurring biofilms at eight processing plants by placing stainless steel coupons in a food processing facility, previously showed to harbor biofilm. They accessed the surface bacteria, through total viable counts, before cleaning, finding a pre-cleaning bacteria mean log of 3.26 ± 1.80 . Cleaning processes did not show a strong impact on eliminating of attached bacteria. Cleaning, without disinfection achieved less than one log reduction while disinfection reduced the growth further by 1.21 logs (Gibson et al. 1999). All total viable counts showed standard deviations exceeding one log. Additionally, their evaluation of laboratory removal of biofilms using pressure washing with either caustic, acid, or neither showed no significant difference in removal of

P.aeruginosa (Gibson et al. 1999) biofilm, which proved more resistant to solutions compared with other biofilms

According to the EPA's procedure for evaluating sanitization action of detergents, in order for a solution to be considered effective there must at least a mean 5-log reduction in planktonic cells after a 30 second contact time on test cultures (EPA 2016). The occurrence of bacteria after cleaning operations has been noticed in dairy processing (Sharma and Anand 2002). Through swabbing of post CIP surfaces, they notices biofilm counts ranging from 6.29-7.97 Log₁₀ cfu/ml. While implementation of iodophore sanitizer post CIP showed log₁₀ reductions ranging from 3.15-5.55, bacterial counts of 1.23-4.74 log₁₀ cfu/mL were still present on processing surfaces. They classified sanitizer efficacy based on a reduction of 3 logs (Sharma and Anand 2002) as the reaction does not occur at the same intensity to attached bacteria (Mosteller and Bishop 1993). Treatment 3 and 5 both involved exposer to an organic acid sanitizer containing a mixture of Hydrogen Peroxide, Peroxyacetic Acid, Octanoic Acid, and other inert components. Peroxide based disinfectants (0.5%) have been shown to be permeable, to *P.aeruginosa* biofilms, meaning they have been shown to penetrate through the outer layer of the biofilm (Wirtanen et al. 2001). All experimental caustic reuse solutions (0-50) resulted in the mean log reduction of at least 5 logs for treatment 3, (6.47-9.07) and for treatment 5 (5.12-8.67) (**Figure 13**). However, the standard deviation varied between 1-3 logs for these two treatments. It is the thought of the author that variation in log₁₀ reduction may be due to the sanitizers' ability to reach the entire biofilm.

Research evaluating cleaning efficacy on biofilms showed *Pseudomonas* species biofilms were superior in survival after cleaning compared to other species biofilms (Hoa et al.

2015). These strong biofilms still exhibited 3-log cfu/cm² of microorganism remain on the surface after treatment.

4. Conclusion:

In food processing, the occurrence of biofilms can be a result of inadequate cleaning measures. On the plant floor, the point of reuse for caustic solution is subjective to visual observations by CIP operators. The physico-chemical quantification of organic load was defined in this research. The increase in reuse cycles is directly related in increases in COD, Turbidity, TSS, TP, and TN. The reduction in surface tension with reuse solution is thought to advance the cleaning ability as hydrolysis of milk components have surfactant properties. Before caustic cleaning solution can be reused, the efficacy must be evaluated. To uphold the integrity of plant safety, a 3 log reduction in attached bacteria should be achieved. CIP operations, including a sanitizer rinse as noticed in treatment 3 and 5 with solutions 0-50N experimental reuse solutions, showed a 3 log reduction in bacterial density of *P.aeruginosa*. Therefore, experimental caustic solutions showed the potential for reuse. To further determine limit of reuse of experimental caustic solutions additional experiments are needed. The mechanical action of CIP operations was not addressed here and should be implemented to observe the efficacy of reuse solutions when mechanical action is performed. Additionally, pilot scale should be implemented to ensure that the final sanitizer still achieved the 3 log reduction when 0-50N solutions were used. Lastly, the microbial efficacy of 0-50N with and without treatment by membrane filtration should be explored to compare the direct role changes in organic load and surface tension effect the log reduction of cleaning operations.

List of References

- Alvarez, N., et al. (2007). "The role of surface tension of re-used caustic soda on the cleaning efficiency in dairy plants." *International Dairy Journal* **17**(4): 403-411.
- Anantheswaran, R., et al. (2014). "CC343 Energy Efficiency in Food Processing."
- Andrade, L., et al. (2014). "Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor." *Separation and Purification Technology* **126**: 21-29.
- ASTM, E. (2004). "1054-08. Standard test method for evaluation of inactivators of antimicrobial agents." Designation. West Conshohocken, PA: ASTM International.
- ASTM international, (2007). Standard Test Method for Quantification of Pseudomonas Aeruginosa Biofilm Grown with High Shear and Continuous Flow Using CDC Biofilm Reactor, ASTM International.
- ASTM International,(2013). ASTM E2871-13 Standard Test Method for Evaluating Disinfectant Efficacy against Pseudomonas Aeruginosa Biofilm Grown in CDC Biofilm Reactor using Single Tube Method, ASTM International West Conshohocken, PA.
- Bauer, D., et al. (2014). "The water-energy nexus: Challenges and opportunities." Washington DC: US Department of Energy.
- Bazilian, M., et al. (2011). "Considering the energy, water and food nexus: Towards an integrated modelling approach." *Energy Policy* **39**(12): 7896-7906.
- Biggs, E. M., et al. (2015). Sustainable development and the water–energy–food nexus: A perspective on livelihoods.
- Bremer, P. J., et al. (2006). "Laboratory scale Clean-In-Place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms." *International journal of food microbiology* **106**(3): 254-262.
- Bridier, A., et al. (2011). "Resistance of bacterial biofilms to disinfectants: a review." *Biofouling* **27**(9): 1017-1032.
- Bridier, A., et al. (2015). "Biofilm-associated persistence of food-borne pathogens." *Food microbiology* **45**: 167-178.
- Brush, A. (2011). "Energy Efficiency Improvement and Cost Saving Opportunities for the Dairy Processing Industry: An ENERGY STAR? Guide for Energy and Plant Managers."
- Buabeng-Baidoo, E., et al. (2017). "Study of water reuse opportunities in a large-scale milk processing plant through process integration." *Chemical Engineering Research and Design* **121**: 81-91.

