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Feasibility, safety, economic and environmental implications of whey-recovered water for cleaning-in place systems: A case study on water conservation for the dairy industry

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FEASIBILITY, SAFETY, ECONOMIC AND ENVIRONMENTAL IMPLICATIONS
OF WHEY-RECOVERED WATER FOR CLEANING-IN PLACE SYSTEMS: A CASE
STUDY ON WATER CONSERVATION FOR THE DAIRY INDUSTRY

by

Yulie E. Meneses-Gonzalez

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FEASIBILITY, SAFETY, ECONOMIC AND ENVIRONMENTAL IMPLICATIONS
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University of Nebraska, 2016

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Several countries around the world are facing the challenge of producing food with limited water resources for a growing population. This reality is forcing all sectors involved in the food supply chain to look for water conservation strategies that contribute to assure global food security. Besides water consumption, the food industry has to deal with wastewater generation; therefore, water reconditioning and reuse is an attractive solution to address both issues. The goal of this research was to demonstrate that high quality water can be recovered from whey, a by-product of the cheese making process, and reused in cleaning-in place (CIP) operations. Technical, economic, safety and environmental feasibility of the proposed intervention was also considered. First, the performance of the water recovery system was evaluated as well as the quality of protein, lactose and water recovered from whey. A combination of ultrafiltration and reverse osmosis allowed a water recovery of 47 % with > 98 % removal of the initial pollutants present in whey. Once spray dried, protein and lactose powder fulfill commercial standards. When applied in CIP systems, the cleaning efficiency of the recovered water was proven to be similar to tap water. Subsequently, a cost analysis was performed for small, medium and high cheese production scales; results demonstrated that the proposed

intervention is economically feasible generating revenues of 0.18, 3.05 and 33.4 million \$/year, respectively. Then, a comparative life cycle assessment was conducted, revealing that the recovery system generate 87.7 % and 18% lower environmental impacts than a wastewater and water production system, respectively. Energy usage was the input causing most of the emissions. Lastly, the risk assessment on the reuse of contaminated-reconditioned water with *L. monocytogenes* in fluid milk processing, indicated low levels of bacteria transferred from the contaminated water to the equipment surface.

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Dedication

I want to dedicate my doctoral dissertation to my baby, who has been the main motivation to complete this professional achievement. Even though we have not met yet, I love you since the very first moment I knew you were growing with me. Now I have someone that will follow my steps and I hope I can be a good example for you. Everything can be achieved with hard work and dedication, just never give up!

Preface

The present doctoral dissertation is a holistic study aimed to provide answers to different aspects associated to water conservation initiatives in the food industry, using the dairy industry as a case study. The work is divided in five complementary components: water recovery and reuse, value-added of the by-products, cost analysis, risk assessment and life cycle assessment; each one of these are described in detail with their corresponding methodology and results in the five chapters encompassed in this dissertation. Chapter 1 provides a comprehensive literature review that highlights current situation and challenges of implementing water reconditioning and reuse in the food industry including regulations, current technologies, food safety aspects, environmental impacts and a perspective about future research needs. Chapter 2 describes the proposed water recovery system to separate protein and lactose from whey and to recover water at the same time. This chapter includes process efficiency parameters, safety aspects of water reuse in cleaning-in-place systems and cost analysis for a small, medium and large scale cheese production. Chapter 3 evaluates other environmental impacts relevant to the dairy industry, as a complementary study to understand how water conservation initiatives affect other environmental categories, especially those related to the water and energy nexus. Chapter 4 simulates a scenario of post contamination of the recovered water with *Listeria monocytogenes* and determines the probability of contamination per package of product processed in the equipment cleaned with the contaminated water. Lastly, Chapter 5 presents a summary of the major findings obtained from this study and proposes some ideas for future research.

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**CHAPTER 1: WATER RECONDITIONING AND REUSE IN THE FOOD
INDUSTRY: CURRENT SITUATION AND CHALLENGES**

Abstract

While the demand for food and water is growing, water shortages are already occurring in many of the world's major food production areas. Irrigation is unarguably the most water demanding operation among the food supply chain, however efforts from different sectors will collectively secure food for the world's population. Food processing is a key component of the food supply chain and its water footprint is of great consideration, not only because of the high quality water used in the manufacturing of products, but also for the significant volumes of pollutant wastewater generated. Different food sectors produce wastewater of different qualities, but for all cases water reconditioning and reuse offer opportunities to reduce water consumption and to contribute to a better water management in the food industry. The factors converging to implement such initiatives including, regulations in place, available technologies, food safety considerations, risk perceptions, water quality, environmental impacts and research needs are discussed herein. The goal of this review paper was to bring to the forefront of the debate the challenges and opportunities that water conservation initiatives offer, in order to produce more food with less water.

Key words: Water conservations, regulations for reuse, membrane technologies

Introduction

A renewable resource is defined as an element that after exploitation, can return to its previous stock level by natural processes of growth or replenishment (OECD, 2001). Water used to be considered a renewable natural resource, but that assumption is not adequate anymore within the reality the world faces today. Water scarcity is already limiting the economic growth of China and India and the current serious drought in California forced the state to limit its agricultural water withdrawals (Morrison et al., 2009). But, why is water becoming scarce in the first place? Climate change is a significant contributor, however population growth and economic development play an important role as well by increasing domestic water demand and driving dietary shifts into higher animal protein consumption.

A meat-based diet has a larger water footprint (36% larger) than a vegetarian diet (Hoekstra, 2012). For example, the volume of 29, 31, 112 L of water are required to produce one gram of animal protein from egg, milk and meat, respectively; while for the production of one gram of cereal protein, 21 L of water are used (Mekonnen and Hoekstra, 2010). Due to the imminent changes in population and eating preferences, FAO projections indicate that between 2000 and 2050, global meat and milk production should increase by 102% and 82%, respectively (Boland et al., 2013), which indicate a higher demand in the water use to meet the increasing need in agricultural commodities.

The challenge of feeding a growing population, is clearly defined. The question is how food production could reach those levels with limited available water, an essential component in agriculture and food processing.

Water reconditioning and recycling, in all sectors of the food supply chain offer potential opportunities to overcome this challenge. Nevertheless, the food industry, especially at the food processing stage, is highly sensitive to this concept, due to the negative non-science based perceptions about the characteristics of this water and potential risks for contamination (Casani et al., 2005). If more scientific information can be made available, this risk perception could be less biased. Unfortunately, there is limited information about the implications of using reconditioned water in food processing settings.

To bring water conservation practices in the food industry to the forefront of the discussion, the current situation of water reconditioning and reuse in the food processing sector, technologies available for wastewater treatment, regulation constraints, tools to evaluate the implications from a holistic approach and opportunities for future research are reviewed.

Before expanding on the discussion, some definitions of the terminology used for water reuse are included in table 1 for clarification.

Table 1. Definitions of selected water reuse terminologies

Reclaimed water	Water that was originally a constituent of a food, has been removed from the food by a process step, and has been subsequently reconditioned when necessary such that it may be reused in a subsequent manufacturing operation
Reconditioning	The treatment of water intended for reuse by means designed to reduce or eliminate microbiological, chemical and physical contaminants, according to its intended use
Recycled water	Water, other than first use or reclaimed water, that has been obtained from a food manufacturing operation and has been reconditioned when necessary such that it may be reused in a subsequent manufacturing operation
Reuse	The recovery of water from a processing step, including from the food component itself, its reconditioning treatment, if applicable; and its subsequent use in a food manufacturing operation

Source: Taken from the proposed draft guidelines for the hygienic reuse of processing water in food plants. Presented to the Codex Alimentarius Commission (Codex Alimentarius, 1999)

Water, its use and importance in food processing

Water is used throughout the food production chain at different stages including irrigation, processing, cooling, heating, and cleaning. Irrigation represents 37% of the total U.S. freshwater withdrawal, while the manufacturing industry accounts for an additional 5-10% of freshwater consumption (EPA, 2013). The food processing industry itself accounts for over 30% of the water used in manufacturing as a whole (Australian Government Department of Agriculture, 2008). Though, the proportion of water usage in the food industry is relatively small, it is important to highlight that food sectors use high quality water and are frequently located in close proximity to urban areas. Therefore, they not only compete with the community for natural resources, but in addition food companies produce a significant amount of effluents, which if not properly handled can cause significant environmental impacts.