Bustillo-Lecompte, C. F. and M. Mehrvar (2015). "Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances." *Journal of Environmental Management* **161**: 287-302.

Bylund, G. (2003). *Dairy processing handbook*, Tetra Pak Processing Systems AB.

National Dairy Council of Canada, . (1997). "Guide to Energy Efficiency Opportunities in the Dairy Processing Industry."

Carpentier, B. and O. Cerf (1993). "Biofilms and their consequences, with particular reference to hygiene in the food industry." *Journal of applied bacteriology* **75**(6): 499-511.

Casani, S., et al. (2005). "A discussion paper on challenges and limitations to water reuse and hygiene in the food industry." *Water Research* **39**(6): 1134-1146.

CDC, C. f. D. C. a. P. (2016). "Estimates of Foodborne Illness in the United States." from <https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html>.

CDC, C. o. D. C. a. P. (2008). "Guideline for Disinfection and Sterilization in Healthcare Facilities ". from <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/glossary.html>.

Chisti, Y. (1999). "Modern systems of plant cleaning." *Encyclopedia of food microbiology* **3**: 1806-1815.

Cuéllar, A. D. and M. E. Webber (2010). "Wasted Food, Wasted Energy: The Embedded Energy in Food Waste in the United States." *Environmental Science & Technology* **44**(16): 6464-6469.

Codex Alimentarius Comission, (1999). "Codex Alimentarius Commission: Codex Committee on Food Hygiene." Proposed Draft Guidelines for the Hygienic Reuse of Processing Water in Food Plants.

DairyDivision(IUF), I. (n.d.). "United States of America Dairy Industry."

Danalewich, J., et al. (1998). "Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal." *Water Research* **32**(12): 3555-3568.

Daufin, G., et al. (2001). "Recent and emerging applications of membrane processes in the food and dairy industry." *Food and Bioproducts Processing* **79**(2): 89-102.

Demirel, B., et al. (2005). "Anaerobic treatment of dairy wastewaters: a review." *Process Biochemistry* **40**(8): 2583-2595.

DOE, U. S. D. o. E. (2017, 12-27-2017). "Electric Power Monthly." Retrieved September, 2017, from https://www.eia.gov/electricity/monthly/epm_table_grapher.php?t=epmt_5_6_a.

Dresch, M., et al. (1999). "Membrane processes for the recovery of dairy cleaning-in-place solutions." *Le Lait* **79**(2): 245-259.

EIA, (2016). "Chapter 7: Industrial sector energy consumption."

EIA, (2017). "Industry Uses Many Energy Sources ".

EIA, (2017). "Natural Gas Prices." from
https://www.eia.gov/dnav/ng/ng_pri_sum_a_EPGO_PIN_DMcf_a.htm.

EIA, (2018). "Electric Power Monthly." from
https://www.eia.gov/electricity/monthly/epm_table_grapher.php?t=epmt_5_6_a.

e-CFR (2018). "Title 40: Protection of Environment PART 405—DAIRY PRODUCTS PROCESSING POINT SOURCE CATEGORY." from <https://www.ecfr.gov/cgi-bin/text-idx?SID=9b2ad535d533c8e67ed84ba64ff900de&mc=true&node=pt40.31.405&rgn=div5>.

EPA, (2016). "Standard Operating Procedure for Germicidal and Detergent Sanitizing Action of Disinfectants Test SOP Number: MB-27-02."

EPA, (2017). "Nutrient Pollution, The Effects: Environment ". from
<https://www.epa.gov/nutrientpollution/effects-environment>.

EPA,. (2018). "National Pollutant Discharge Elimination System (NPDES)." from
<https://www.epa.gov/npdes>.

FAO, (2011). "ENERGY-SMART FOOD FOR PEOPLE AND CLIMATE Issue Paper."

Fernández, P., et al. (2010). "Nanofiltration regeneration of contaminated single-phase detergents used in the dairy industry." *Journal of Food Engineering* **97**(3): 319-328.

Finley, J. W. and J. N. Seiber (2014). "The Nexus of Food, Energy, and Water." *Journal of Agricultural and Food Chemistry* **62**(27): 6255-6262.

Gésan-Guiziu, G., et al. (2002). "Nanofiltration for the recovery of caustic cleaning-in-place solutions: robustness towards large variations of composition." *Journal of dairy research* **69**(4): 633-643.

Gibson, H., et al. (1999). "Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms." *Journal of applied microbiology* **87**(1): 41-48.

Gulati, M., et al. (2013). "The Water–energy–food Security Nexus: Challenges and Opportunities for Food Security in South Africa." *Aquatic Procedia* **1**(Supplement C): 150-164.

Hach Company (2014). "Photometric Method1 Method 8006." from
<file:///C:/Users/cadams21/Downloads/DOC316.53.01139.pdf>.

HACH Company (2016). "Ascorbic Acid Method Method 10209/10210." from
[file:///C:/Users/cadams21/Downloads/DOC316.53.01126%20\(1\).pdf](file:///C:/Users/cadams21/Downloads/DOC316.53.01126%20(1).pdf).

- Hach Company (2018). "Persulfate Digestion Method Method 10208." from <https://www.hach.com/nitrogen-total-tnplus-vial-test-lr-1-16-mg-l-n/product-parameter-reagent?id=7640209878&callback=qs>.
- Hafez, A., et al. (2007). "Wastewater treatment and water reuse of food processing industries. Part II: Techno-economic study of a membrane separation technique." *Desalination* **214**(1-3): 261-272.
- Hamilton, M. (2011). The log reduction (LR) measure of disinfectant efficacy, KSA-SM-07.
- Hanlon, P., et al. (2013). *Food, Water and Energy: KNOW THE NEXUS*. G. C. Foundation. New York, NY, USA.
- Hoa, B., et al. (2015). "Efficacy of sanitizers on *Listeria*, *Salmonella*, and *Pseudomonas* single and mixed biofilms in a seafood processing environment." *Agriculture, Food & Analytical Bacteriology* **5**(1): 15-28.
- IFC, I. F. C. (2007). "Environmental, Health, and Safety Guidelines for Dairy Processing." World Bank Group.
- Industrial Water World (2011). "SURVEY EXAMINES WASTEWATER TREATMENT COSTS." from <https://www.waterworld.com/articles/iww/print/volume-11/issue-1/feature-editorial/survey-examines-wastewater-treatment-costs.html>.
- IRENA, (2015). *Renewable Energy in the Water, Energy & Food Nexus*. I. R. E. Agency: 12-21.
- Irie, Y., et al. (2010). "Pseudomonas aeruginosa biofilm matrix polysaccharide Psl is regulated transcriptionally by RpoS and post-transcriptionally by RsmA." *Molecular microbiology* **78**(1): 158-172.
- Kirby, R. M., et al. (2003). "Water in food production and processing: quantity and quality concerns." *Food control* **14**(5): 283-299.
- Klemes, J., et al. (2008). *Handbook of water and energy management in food processing*, Elsevier.
- Koyuncu, I., et al. (2000). "Application of low pressure nanofiltration membranes for the recovery and reuse of dairy industry effluents." *Water Science and Technology* **41**(1): 213-221.
- Kumar, C. G. and S. Anand (1998). "Significance of microbial biofilms in food industry: a review." *International journal of food microbiology* **42**(1-2): 9-27.
- Kushwaha, J. P., et al. (2011). "An overview of various technologies for the treatment of dairy wastewaters." *Crit Rev Food Sci Nutr* **51**(5): 442-452.

- Lampi, M. (2001). Best available techniques (BAT) for the Nordic dairy industry, Nordic Council of Ministers.
- Luo, J., et al. (2012). "Treatment of dairy effluent by shear-enhanced membrane filtration: The role of foulants." *Separation and Purification Technology* **96**(Supplement C): 194-203.
- Luo, J., et al. (2011). "A two-stage ultrafiltration and nanofiltration process for recycling dairy wastewater." *Bioresour Technol* **102**(16): 7437-7442.
- Marie Furic, A. E., Walid Blel and Olivier Sire (2015). "Regeneration of Lyes for Cleaning-In-Place (CIP) Processes in Dairies: Comparison of Recycled Potash and Soda Solutions Cleaning Properties " *American Journal of Applied Sciences*.
- Masanet, E., et al. (2014). "Energy efficiency opportunities in the US Dairy processing industry." *Energy Engineering* **111**(5): 7-34.
- Meneses, Y. E. and R. A. Flores (2016). "Feasibility, safety, and economic implications of whey-recovered water in cleaning-in-place systems: A case study on water conservation for the dairy industry." *J Dairy Sci* **99**(5): 3396-3407.
- Meneses, Y. E., et al. (2017). "Water reconditioning and reuse in the food processing industry: Current situation and challenges." *Trends in Food Science & Technology*.
- Milmoe, N. A. P. H. (n.d.). "The Food Processing Industry at a Glance."
- MOSTELLER, T. M. and J. R. BISHOP (1993). "Sanitizer Efficacy Against Attached Bacteria in a Milk Biofilm." *Journal of food protection* **56**(1): 34-41.
- Munavalli, G. R. and P. S. Saler (2009). "Treatment of dairy wastewater by water hyacinth." *Water Sci Technol* **59**(4): 713-722.
- NPDES, N. P. D. E. S. (2017). "Number and Percent of Major Municipal Sewage Treatment Facilities with Numeric Effluent Limits for Nitrogen (N) and/or Phosphorus (P)." from <https://www.epa.gov/npdes/status-nutrient-requirements-mpdes-permitted-facilities>.
- O. Monroy H . F. Vhquez M., J. C. D. a. J. P. G. (n.d.). "ANAEROBIC-AEROBIC TREATMENT OF DAIRY WASTEWATER WITH NATIONAL TECHNOLOGY IN MEXICO: the case of "El Sauz".".
- Ölmez, H. and U. Kretzschmar (2009). "Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact." *LWT-Food Science and Technology* **42**(3): 686-693.
- Owen, G., et al. (1995). "Economic assessment of membrane processes for water and waste water treatment." *Journal of membrane science* **102**: 77-91.
- Özbay, A. and G. N. Demirer (2007). "Cleaner production opportunity assessment for a milk processing facility." *Journal of Environmental Management* **84**(4): 484-493.