Together with the fact of water scarcity, stricter environmental regulations and the increasing cost of municipal water and wastewater treatments, all become determining factors that motivate food businesses to look for alternative ways to produce food efficiently and in a sustainable framework (Maguire, 2015).

Some processing wastewater streams are reasonably clean, examples of such streams include, but are not limited to, cheese whey (Rektor and Vatai, 2004), condensed water from evaporation processes (Vourch et al., 2008), rinse water from operations start up and final produce rinse water (Balannec et al., 2002). Water from these streams can be recovered and treated (reconditioned) to reach any quality level, for reuse in the same or other processes. In order to achieve a significant reduction in water usage, it seems logical that recovered water be reused in high water demanding operations identified throughout the processing flow. Yet, information about water usage during specific process operations is not openly available from the U.S. food industries. This fact is a significant hindrance in conducting studies on water conservation alternatives, since the knowledge about potential streams for water recovery and water quality requirements for different operations is limited and therefore does not allow for improvements in the most significant water consuming operations. Cooperation among industry, academia and regulatory agencies is fundamental to strengthen the culture of water conservation and sustainable production in the food industry.

Regulations

The U.S. Environmental Protection Agency (EPA) has published some guidelines for water reuse (EPA, 2012 a), although official federal regulations are not in place. In the

U.S., standards regarding water reuse is the responsibility of each state and their local agencies.

The idea of reusing processing water in food plants is not new, in fact the United States with the assistance of Australia, Netherlands, India, Germany, France and the International Dairy Federation prepared and proposed a revised Draft Guidelines at the 31st session of the Codex Alimentarius Commission (CAC) (Codex Alimentarius, 1999). Even though all delegates agreed on the importance of water conservation initiatives for the food industry, the decision about the inclusion of these guidelines was deferred on several consecutive sessions, until the 34th session when the decision to discontinue the consideration of the Proposed Draft Guidelines was made (Codex Alimentarius, 2004). One lesson learned from these sessions is a general guidance for all operations may not be available. Instead, guidelines for water reuse should be developed for specific commodities due to the fact that the practice of water reuse varies widely depending on the type of industry (Codex Alimentarius, 1998).

Currently regulations require that potable water or equivalent must be used for food contact applications, but other water qualities are acceptable for non-food contact applications (Casani et. al., 2015). Both situations open the door for water reconditioning and reuse practices, as long as the water quality requirements are satisfied and the safety and quality of the final products is not compromised.

Within the food industry, a few sectors have allowed the use of reclaimed water in their manufacturing practices including dairy (FDA, 2015), poultry (Codex Alimentarius,

2007), vegetables and fruits (Codex Alimentarius, 2013) industries. In all cases continuous monitoring, audits and frequent sampling of the water are required.

Interestingly enough, water recycling projects have been successfully implemented in many places, where water scarcity problems started years ago such as Singapore (Singapore Government, 2002), Australia , Israel, China, and Florida and California among the states from the U.S. (Anderson, 2003). These projects provide evidence about the potential for the implementation of water conservation initiatives throughout the food supply chain.

The current global situation depletes natural resources and drives to produce food in a dynamic system, where water and energy are not everlasting resources. In order to face such big challenge, it is essential to provide scientific-based knowledge about the beneficial implications (safety, economic, and environmental) related to any water conservation initiative, which unfortunately is still lacking today. Such valuable results could be translated into new regulations and guidelines, for specific food sectors introducing the concept of water reconditioning and reuse to the food industry. It is important to keep in mind that water-conservation initiatives are most likely to be implemented if win-win solutions are provided.

Water and wastewater quality characterization

For potable and drinking water, the World Health Organization (WHO) (WHO, 2008) and the EPA (EPA, 2012 b) require testing for a long list of chemicals, microbial and sensory parameters. Table 2 shows examples of microbial organisms associated to water

and foodborne outbreaks. Recovered water differ from fresh potable water in its stored in either open or underground locations where all types of contamination are possible. Recovered water coming from a processing operation may have contaminating agents related to food product quality, the processing operation generating the wastewater, treatment method chosen for reconditioning, and the processing plant environment. Testing of all these parameters for water recovered in a food processing plant is overwhelming, expensive and in some cases impractical. Water recovery treatment should be designed to target these hazardous organisms.

Therefore, a structured quality assessment of the wastewater and recovered water is important to provide the baseline information of possible contaminants associated with these water sources, which facilitate selecting a suitable reconditioning treatment and evaluating its performance. Some common parameters of water quality assessment include Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Organic Carbon (TOC), Total Kjeldahl Nitrogen (TKN), ammonia, nitrate, nitrite, pH, conductivity, total solids, Aerobic Plate Counts (APC), and coliforms/*E.coli*. Standard methods are available for all these analyses (APHA, 2005). Depending on the composition of the wastewater source, other analyses to determine lipid, protein, lactose/sugar, and minerals removal could be considered as well.

The quality characteristics and amounts of other by-products generated during the reconditioning process should be monitored in order to identify potential use, recycling or further treatment. This is a key factor to reach the superior objective of zero plant discharge to the environment.

Table 2. Pathogenic and food spoilage microorganisms and their significance for food and drinking water safety.

Microorganism	Pathogenicity ^a	Transmission ^a	Infective dose ^a	source ^a	Persistence in W or DW ^a	Information related to foodborne outbreaks surveillance			
						Food category ^{b, c}	Outbreaks and (%) ^d	Illness severity ^{f, g}	Hospitalization (and death) (%) ^{g, h}
<i>Campylobacter jejuni</i>	Pathogen	Ingestion	Low-moderate	A, F, W, E	Moderate	DR	140 (2)	mild diarrhea illness, fever	15 (6)
<i>Legionella</i> spp.	Opportunistic	Ingestion	Low	W, DW, E	May multiply	NR	21 (65.6) ^e	cough, high fever ⁱ	89 ^e
<i>Salmonella</i> spp.	Pathogen	Ingestion	High	H, A, F	Moderate	PO, PK, V, B	1,449 (18)	acute gastroenteritis	35 (28)
Norovirus	Pathogen	Ingestion and inhalation	Low	H, F	Uncertain	B, DR, F	3,444 (43)	acute gastroenteritis	26 (11)
<i>E.coli</i>	Pathogen	Ingestion	High	H, A, F, W	Moderate	B, V, G	308 (4)	diarrhea illness	4 (2)
<i>Pseudomonas aeruginosa</i>	Opportunistic	Contact or inhalation	High	H, W, E	May multiply	NR	NR	food spoilage	NA
<i>Listeria monocytogenes</i>	Pathogen	Ingestion	High	H, A, F, E	Long	DR, PO	25	fever, invasive infection	91 (21)

Source : a) (Casani and Knøchel, 2002), b) (CDC, 2006) c) (CDC, 2013e), d) (CDC, 2013d), e) (CDC, 2013f) f) (CDC, 2013b), g) (CDC, 2014), h) (CDC, 2013c), i) (CDC, 2013a)

H: Human; A: Animal; F: feces or intestinal tract; W: water; E: environment; DR: dairy; PO: poultry, PK: pork, V: vegetables; F: fruits, G: grains, B: Beef
W: water, DW: Drinking water; NA: Not available; NR: No related

Reconditioning treatments

When the recovered water is intended to be used in operations that require high-quality water (e.g. potable water is currently required to be used for equipment and surfaces in contact with food), the microbial and chemical quality parameters should at least be equal to those of the tap water, to assure safety and quality of the final product. In that regard, the selection of the reconditioning treatment becomes critical to supply water with the required quality characteristics (Casani et al., 2005).

Chemical, physical, or a combination of both treatments are currently available to lower the microbial load and remove hazardous chemicals. The advantages and limitations of chemical treatment methods for food process water have been discussed by Casani and colleagues; including processing aids such as chlorine, chlorine dioxide, chloramines, ozone, hydrogen peroxide and peracetic acid (Casani et al., 2005). Whereas for physical treatments, the membrane filtration system offers attractive opportunities to the food industry due to the valuable byproducts that can be recovered from wastewater streams, such as protein and lactose from whey.

The cost associated with membrane systems has frequently been considered the downside of this technology, but the development of more efficient and cost effective membranes has increased the interest in water reuse and recycling (Sarkar et al., 2006).

Membranes filtration for water reconditioning and reuse

The chemical process industry is a pioneer in the use of membrane separation systems. However, the food industry has successfully applied these principles to manufacture high quality and environmentally friendly products with great flexibility in the system design (Ahmad and Ahmed, 2014).

The general objective of a membrane system of any type is to generate two streams, a concentrate and a permeate. Selection of the membrane system is based on different considerations to obtain the desired characteristics on the final product. These include: the final concentration, product quality, flux, operating cost, capital investment and energy consumption (Porter, 1989). Therefore, it is frequently found that different types of membranes are combined in the same processing systems and even with other technologies to be able to optimize the performance of the entire system.

Dairy, beverage, and ingredient industries are among the food sectors that take advantage of membrane technologies; while meat, poultry and fruit industries are making inroads with this technology, especially to treat wastewater generated from the production process. The objectives in these last cases are to produce purified water for recycling and to recover valuable by-products.

In the following sections, the factors and characteristics affecting membranes performance are introduced including: material composition, physical structure and design.

Membrane chemistry. Membrane material for a particular application is selected considering its resistance to pressure, temperature, pH, chemical compatibility and cost (Girard et al., 2000). Membranes can be fabricated from a wide variety of organic (e.g. polymers) and inorganic (ceramic) materials.

Table 3. Membrane materials and their membrane pore size and module design used in food applications

Organic	Membrane	Module ^b	Max. Temp. (°C) ^a	pH range ^b
Hydrophilic				
polysulfone	NF, UF, MF	PF, TU, SW	80	1.5-12
polyethersulfone	NF, UF, MF	PF, SW	80	1.5-12
cellulose acetate	NF, RO	PF, TU, SW, HF	30-60	2-7.25
polyamide	RO, NF	PF, TU, SW, HF	60	1.5-9.5
polycarbonate	MF			
Hydrophobic				
polyethylene	MF			
polypropylene	MF	PF, HF		
polytetrafluoroethylene	MF	PF, TU, SW		
poly (vinylidene fluoride)	UF, MF		80	1.5-12
Inorganics				
aluminum oxide	MF, UF	TU	300	0-14
zirconium oxide	UF	TU	300	0.5-13.5

PF: plate and frame; TU: tubular; SW: spiral wound; HF: hollow fiber

MF: microfiltration, UF: ultrafiltration, NF: nanofiltration, RO: reverse osmosis

Source : Adapted from a) Ahmad and Ahmed (2014), b) Girard et al. (2000)

Today, commercially available membranes are mainly made from polymers (Khulbe et al., 2007), since they show high chemical stability, high packing density, high permselectivity and are less expensive (Jiansheng et al., 2005, Khulbe et al., 2007, de

Morais Coutinho et al., 2009). The membranes made of inorganic ceramics present higher thermal stability than polymer, but are significantly more expensive (Jiansheng et al., 2005). Table 3 summarizes some membrane materials and the module on which they are regularly applied.

Separation by size. Membranes can also be categorized by size-based separation, including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). Separation by size is performed by solid matrixes, where the determining factors are the pore diameter in membrane and the size of the particle of interest (Van Der Bruggen et al., 2003), but they also share the characteristic of using pressure difference as the driving force to transport the solvent through the membrane (Cheryan, 1998, Van Der Bruggen et al., 2003, Cassano et al., 2014). The difference in performance between them are given by the ranges in pore size, the bigger the pore size, the higher the permeability and lower the pressure requirement. Table 4 summarizes the characteristics of the different membranes based on pore size.

Module design. The module design for a membrane filtration system is determined by the configuration of the membrane. In the food industry, four configurations are commonly found a) plate and frame b) spiral wound c) tubular and d) hollow fiber (Mallevialle et al., 1996, Alkhudhiri et al., 2012). The module design for a specified membrane aims to maximize the packing density and reduce fouling as much as possible. Table 5 shows a comparison among the different modules designs mentioned above.

Table 4. Summary of different membrane technologies categorized by pore size and their characteristics

Membrane	Pore size	Pressure (bar)	MWCO (KDa)	Permeability (l/h.m ² .bar)	Separation mechanism	Concentrate
Microfiltration	0.1-10 μ m	0.5- 4	100-500	>1000	sieving	Bacteria, fat oil, colloids, organic microparticles
Ultrafiltration	2-10 nm	2.0-10	20-150	10-1000	sieving	Macrosolutes and colloids
Nanofiltration	1-3 nm	3.0-30	2.0-20	1.5-30	sieving +charge effects	High molecular weight compounds and multivalent ions
Reverse osmosis	0.1- nm	10-100	0.2-2	0.05-1.5	solution-diffusion	Salts, sodium chloride and inorganic ions

Source: Adapted from Cassano et al. (2014), Van Der Bruggen et al. (2003) and Muro et al. (2012).

Table 5. Comparison among different characteristics of membrane module designs

Configuration	Packing density (m ² /m ³)	Membrane	Fouling	Cost /area	Pre treatment	Backflushing
Plate and frame	moderate (200-400)	RO	Medium	high	no	no
Spiral wound	moderate (300-1000)	UF, RO , FO	Medium	low	yes	no
Tubular	low (100-300)	UF	Low	high	little	no
Hollow fibre	high (1000-10000)	MBR	High	low	yes	yes

Source: Adapted from Girard et al. (2000)

Other membrane technologies. The ongoing research in membrane science allows for the development of new technologies and other more complex systems that offer alternatives for water reconditioning in diverse food sectors, such as membrane distillation, osmotic distillation (Drioli et al., 2011), pervaporation (Karlsson and

Tragardh, 1996), membrane bioreactors (Judd, 2010), and ion-exchange membranes (Strathmann, 2010).

Membrane technology continues to be an important research area, since it offers potential solutions to minimize loss, reduce energy, reduce water consumption, preserve nutritional characteristics, improve quality in food products, and to manage industrial wastewater; which are all important challenges today for the academy and the food industry. Membrane fouling remains to be an important limiting factor in terms of cost efficiency, but as investigations progress, better overall performance and innovative integrated systems are expected.

Water conservation initiatives, evaluated from a holistic perspective

The adaptation of water conservation practices in the food industry requires a multi criteria analysis that incorporates economical, safety and environmental assessments. Findings from these types of studies will provide key information for food companies to perceive water reconditioning and reuse as a promising option to reduce their water footprints.

Risk perception

One of the biggest barriers for water recycling initiatives is the consumer perception about increasing food safety risk (Australian Industry Group, 2008, International Life Science Institute, 2008). Consumers' risk perception regarding using recycled water in the food industry mainly results from reported food safety issues without questioning the true contamination sources in detail. For example, although potable water is currently

used for food processing, 48 waterborne outbreaks were reported in the U.S. from January 2007 to December 2008 (CDC, 2011), 14 outbreaks in Europe in 2010 (European Center for Disease Prevention and Control, 2012), and 51 in Australia in 2012 (The Institute of Environmental Science and Research, 2012). Rather than potential contamination originating in water production systems, inappropriate storage, leakage from dirty water systems into a clean water system or presence of contaminants in the distribution systems are more likely causes to introduce hazards into even the cleanest potable water (International Life Science Institute, 2008). Thus, in order to minimize these concerns and to better evaluate the actual risk of using reconditioned water in the food industry, comprehensive assessments of the risk factors affecting the water recovery system and the recovered water should be implemented, independently of the water source. Also, suitable control and monitoring plans for those systems should be developed.