- Painter, J. A., et al. (2013). "Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008." *Emerging infectious diseases* **19**(3): 407.
- Pouliot, Y. (2008). "Membrane processes in dairy technology—From a simple idea to worldwide panacea." *International Dairy Journal* **18**(7): 735-740.
- Powell, K. D. R. G. M. (1997). "Dairy Processing Methods to Reduce Water Use and Liquid Waste Load." *K-State Research and Extension*(MF-2071).
- Rad, S. J. and M. J. Lewis (2014). "Water utilisation, energy utilisation and waste water management in the dairy industry: a review." *International Journal of Dairy Technology* **67**(1): 1-20.
- Ramirez, C., et al. (2006). "From fluid milk to milk powder: Energy use and energy efficiency in the European dairy industry." *Energy* **31**(12): 1984-2004.
- Ryther, R. (2014). Chapter 27 - Development of a Comprehensive Cleaning and Sanitizing Program for Food Production Facilities. *Food Safety Management*. Y. Motarjemi and H. Lelieveld. San Diego, Academic Press: 741-768.
- Sarkar, B., et al. (2006). "Wastewater treatment in dairy industries — possibility of reuse." *Desalination* **195**(1): 141-152.
- Scanlon, B. R., et al. (2017). "The food-energy-water nexus: Transforming science for society." *Water Resources Research* **53**(5): 3550-3556.
- Sharma, M. and S. K. Anand (2002). "Biofilms evaluation as an essential component of HACCP for food/dairy processing industry – a case." *Food control* **13**(6): 469-477.
- Siebert, S., et al. (2010). "Groundwater use for irrigation—a global inventory." *Hydrology and Earth System Sciences* **14**(10): 1863-1880.
- Sun, A., et al. (2012). *Energy Use in Wisconsin’s Dairy Industry and Options for Improved Energy Efficiency*.
- Trägårdh, G. and D. Johansson (1998). "Purification of alkaline cleaning solutions from the dairy industry using membrane separation technology." *Desalination* **119**(1-3): 21-29.
- United Dairymen of Idaho (2014). "U.S. Dairy Industry." Where Good Comes From. from <http://idahodairy.com/fact-sheet/u-s-dairy-industry/>.
- UW Soild and Forage Lab, (2009). "Total Solids (Dry Matter Content) , Standard Method 2540c."
- USDA (2017). *Milk Production*. USDA.
- USGS (2016). "The World's Water."

Uzi MERINA, G. G.-G., Evelyne BOYAVAL b, Georges DAUFIN b., (2002). "Cleaning-in-place in the dairy industry: criteria for reuse of caustic (NaOH) solutions." INRA, EDP Sciences,.

Vourch, M., et al. (2008). "Treatment of dairy industry wastewater by reverse osmosis for water reuse." *Desalination* **219**(1): 190-202.

WHO (2011). "Guidelines for drinking-water quality." *WHO chronicle* **38**(4): 104-108.

WHO, (2017). *Drinking - water*, World Health Organization.

Wirtanen, G., et al. (2001). "Microbiological methods for testing disinfectant efficiency on *Pseudomonas* biofilm." *Colloids and Surfaces B: Biointerfaces* **20**(1): 37-50.

Xu, T. and J. Flapper (2009). "Energy use and implications for efficiency strategies in global fluid-milk processing industry." *Energy Policy* **37**(12): 5334-5341.

Chapter 5: SUMMARY

Current knowledge on the FEW Nexus during secondary food production is limited. It is believed that to optimize the FEW Nexus within food processing, baseline consumption must be determined. In addition to the baseline resource, data on milk processing, and wastewater must be known. Providing dairy processors with baseline information will help individual plants judge their efficiency. From here, processors can work to identify where improvements can be made and where or not they fall within their fiscal year.

This research determined that for every gallon of finished milk 0.13 kWh, 0.01 Therms, energy and 0.87 gallons freshwater are needed. A processing plant producing a variety of fluid milk products including unflavored, chocolate, and strawberry varieties of whole, 2%, 1%, skim, half & half, and occasionally ice cream base that daily produced 7,218 gallons of finished fluid milk can use these findings as a reference for efficiency.

The recovery and treatment of wastewater must be approached in a way that is plausible for all members of the industry. It is thought that a producer's decision to recover wastewater must be validated with stability in product safety and incentivized by the potential benefit to their bottom line. The desire of a producer to become a "good steward" of resources may not be enough to implement beneficial changes to the FEW Nexus within a milk processing facility.

The cleaning operation accounted for the majority of water and 24% of the electricity needed for the processing of fluid milk. Water reuse, focused on cleaning operations need to address both mechanical and microbiota cleaning efficiency. Failure to address microbial efficacy in relation to their physio-chemical characterization can lead to

insufficient cleaning operations. Water reuse can concern food processors due to the organic load and potential of bacterial contamination in used processing water. The reuse of caustic solution showed increase in organic load and a decrease in surface tension. Experimental caustic solutions showed no significant difference when implemented into a complete CIP system. The definition of efficacy, in relation to reuse of cleaning solutions should be further evaluated to better understand the reduction in biofilm density as it relates to surface tension and organic load.

It is the hope that these findings will catalyze producers to reuse process wastewater with confidence in its' safety, while also providing financial and environmental benefits to the production.