Microbial risk assessment, a tool for safety. Risk Assessment is one element of risk analysis, a more complex investigation that can include other phases like planning, data collection, management and communication (Schroeder et al., 2007). Governments worldwide use risk assessment to support human health related regulatory decision-making (EPA, 2014).

Risk assessment can be performed from a qualitative or quantitative methodology (EPA, 2014), and it serves a science-based approach to evaluate safety, which allows estimation of the likelihood and severity of a particular unwanted outcome, given a well-defined scenario (Schroeder et al., 2007).

Quantitative risk assessment is preferred since in this approach distributions are generated for predictions, allowing a better understanding of uncertainty while at the same time the variability related to contributing factors can be considered (Schroeder et al., 2007).

In recent years, Quantitative Microbial Risk Assessment (QMRA) has been applied in food and water safety risk management systems to evaluate pathogen risk and achieve the ultimate goal of public health protection (FAO, 2006, FAO/WHO, 2006, CAC/GL, 2007, Smeets et al., 2010b, Schijven et al., 2011b, Zhou et al., 2014). Information availability, accurate identification of microbial foodborne pathogens and the advance on mathematical techniques have permitted QMRAs to gain greater international credibility (EPA/USDA, 2012). In QMRA, the modular process risk model (MPRM) methodology is usually applied to split the food supply chain into basic modules according to food-handling steps, which are then linked into a chain model (Nauta, 2005). Therefore, a properly designed QMRA is able to objectively and systematically collect relevant scientific evidence that takes into consideration all possible risk factors and evaluate efficacy of potential control measures that can be applied at each step of the food supply chain.

QMRA has allowed for the establishment of an acceptable risk level of 1 infection per 10,000 people per year as a safe risk level for drinking water (WHO, 2011). The same tool has been applied to evaluate the risk factor associated to the reuse of wastewater in irrigation (Stine et al., 2005). However, to the best of our knowledge, QMRA has not

been applied to evaluate the risk associated with water reconditioning and reuse in the food industry.

The studies developed for managing drinking water systems can provide strong scientific foundations for food processing operations, willing to undertake water reconditioning and reuse activities. Even though the type of hazards and treatments might vary, the objectives in both scenarios are the same: i) collection of data related to the fate of the microorganism of concern and the prediction of human exposure of the target organism through food consumption, ii) evaluation of the efficacy of existing and potential control measures in reducing contamination, iii) providing a risk outcome in relation to regulatory standards (Schijven et al., 2011a).

Risk perception is an important hurdle for water reconditioning and reuse in the food industry. QMRA can help to diminish the negative awareness by providing scientific information about how water reconditioning and reuse can be done in a safe manner. As described by Smeets et al. (Smeets et al., 2010a), QMRA can estimate how safe the water is, how much the safety of the water varies and how certain the estimate is. Similar management questions can be expected for reconditioned water intended for reuse in the food industry. Consequently; QMRA studies targeting the following areas and are critical to support further implementation of water conservation initiatives.

- evaluation of different levels of safety associated to the type and complexity of the reconditioning treatment or system
- risk related to post-reconditioning treatment contamination

- risk factors associated with the occurrence of special events (treatment or equipment failure)
- determination of monitoring frequency, sampling quantity based on the number of the barriers included in the system and its efficacy

The results obtained from a QMRA will help to strengthen HACCP plans for reconditioned and recycled water. This approach allows for a wider characterization and quantification of the risk associated with a particular recycling activity in any product and process.

Environmental impact/ Life Cycle Assessment

When water conservation initiatives are undertaken, it is obvious to anticipate reduction on environmental impacts related to water depletion. However, the impacts on other environmental categories, such as energy consumption, remain unknown. For that reason, today there is high interest on looking for assessment tools that incorporate a broader range of environmental categories that can be affected by the implementation of a particular activity or process.

Life Cycle Assessment (LCA) is a standardized methodology, widely used to evaluate the environmental impacts at each stage of the production chain including consumption, disposal and recycling, if applicable, without assessing economic or social impacts (Morawicki, 2011). According to the ISO norms 14040:2006 and 14044:2006, the elements of a LCA include definition of goal and scope, life cycle inventory analysis, life cycle impact assessment and interpretation; which are adjusted and reviewed to fulfill the

guidelines established in the standard. LCAs are critically important in managing food systems that resources are deployed sustainably, while mitigating the excessive use of inputs, including land, water, energy, fertilizers, and other tools (Ramaswamy, 2015).

Life cycle impact assessment, translates resources use and emissions that occur in the life cycle of a product or service, into potential impacts on the environment (including human health). While the life cycle impact assessment methodology is under development for toxic effects of chemicals on human health (i.e., human toxicity) and ecosystems (i.e. eco-toxicity), the effects of pathogens are not currently considered in LCA. Nonetheless, Harder et al., 2014 suggested that QMRA results can be integrated in LCA framework to provide a more accurate evaluation of all possible factors affecting human health. Both LCA and QMRA results can be expressed in terms of the Disability-Adjusted Life Year (DALY), a concept developed by the World Health Organization (WHO) as a way to evaluate potential impacts on human health (WHO, 2015).

The consideration of an LCA for a rounded evaluation of a water conservation initiative, bring significant input to the decision making. This methodology makes it possible to compare different water reconditioning treatment options, the effect of the water recycling intervention in the overall environmental footprint of the particular product or process of interest, allows food producers to use claims showing their efforts towards sustainable production, and expose hotspots in the system that have potential for improvement.

Research needs

Much needed water conservation initiatives for the food industry have been discussed in this dissertation. Although detailed scientific information can contribute to decision making processes, the lack of such information remains an important constraint.

Unarguably more research studies targeting areas of process efficiency, food safety, economic feasibility, and environmental impacts related to water reconditioning and reuse are necessary to strengthen this initiative. Thus, some suggested research areas that need attention include:

- **Assessment on the amount of water used by the food industry in the United States**

In order to evaluate the efficacy of any intervention for the reduction of water consumption, it is necessary to determine the amount of water being currently used on different food processing sectors in the United States, and if possible, in each of the processing operations within each sector. Limited information is available from the U.S. food industry in this regard. These studies are valuable to identify wastewater streams with potential for recondition, and to select operations in which water reuse will generate a noteworthy reduction in water depletion.

- **Sanitation operations**

The first step to reduce water use is to identify stages in the process where water is not required or at least not in the amount currently used. Cleaning operations

could be an option, since these activities are usually performed on a routine basis, following general standard procedures or using cleaning-in-place (CIP) systems that run under set conditions. Further evaluation of the type and amount of organic load that needs to be removed from the target equipment will help to determine how often these cleaning operations need to be implemented, as well as the amount of water and chemicals that are sufficient to reach the target level of cleanliness and sanitization.

- **Water quality characterization for use in the food industry**

Potable quality water is not required for every single operations in a food processing facility. Therefore, wastewater generated in some processes could be recycled in others, with or without additional treatment, depending on the water quality requirements for the specific reuse. Those streams should be well defined and characterized in terms of microbial load, chemical composition, and water quality parameters (described previously in section 4).

- **Water treatment, fit for purpose**

Water reconditioning can represent technical and financial challenges. Nevertheless, deeper understanding on current and emerging treatment options (chlorination, ozone, UV, membranes filtration, ion exchange and biological treatments, etc.) offer opportunities to select the treatment combination that can efficiently and cost-effectively achieve the desired water quality required for the intended application.

- **Risk assessment studies to evaluate impacts on product safety**

Microbiological experiments combined with quantitative microbial risk assessment have potential to provide relevant information regarding the likelihood of contamination with a microorganism of interest in the final product, when reconditioned water has been used in the manufacturing process. Furthermore, post-contamination scenarios can be modelled to implement better controls in the reconditioning treatment. Results from such studies provide the knowledge to establish the maximum acceptable levels of microbial hazards that can be present in a particular water type, as well as the likelihood of people becoming ill by consuming a food product manufactured in processing operations where reconditioned water have been used.

Water reconditioning and reuse could be more attractive for some food plants than others; depending on the production scale, location, technology available, regulations in place, and wastewater treatment cost. But, alternatives for the reduction of water footprints must be evaluated in all sectors, if these companies are willing to continue on the business.

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**CHAPTER 2: FEASIBILITY, SAFETY AND ECONOMIC IMPLICATIONS OF
WHEY-RECOVERED WATER IN CLEANING-IN-PLACE SYSTEMS: A CASE
STUDY ON WATER CONSERVATION FOR THE DAIRY INDUSTRY**

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Abstract

Water scarcity is threatening food security and business growth in the U.S. In the dairy sector, most of the water is used in cleaning applications, therefore; any attempt to support water conservation in these processes will have a considerable impact on the water footprint of dairy products. This study demonstrates the viability for recovering good quality water from whey, a highly pollutant cheese making by-product, to be reused in cleaning in place (**CIP**) systems. The results obtained in this study indicate that by using a combined ultrafiltration (**UF**) and reverse osmosis (**RO**) system 47 % water can be recovered. This system generates protein and lactose concentrates, by-products that once spray-dried fulfill commercial standards for protein and lactose powders. The physicochemical and microbiological quality of the recovered permeate was also analysed, suggesting suitable properties to be reused in the CIP system without affecting the quality and safety of the product manufactured on the cleaned equipment. A cost analysis was conducted for three cheese manufacturing levels, considering an annual production of 1, 20 and 225 million L of whey. Results indicate the feasibility of this intervention in the dairy industry, generating revenues of 0.18, 3.05 and 33.4 million \$/year, respectively. The findings provide scientific evidence to promote the safety of reuse of reconditioned water in food processing plants, contributing to building a culture of water conservation and sustainable production throughout the food supply chain.

Key Words: water reconditioning, water optimization, food industry, membrane filtration

Introduction

Water and food production have such inextricable relation that water scarcity is adversely affecting U.S. agriculture with potential implications for decreasing the food supply and raising food prices (USDA, 2014a). Water shortages and the impact of climate change are risk factors for food security along with the increasing population estimated to reach 9 billion people by 2050 (de Fraiture and Wichelns, 2010). Therefore, water availability for food production will increasingly rely on the sustainable management and use of water in all sectors.

Detailed data on water usage in U.S. dairy processing is not widely available. Nevertheless; published reports from other countries, where water scarcity became a top priority years ago (e.g. Australia), indicate that food industry alone is responsible for 30% of water consumption in all manufacturing combined (Australian Government Department of Agriculture, 2008). Food processing uses only high quality fresh water as an ingredient and for processing steps such as washing, cooling, heating, transportation, and cleaning. The amount of water used in a particular food processing plant varies depending on the size, efficiency of the equipment, plant layout, and culture. The dairy industry uses, 1 to 60 liters of water per kg of processed milk, mainly for cleaning in place applications (28% of total water usage) (Rad and Lewis, 2014).

Proper reconditioning (treatment of water intended to be reused) and reuse of wastewater in the food industry is a promising alternative to current practices of discharging these streams in places where they can negatively affect the environment.

The authors firmly believe that wastewater recondition, using technologies already available for the food industry, can contribute to conservation initiatives without compromising the safety and the quality of the final product.

Current regulations on food hygiene indicate that only potable water can be used for food contact surfaces and equipment cleaning (FDA, 2013, Alimentarius, 2014); whereas the use of reconditioned water is restricted to initial cleaning of vegetables and fruits, and to the scalding water for meat and poultry (USDA-FSIS, 2012). However; processors are willing to expand the applications for reconditioned water to reduce the consumption of this natural resource and minimize environmental impacts (Casani and Knøchel, 2002).

The lack of published data about the implications of using reconditioned water in food processing plants, represents a barrier for water recycling; such information is key to motivate implementation of water conservation initiatives. For that reason, the present study was developed as a holistic approach to provide evidence on the advantages and restrictions of wastewater recondition and reuse; based on the three pillars of sustainability (economic, environmental, and social). The main objective was to demonstrate that high quality water can be recovered from cheese whey, with potential for water reuse in CIP operations. First the performance of the UF and RO system was evaluated based on permeate flux, pressure changes, volume reduction ratio, flux decline, filtration time, rejection and retentate solid content. The cleaning efficiency of the recovered water versus potable water was assessed and finally a cost analysis, for different cheese production scales, was considered to evaluate the feasibility of this

proposed approach in the dairy industry. A diagram of the water recovery system and whey powder production is presented in Figure 1.

Materials and Methods

Water Recovery System Configuration and Operating Conditions

Membrane Filtration. Cheddar cheese whey, produced from standardized whole milk (3.6 % fat), was collected from three different cheese batches (276.5 ± 11 L each time). Whey was collected from a processing plant located in Lincoln, NE, USA throughout the months of September and October 2014. Once collected, the whey was immediately fed to the filtration system, to avoid additional heating or pH changes (initial temperature 33 ± 2 °C). UF and RO filtrations were performed in the model R pilot scale membrane filtration system from GEA Group (Hudson, WI), made entirely of 316 stainless steel. For UF, a semi-permeable polyethersulfone spiral membrane with a molecular weight cut off (**MWCO**) of 10,000 Da and effective area of 5.4 m^2 manufactured by KOCH (Wilmington, MA) was used; with an initial cross-flow rate of 270 L/h and a pressure of 0.3 MPa (3 bar). For RO, a spiral high rejection (98%) membrane manufactured by Filmtec TM membranes (Santa Ana, CA) (RO-3838/30-FF) with an effective area of 7.4 m^2 was used; applying an initial cross-flow of 230 L/h and pressure of 3 MPa (30 bar). The filtration system was set up in a concentration mode (retentate returned to the feed tank), whey was the feed material for UF; while the UF permeate was the feed material for the RO membrane. The filtration times were 60 and 40 minutes for UF and RO, respectively.

For membrane cleaning, the membrane manufacturers' recommendations were followed using a cleaning regime that included enzymatic (Ultrasil 67 by Ecolab®, 11 ml/gal) and alkaline (Ultrasil 110 Ecolab®, 3 ml/gal) washes at 25 °C. Cleaning efficiency was verified by monitoring pH in final water rinses and by comparing water flow rates before membrane use and after cleaning.