APPENDIX

Month	Electricity				Natural Gas				Water usage			
	kw/h	Revenue	Cycle (in days)	kWh./day	Therms	Cost	Cycle (in days)	Therms/day	Gallons	Revenue	Cycle (in days)	Gallons/day
Jul-16	17151.00	\$2,191.47	30	571.70	1815	\$1,166.22	33	55.00	8400	\$298.57	31	2709.68
Jun-16	17307.00	\$2,211.11	34	509.03	1405	\$823.68	30	46.83	107600	\$373.26	35	3074.29
May-16	12412.00	\$1,284.92	27	459.70	1741	\$1,044.44	32	54.41	75300	\$271.03	28	2689.29
Apr-16	21407.00	\$2,173.15	31	690.55	2287	\$1,456.09	31	73.77	116200	\$400.48	28	4150.00
Mar-16	17738.00	\$1,810.83	32	554.31	2156	\$1,430.52	32	67.38	127800	\$437.19	31	4122.58
Feb-16	15564.00	\$1,596.16	29	536.69	2440	\$1,659.55	29	84.14	101500	\$353.95	29	3500.00
Jan-16	15138.00	\$1,554.09	29	522.00	2399	\$1,625.49	30	79.97	106850	\$370.89	31	3446.77
Dec-15	16008.00	\$1,636.31	33	485.09	2032	\$1,353.66	33	61.58	109400	\$378.96	30	3646.67
Nov-15	15402.00	\$1,576.47	30	513.40	1832	\$1,202.50	28	65.43	126850	\$434.19	30	4228.33
Oct-15	18088.00	\$1,841.69	30	602.93	1958	\$1,269.78	29	67.52	135700	\$462.20	32	4240.63
Sept-15	24657.00	\$3,066.96	32	770.53	2000	\$1,322.28	29	68.97	145450	\$493.05	30	4848.33
Aug-15	21093.00	\$2,631.88	29	727.34	1519	\$1,055.47	31	49.00	129850	\$443.68	31	4188.71
Jul-15	17797.00	\$2,229.51	30	593.23	1340	\$927.89	30	44.67	90950	\$320.56	30	3031.67

Figure 12: Utility Consumption Based on Consolidated Billed Data

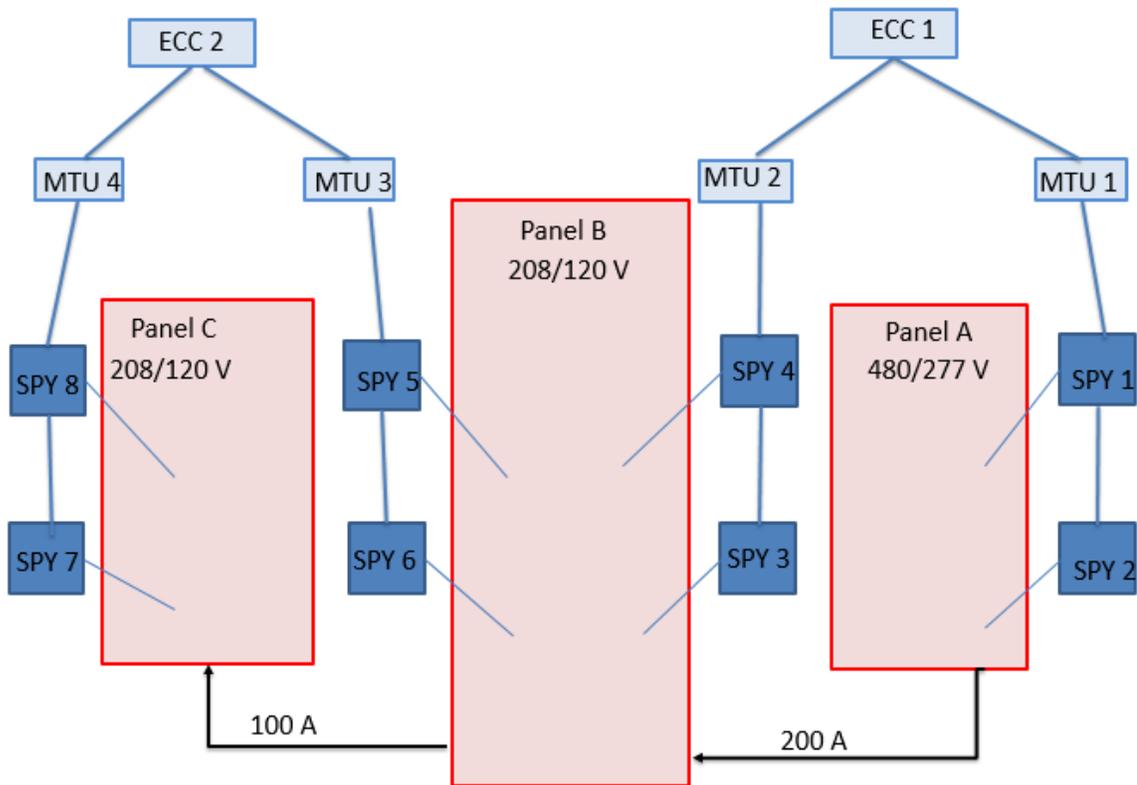


Figure 13: Diagram of Installed TED Pro 4003, 3-Phase Amp Meter

Table 7: Variation in Electrical Monthly Consumption by Three Methods Chosen

Month	Total monthly kWh, hourly meter	Total monthly kWh, billed data	Total monthly kWh, TED DATA
Sep-16	23,028	21,134	6,238
Oct-16	15,790	15,028	18,355
Nov-16	16,058	16,585	18,204
Dec-16	14,299	14,887	16,333
Jan-17	16,267	15,472	17,091
Feb-17	17,082	17,174	18,863