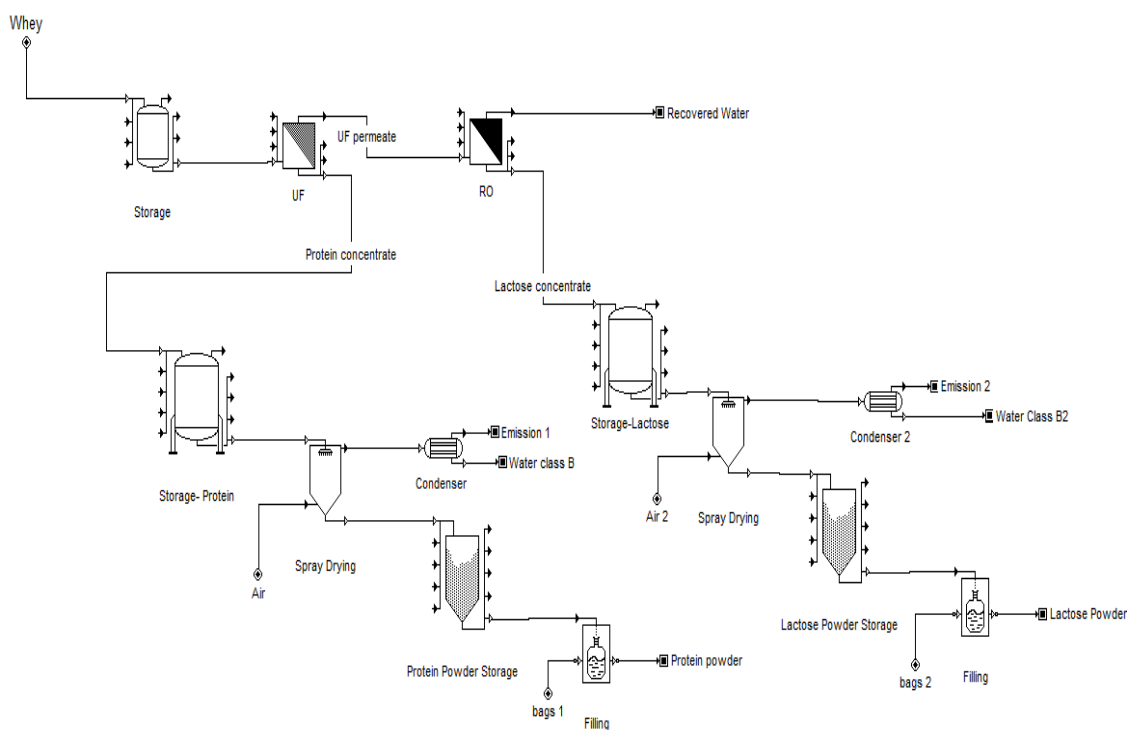


Figure 1. Water recovery system using UF/RO membranes with protein and lactose powder production. Water class B and B2 (condensed water obtained during the spray drying steps), emission 1 and 2 (air leaving the spray drying system)

Filtration Efficiency. Several parameters were monitored during filtration for both UF and RO, including solid content, pressure changes, volumetric water permeate, water flux (J_w), volumetric reduction ratio (VRR), rejection (R), and water recovery (WR). J_w , VRR , R , and WR were determined using equations 1, 2, 3 and 4, respectively.

$$J_w = \frac{1}{A_m} \cdot \frac{\Delta V t}{\Delta t} \quad \text{Eq. (1)}$$

Where, A_m represents the membrane area, t the time, and Vt is the volumetric water permeation at t time.

$$VRR(t) = \frac{V_0}{V_{r(t)}} = \frac{V_0}{V_0 - V_{p(t)}} \quad \text{Eq. (2)}$$

Where V_0 is the initial volume of solution; $V_{r(t)}$ and $V_{p(t)}$ represent the retentate and permeate volume respectively at t time.

$$R_i(\%) = \left(1 - \frac{C_{p,i}}{C_{R,i}}\right) \cdot 100 \quad \text{Eq. (3)}$$

Where, $C_{p,i}$ and $C_{R,i}$ are the concentration values of the i contaminant measured in permeate and retentate, respectively.

$$WR(\%) = \left(\frac{V_p}{V_f}\right) \cdot 100 \quad \text{Eq. (4)}$$

Where, V_p and V_f are the volumes measured in permeate and feed, respectively.

Spray Drying. The UF and RO concentrate streams were further spray dried to obtain protein powder and lactose, respectively. The operating conditions for the pilot scale spray dryer (Henningsen, Model T-20) were the following: feed flow of 0.16 L/min, air pressure of 0.17 MPa (25 psig), furnace temperature of 310 °C and outlet air temperature of 105 °C. Total solid, fat, protein, lactose, water activity and moisture were evaluated on the powders obtained, following the methods described below.

Analyses. Physicochemical analyses were performed on the initial feed, permeate and concentrate UF/RO streams. All samples were analyzed using the American Public

Health Association (**APHA**) recommended methods as described by Rice et al. (2012). Chemical oxygen demand (**COD**) was digested using the closed reflux method and analyzed on a Perkin Elmer Lambda 25 spectrophotometer while total organic carbon (**TOC**) was measured on preserved samples using hot persulfate oxidation on an OI Analytical model 1020 TOC analyzer. Conductivity was measured using a Fisher Accumet meter. Nitrate and nitrite were measured using the Cd-reduction method and ammonia by phenate colorimetry using a Seal Analytical AQ2 discrete chemistry auto analyzer. All instruments were calibrated immediately before analysis, and quality verified using analysis of laboratory duplicates, fortified blanks and method blanks.

Proximal composition was determined by measuring total solids (Ahn et al., 2014), fat, protein (Nitrogen analyzer- LECO F528) and lactose (following manufacturer instructions Sigma-Aldrich MAK017). The fat content was determined by adapting the method from Hildebrandt et al. (2011) with a variation on sample preparation, where 20 μ l of sample were mixed 980 μ l of tween 0.5% solution, then 50 μ l of the mixture was added to the 96 well plate.

Finally, aerobic plate counts (**APC**) and *E.coli*/coliform testing were performed on the initial whey and RO permeate (recovered water). APC was performed by plating samples onto Standard Methods Agar (**SMA**) (Acumedia, Lansing, MI) plates, using the spread technique and incubated for 48 h at 32 °C. The number of viable *E.coli* was determined by plating onto *E.coli*/coliform Petrifilm™ (3M, St. Paul, MN) following and incubation period of 24 h at 37 °C.

Biofilm Formation and Water Reuse in CIP

A CIP regime was simulated to compare the cleaning effectiveness of the recovered water against potable water. For these experiments, a constant biofilm of *Pseudomonas aeruginosa* (# 1063/2783 FPC microbiology laboratory collection) was formed by inoculating the bacteria into a CDC bioreactor (Biosurface Technologies Corp, Bozeman, MT) containing 316 stainless steel coupons, following a standardized procedure (ASTM International, 2012). Contaminated coupons were cleaned following a *standardized CIP regime* (described later); cleaned coupons were then sampled for bacterial counts, before (3 coupons) and after (6 coupons) the CIP procedure. Bacteria counts were done by aseptically removing the coupons from the holders, and placing them into 9 ml dilution water tubes to eliminate any planktonic bacteria; coupons were immediately transferred to new 9 ml dilution water tubes and sonicated for 4 minutes, using an ultrasonic cleaner (Branson®[®], Model 1210). After sonication, tubes were mixed and samples were aseptically plated on SMA using the spread technique and incubated for 48 h at 37 °C. This experiment was performed three times for each water type. Student's *t*-test, assuming unequal variance, was used to compare the mean levels of bacteria enumeration before and after CIP regime. The significance level of $\alpha = 0.05$ was chosen for these tests.

Additionally, scanning electron microscopy (**SEM**) images were taken on stainless steel coupons before and after CIP, to obtain a closer observation of the biofilm and the effect of the CIP procedure on the surfaces. The Karnovsky's Fixative solution (EMS, Hatfield, PA) was used to prepare the coupons for SEM imaging. Coupons samples were

fixed by 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and further fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 hour. Samples were dehydrated in a graduated ethanol series to 100%. The specimens were subjected to critical point drying in a critical point dryer (Samdri-795), coated with gold/palladium in a sputter-coating apparatus (Technics Hummer Sputter Coater) in order to be observed under the scanning electron microscopes (Hitachi S3000N) at the UNL's Microscopy Core Facility.

Basic water quality analyses including hardness, alkalinity, total and free chlorine as well as APC were performed to monitor and compare the initial quality of the potable and recovered water used on these experiments. For water quality analyses, AquaCheck test strips (HACH, Loveland, CO) were used.

Standardized CIP Regime. The CIP regime consisted of an initial 5 min water rinse (25 °C), a 10 min wash with caustic cleaner (30 g/L, 65 ± 1 °C) (Spartan ®), followed by a 5 min water rinse (25 °C), a 10 min wash with an acidic solution (6 g/L, 65 ± 1 °C) (Spartan ®), and a final 5 min water rinse (25 °C).

Cost Analysis

In order to provide insight about the investment, revenue, and savings that the proposed water conservation initiative could represent to cheese manufactures, a cost analysis was performed. The entire process was simulated using the SuperPro Designer® v9.0 software (Intelligen, Inc., Scotch Plains, NJ), including the UF/RO system for water recovery, spray driers for powder production and packing equipment (Figure 1).

Membrane cleaning operations were not considered for this analysis. A batch operating mode and an annual operating time of 7920 h were selected for the analysis. The simulation was performed for three cheese production levels (0.2, 4 and 43 million pounds/year) generating 3,521; 62,815 and 687,412 L of whey/day, which correspond to real Cheddar cheese production levels in Wisconsin (USDA, 2013), considering 10% cheese yield. Input data necessary to run the simulation regarding permeate flux, and recovery (permeate/feed) were 16.0 L/m²h and 73.80% for UF and 12.80 L/m²h and 64.8% for RO respectively; all data were obtained from the experimental results generated in this study. To run the material and mass balances, the software requires the composition of a stock mixture (whey); such data were obtained from the proximal analyses performed on whey samples used for each filtration (proximal composition reported later). Software default costs were used for equipment, whereas membrane prices were obtained from manufacturers (65 and 44 \$/m² for UF and RO, respectively).

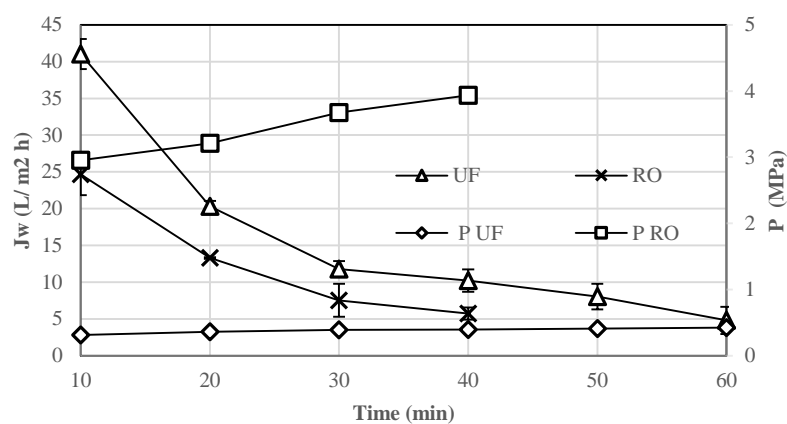
Additional price data for protein and lactose were obtained from published reports (USDA, 2014b); while whey cost (0.07 \$/L), water (0.5 \$/m³), energy (0.1 \$/kW-h), steam (12 \$/MT), chilled water (0.4 \$/MT) and wastewater treatment (0.01\$/L) were obtained from local providers in Lincoln, NE. The total cost estimation includes only items related to direct fix cost (**DFC**) (piping, instrumentation, insulation, electrical facilities and equipment installation). Other costing related to construction, yard improvements, buildings, contractors' fee, and contingency were not included; since the proposed intervention is aimed to be applied on existing plants. Whereas the annual operating cost, working capital, and start-up cost were estimated by the software based on

labor, facility, consumables, and utilities costs. Membrane life used for the simulations were 1,000 and 2,000 operating hours for UF and RO; respectively. The estimated annual revenue resulting from the protein and lactose powder sales (\$ 87.19 and \$ 27.74 per 25 kg package, respectively) and recovered water (0.50 \$/ m³). The reports generated by the software also include the internal revenue rate (**IRR**), payback time (**PBT**), and net present value (**NPV**) among other financial indicators; these values were determined by the cash flow analysis at the base of 15-year project lifetime, 4% inflation, direct fix cost (**DFC**) outlay of 30%, 40%, 30% for the first three years of the project; respectively. All these economic parameters correspond to software default values for the version used. Additional information about the model design steps has been described in a book chapter by Petrides (2014).

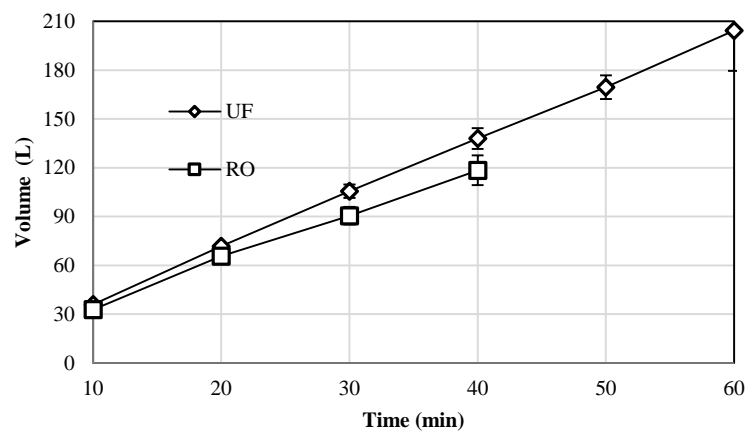
Results and discussion

Process Efficiency

Different parameters such as permeate water fluxes, volumetric water permeation, pressure changes, total solid content were monitored to evaluate the efficiency of the UF/RO membrane system for water recovery and for protein and lactose concentration. Results are shown in Figure 2 (a), (b), (c).

a) Permeate water fluxes (J_w) and pressure (P) changes

b) Volumetric water permeation



c) Volumetric water permeation

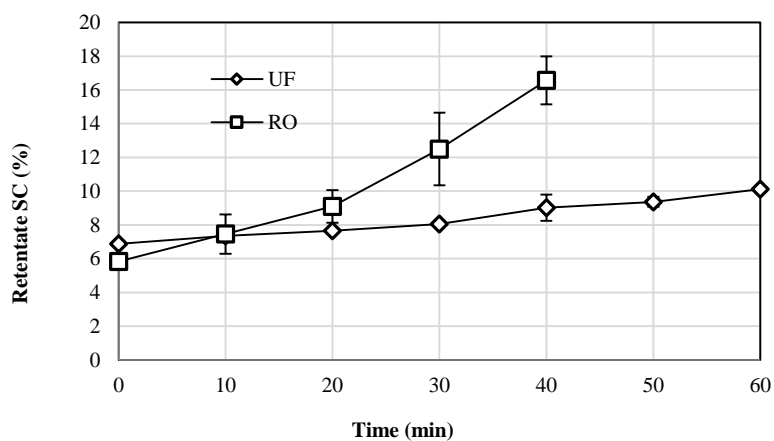


Figure 2. Process efficiency parameter control during filtration. a) Permeate water fluxes and pressure changes, b) Volumetric water permeation, c) Retentate solid content

a) Permeate Water Fluxes and Pressure Changes. Reduction in water fluxes and pressure increments observed in Fig.2(a) are direct result of concentration polarization and membrane fouling (Luo et al., 2012). Concentration polarization is the reversible accumulation of solute molecules in the solution near the membrane surface; whereas fouling is irreversible (Dickson, 2015). Membrane properties such as pore size and materials play a role on flux decline at the beginning of the filtration, however; as the filtration progresses the flux decline is controlled by the deposition of foulants (fat, proteins, lactose and minerals) and their interaction within the membrane (Carić et al., 2000). Whey proteins can easily bind calcium phosphates to form complex organic-inorganic aggregations, thus when both elements are present in the feed material these complex bridges are formed resulting in flux decline and can be associated with the continued concentration polarization and fouling (Luo et al., 2012). Given that UF removes whey proteins from the feed, the flux decline for the RO filtration can be associated to the continue concentration of lactose in the retentate stream. As shown in Fig.2(a) the final water flux for UF was $4.80 \pm 1.85 \text{ L/m}^2\text{h}$ at minute 60; while for RO was $5.71 \pm 0.82 \text{ L/m}^2\text{h}$ at minute 40. The average water flux for the entire UF filtration was $16.03 \pm 1.50 \text{ L/m}^2\text{h}$, while for RO the average was $12.80 \pm 1.51 \text{ L/m}^2\text{h}$. Both values were used later on as inputs for the cost analysis.