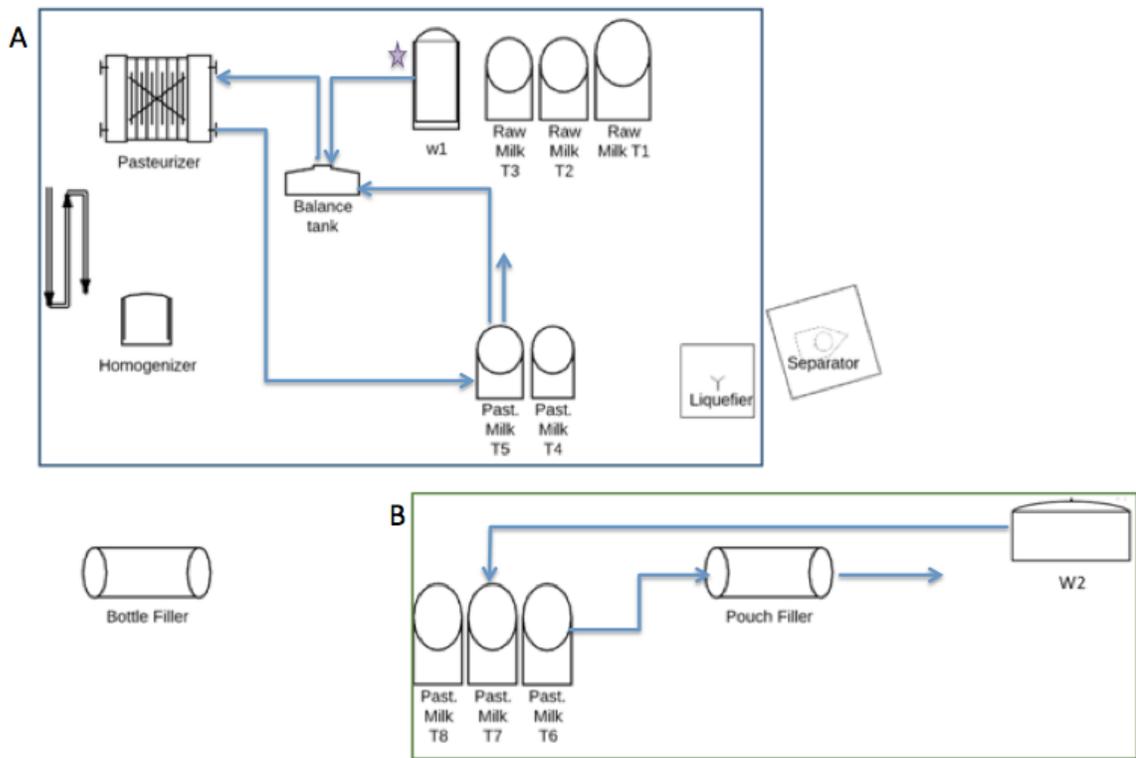


Figure 14: Cleaning in Place Operations for Fluid Milk Processing Facility

Table 8: Average Electrical Consumption for Processing Days Using the AMI Electrical Meter

Processing days	Averages	kWh			% cleaning of total
		Processing	Cleaning	Total	
September	Monday	867.86	278.02	1145.88	24.67
	Tuesday	708.16	186.47	894.62	21.21
	Wednesday	660.66	243.20	903.86	25.66
	Thursday	931.26	290.60	1221.86	23.53
October	Monday	711.73	209.45	921.18	22.13
	Tuesday	600.50	184.30	784.80	23.54
	Wednesday	505.05	164.92	669.97	24.72
	Thursday	618.33	172.09	790.41	21.68
November	Monday	693.96	244.02	937.98	25.85
	Tuesday	597.22	171.31	768.53	22.30
	Wednesday	526.09	149.18	675.27	22.08
	Thursday	522.60	149.16	671.76	25.30
December	Monday	662.41	199.79	862.20	22.25
	Tuesday	374.71	130.85	505.55	28.35
	Wednesday	498.26	143.00	641.26	24.06
	Thursday	640.79	192.26	833.05	23.08
January	Monday	643.00	262.82	905.82	28.84
	Tuesday	543.63	163.39	707.02	23.20
	Wednesday	552.31	176.23	728.53	24.04
	Thursday	670.41	220.43	890.83	24.75
February	Monday	674.00	273.21	947.21	28.54
	Tuesday	543.06	163.37	706.42	23.11
	Wednesday	617.29	174.61	791.90	22.45
	Thursday	671.32	205.95	877.27	23.44

Table 9: Average Electrical Consumption for Non-Processing Days Using the AMI Electrical Meter

	kWh
Non Processing days	Total
Friday	544.47
Saturday	409.22
Sunday	195.53
Friday	168.90
Saturday	127.62
Sunday	128.37
Friday	330.62
Saturday	37.80
Sunday	36.87
Friday	154.61
Saturday	145.54
Sunday	149.19
Friday	150.12
Saturday	123.96
Sunday	125.73
Friday	287.82
Saturday	460.87
Sunday	102.68

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Table 10: Average Daily Finished Milk Production

Averages		gallons of gallons processed
Processing days	Lbs. of gallons processed	
Monday	72608.00	8406.14
Tuesday	43079.25	4987.47
Wednesday	52648.00	6095.28
Thursday	90777.40	10509.68
Monday	72613.40	8406.76
Tuesday	45266.25	5240.67
Wednesday	39175.25	4535.48
Thursday	54388.25	6296.76
Monday	74285.00	8600.29
Tuesday	48207.60	5581.20
Wednesday	49000.00	5672.94
Thursday	60813.67	7040.66
Monday	70822.75	8199.45
Tuesday	34854.33	4035.23
Wednesday	56172.67	6503.35
Thursday	65182.40	7546.44
Monday	75994.20	8798.17
Tuesday	46153.80	5343.42
Wednesday	56497.12	6540.91
Thursday	75003.75	8683.50
Monday	85898.75	9944.86
Tuesday	48362.00	5599.07
Wednesday	62994.50	7293.14
Thursday	73159.25	8469.96

Table 11: Daily averages of reported in and out MILK per Day in the Month

	August	September	October	November	January	February	March
Lbs. of Raw milk Received	74784.74	69199	58313.06	59386.67	77095.22	73,238.90	71202.35
Cream	-	2787.4	2729.29	2990.88	7670.17	6,295.09	4866.67
Lbs. of Gallons produced	70425.6	64762.05	54022.71	55389.83	63152.42	66,214.60	66004.12
Gallons of Gallons produced	8189.02	7530.47	6281.71	6440.68	7343.30	7699.37	7674.90
Shrink in lb.	1280.52	1473.42	1561.06	1148.78	6272.63	821.94	904.12

Table 12: Daily Average Water Consumption Determined by Inline Water Meters

	Boiler	Other	Total	Boiler std dev	Other std dev	total std dev
Monday	619.2	4783.8	5403.0	104.9	421.9	495.0
Tuesday	515.9	4734.3	5250.2	175.3	1580.8	1740.7
Wednesday	589.6	4470.7	5060.3	84.7	472.0	479.8
Thursday	604.2	4724.2	5328.4	65.3	440.1	438.7
Processing Day Average	582.2	4678.2	5260.5	45.8	140.8	147.3
Friday	71.7	250.1	321.8	18.9	244.5	261.9
Saturday	61.0	8.6	69.6	9.7	2.2	9.9
Sunday	45.8	7.1	52.9	6.3	1.5	6.3
Non-Processing Day Average	59.5	88.6	148.1	13.0	139.9	150.6

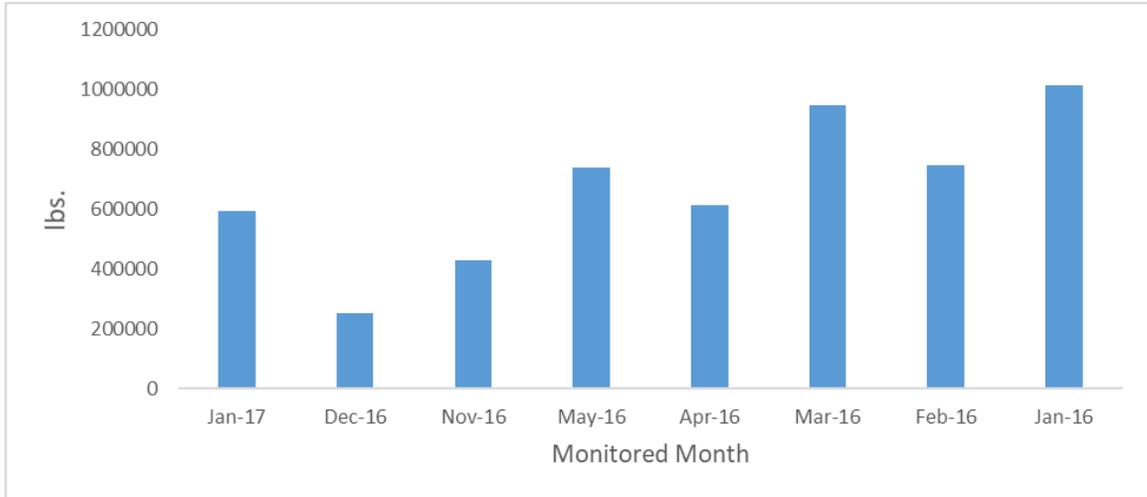


Figure 15: Wastewater Haul Weight Reported

Table 13: Inputs of Processing Units

Unit Operation	raw skim milk	raw cream	hot water	cold water	steam	glycol	Freon	Past. Milk
Receiving tank	+	-	-	-	-	-		-
Separator	+	+	+	-	-	-		-
cream tank	-	+	-	-	-	-	-	-
liquefier	+	+	-	-	-	-	-	-
balance tank	+	+		+	-	-	-	-
pasteurizer	+	+	+	-	-	+	-	-
homogenizer	+	+	-	+	-	-	-	-
bottle filler	-	-	-	+	-	-	-	+
pouch filler	-	-	-	+	-	-	-	+
T1	+	+	+				+	-
T2	+	+	+				+	-
T3	+	+	+				+	-
T4	-	-	-	-	-	-	+	+
T5	-	-	-	-	-	-	+	+
T6	-	-	-	-	-	-	+	+
T7	-	-	-	-	-	-	+	+
T8	-	-	-	-	-	-	+	+
COP tank								-
hot water tank	-	-	-	+	+	-	-	-
glycol/chiller	-	-	-	+	-	+	-	-
water reservoir	-	-	-	+	-	-	-	-

Table 14: Unit operations consuming greater than 2%

Unit	Std	Sept	Std	Oct	Std.	Nov	Std.	Dec.	std.	Jan.	Std.	Feb	Ave
	Dvt	Ave.	Dvt	Ave	Dev	Ave	Dev	Ave	dev	Ave.	dev	Ave	
Water Heater	0.51	3.83	0.58	4.59	0.01	4.26	0.52	3.92	0.61	4.10	0.04	2.37	3.85
Bottle/Cap Filler Conveyor & Pump	2.45	12.64	1.44	11.25	0.01	11.70	0.90	9.91	0.99	11.14	0.00	0.01%	9.44
Separator	1.41	4.19	1.49	5.03	0.01	6.04	1.81	5.74	1.68	5.33	0.18	2.52	4.81
Air Conditioner Process Room	0.22	3.38	0.35	3.93	0.0	4.47	0.23	3.62	0.32	1.88	0.00	0.00	2.88
Truck Wash Pump	0.11	2.11	0.03	2.62	0.0	2.95	0.28	2.95	0.14	2.77	0.58	7.61	3.50
Chiller Unit Inside	0.88	15.36	3.18	18.35	0.02	20.06	3.32	17.91	3.33	21.55	0.27	0.13	15.56
Tank 1 Compressor	0.0	0.0	0.0	0.0	0.0	0.03	0.0	0.00	0.0	0.0	3.27	20.7	3.46

Pouch filler & Conveyor & Pump	0.29	1.61	0.11	1.82	0.0	1.42	0.28	1.24	0.18	1.48	0.46	5.57	2.19
Chiller Unit Outside unit	8.28	10.44	0.27	1.41	0.00	0.07	0.09	0.12	0.05	0.09	0.00	0.00	2.02
Homogenizer	0.49	21.07	2.94	22.62	0.02	20.76	3.33	16.80	3.42	20.79	0.24	0.72	17.13
Compressor (reported as off)	0.50	5.51	0.44	6.81	0.01	6.92	0.19	5.89	1.37	5.35	0.29	7.60	6.35
Tank 1 Agitator	0.61	2.45	0.55	2.21	0.0	1.40	1.06	2.53	0.60	1.56	0.43	2.04	2.03
Tank 2,3,4 Agitator	0.17	1.98	0.43	2.02	0.00	1.37	0.26	1.96	0.47	1.79	0.54	3.46	2.10
pts above 2.0 %	sum	83.01	Sum	81.44	sum	81.36	sum	79.48	sum	73.46	sum	83.06	75.31

Statistical Analysis Model and Assumptions:

Experimental Design:

RS – 0X	RS – 5X	RS – 10X	RS – 15X	RS – 20X
Control	Control	Control	Control	Control
Caustic	Caustic	Caustic	Caustic	Caustic
Caustic/Acid	Caustic/Acid	Caustic/Acid	Caustic/Acid	Caustic/Acid
Caustic/Acid/ Sanitizer	Caustic/Acid/ Sanitizer	Caustic/Acid/ Sanitizer	Caustic/Acid/ Sanitizer	Caustic/Acid/ Sanitizer
Caustic/ Rinse/Acid	Caustic/ Rinse/Acid	Caustic/ Rinse/Acid	Caustic/ Rinse/Acid	Caustic/ Rinse/Acid
Caustic/Rins e/ Acid/Rinse/ Sanitizer	Caustic/Rins e/ Acid/Rinse/ Sanitizer	Caustic/Rins e/ Acid/Rinse/ Sanitizer	Caustic/Rins e/ Acid/Rinse/ Sanitizer	Caustic/Rins e/ Acid/Rinse/ Sanitizer
Day 1	Day 2	Day 3	Day 4	Day 5

ANOVA Table:

Source	DF	In symbol
Reuse solution (A)	$5-1 = 4$	a-1
Block*A – Whole Plot error	$(3-1)*5 = 10$	$(r-1)*a$
Treatment (B)	$6-1 = 5$	b-1
A*B	20	$(a-b)(b-1)$
Split Plot error A*(rep*B)	$5*2*5 = 50$	$a(b-1)(r-1)$
Sampling Error	$2*3*30 = 180$	$(s-1)rab$
Total	$270-1 = 269$	rabs-1

Where r is the number of replications (experiment repeated 3 times)

a is the number of reuse solution (5 reuse solutions – 0X, 5X, 10X, 15X, 20X)

b is the number of treatments (6 treatments – caustic, control, ...)

s is the number of sampling (3 sample were taken from each experiment in one day)

Model: $y_{ijk} = \mu + \alpha_i + \delta_{ij} + \beta_k + \alpha\beta_{ik} + \epsilon_{ijk} + \rho_{ijkl}$

Where y_{ijk} is the response of i th reuse solution treatment, j th whole plot unit and k th treatment

μ is the overall mean

α_i is the number of reuse solution effect

δ_{ij} is the whole plot error

β_k is the treatment effect

$\alpha\beta_{ik}$ is the interaction of reuse solution and treatment

ϵ_{ijk} is the error term

ρ_{ijkl} is the sampling effect.

Assumptions: $\delta_{ij} \sim N(0, \sigma_\delta^2)$, $\epsilon_{ijk} \sim N(0, \sigma_\epsilon^2)$, $\rho_{ijkl} \sim N(0, \sigma_\delta^2)$ all independent among and within each other.