Pressure levels were kept within optimal ranges 0.3-0.5 MPa (3-5 bar) for UF and 3-4 MPa (30-40 bar) for RO, as recommended in the literature (Rektor and Vatai, 2004, Vourch et al., 2008, Luo et al., 2011). On our preliminary observations (data not shown) it was detected that exceeding the upper pressure limit increased the solid content on the

permeate (5.6 times higher solid content than when operated within established conditions), which affected the performance of the next filtration. This phenomenon is explained by (Luo et al., 2010) as the transport by diffusion of salt ion through the membrane. Diffusion is higher when salt concentration in the membrane is higher, which results from the accumulation of solutes in the feed, resulting in lower permeate fluxes, higher VRRs and higher pressures. Since quality of the recovered water (RO permeate) was of special importance for this study, permeate fluxes and pressure were critical factors to control during filtration processes.

b) Volumetric Water Permeation. Permeate volumes for UF were always higher than for RO, due to the difference in pore size. The final volume recovered for UF was 204.4 ± 24.78 L, while for RO 118.5 ± 9.21 L were collected; representing a recovery of 73.80 ± 6.81 % and 64.77 ± 7.43 % respectively, with respect to the initial feed material (whey for UF and UF permeate for RO). At the end of the filtration a VRR of 5.47 ± 1.49 and 5.18 ± 2.91 were calculated for UF and RO, respectively. The final recovery for the UF/RO filtration system resulted on 47.03%.

c) Retentate Solid Content. To study the concentration effect on the retentate, the solid content was monitored at different time points during the filtration, as shown in Fig. 2(c). Time 0 represents the solid content of the feed material, for UF the solid content on whey was 6.87 ± 0.02 %; while for RO the solid content on the UF permeate used as feed was 5.83 ± 0.02 %. The final concentration reached on the retentate streams were 10.11 ± 0.17 % and 16.57 ± 1.42 %, respectively.

The process efficiency parameters presented above, together with the water quality and microbial analyses indicated in Table 1 demonstrate that the procedures and operating conditions applied on the UF/RO filtration system described herein, effectively concentrate whey proteins and lactose while allowing water recovery with high quality characteristics.

Results shown in Table 1 point out the pollutant potential of whey, which presents high values of conductivity, TOC and COD due to the presence of protein and lactose. As it can be expected, the values of these parameters increased on the retentate streams and decreased on the permeates. However, the initial COD on whey was only reduced by 28.3% by the UF filtration, this is attributable to the membrane pore size which retains proteins, but is not effective in lactose rejection (Rosenberg, 1995). The combined effect of the RO membrane allows to reach a rejection level of 98.1% and 99.7% for conductivity and TOC, respectively.

Table 1. Water quality analyses for whey, concentrates and recovered water obtained from the UF/RO system

Parameter ²	Unit	Whey ¹	UF ¹			RO ¹		
			Retentate	Permeate	R (%)	Retentate	Permeate	R (%)
Conductivity	μS/cm	4,287	3,753	4,003	6.61	7,380	79.9	98.1
Ammonia (NH ₄ -N)	mg/L	36.2	79.7	9.95	72.5	21.4	0.19	99.5
Nitrate (NO ₃)	mg N/L	0.49	1.19	0.07	85.6	0.16	0.01	97.6
Nitrite (NO ₂)	mg N/L	0.15	0.10	0.04	70.8	0.04	0.02	84.7
TOC	ppm	23,637	36,118	12,640	46.5	35,057	71.7	99.7
COD	mg/L	84,022	159,583	60,267	28.3	164,800	-	-
Microbial quality								ROPC
APC	Log ₁₀ (cfu/mL)	7.2	7.7	3.0		3.5	1.5	<1
Experimental water recovery (permeate/feed)								
		UF	RO	Recovered water (UF/RO ³)		UF/RO and condensed water ³		
	%	73.8 ± 6.81	64.8 ± 7.43	47.0 ± 1.10		85.6 ± 10.7		

¹Data presented in the table represent the mean values of samples collected from three filtrations.

²APC: Aerobic Plate Counts, COD: Chemical Oxygen Demand, R: Rejection, RO: Reverse Osmosis, TOC: Total Organic Carbon, UF: Ultrafiltration, ROPC: RO Permeate Chlorinated.

³ Values calculated from the total permeate of the referenced operations and the initial whey

Similar results have been reported earlier, suggesting recovering water of good quality; nevertheless implications of water reuse was not assessed (Aydiner et al., 2014). The high initial APC counts (7 log CFU/ml) found in whey samples were expected, due to the starter bacteria added during cheese manufacture. UF membrane was able to reduce the bacteria load by 3 log CFU/ml, and after the RO filtration 1.5 log CFU/ml were detected in the permeate. These results are consistent with another water recovery study (Singapore Government, 2002); however there is also vast literature indicating the use of membranes for complete bacterial removal (Daufin et al., 2001, Madaeni et al., 2011),

differences might be due to the membrane type and pressure ranges used during filtration. The water recovered in the first phase of the study (RO permeate) was then used for the CIP experiment described later, therefore chlorination (1ppm) within the WHO (2003) and EPA (1999) standards for drinking water was necessary to assure complete disinfection of the recovered water, for a final APC count of $< 1 \log \text{CFU/ml}$. Initial whey and recovered water were also tested for *E.coli*/coliforms presence, but the results were $< 1 \log \text{CFU/ml}$ in both cases. It is important to highlight that only cheese-whey originated from pasteurized milk was used in the present study, therefore pathogenic bacteria were not present on the evaluated samples, as demonstrated by the microbial results.

Spray Drying

The concentrates obtained from the UF/RO filtration system, were spray dried to produce protein powder, lactose powder and to determine the amount of condensed water that can be recovered from this processing step. Table 2 summarizes the values obtained from the proximate analyses for whey, concentrates and powder samples. The 29.5% protein content found in the protein powder falls within the Codex Alimentarius (2010) and USDA (2003) standards for whey protein concentrate, as parametric values of pH, moisture, and fat. The reference lactose content for whey powder, according to the Codex Alimentarius, is higher (61%) than the 41% observed in the sample. For the present study the RO concentrate (mainly lactose) was not mixed with the UF concentrate for the drying step. Therefore, a reduced level of lactose was found in the protein powder, while

63.7% was observed in the lactose powder. These powders presented a final moisture content of 2.13 % and 1.55 %, respectively. The water removed from the concentrated streams in the drying operation, contributed to an overall water recovery of 85.65% for the entire system (UF/RO and spray drying).

Table 2. Proximate composition of whey, concentrate, protein and lactose powders

Parameter	Units	Whey ¹	UF ¹	RO ¹	Protein powder	Lactose powder	Codex	USDA
pH	-	6.56	6.49	6.35				
Fat	%	0.02	0.06	ND	0.08	0.02	2	<10
Total protein	%	0.49	2.46	16.6	29.5	2.60	>10	>25
Lactose	%	5.62	9.71	15.0	41.0	63.7	61	NI
Total dry matter	%	6.87	10.1	16.6	97.9	98.5	NI	NI
Water activity	-	-	-	-	0.07	0.06	NI	NI
Moisture	%	93.1	89.9	83.4	2.13	1.55	<5	<5
Density	g/ cm ³	1.03	1.03	1.07	-	-	NI	<7

¹Average values obtained from samples collected from the concentrate streams at the end of the filtration. UF: Ultrafiltration, RO: Reverse Osmosis NI: Not Indicated

Protein and lactose powders produced from UF/RO concentrate seem to have potential for commercialization as ingredients, while the condensed water (water class B and B₂) can be reused for outside plant applications (cleaning trucks, outside floors and walls), or reconditioned by an additional polishing RO step and chlorination to be reused in other operations demanding higher water quality.

Reuse of Whey-recovered Water in CIP Operations

The risk of contamination is an important hurdle for water reconditioning and reuse initiatives in processing plants, especially when food-contact surfaces are involved. Thus,

the next objective of this study was to determine the cleaning efficiency of chlorinated recovered water obtained from the UF/RO system, when used in CIP operations. Figure 3 shows the results comparing CIP performed with potable water and recovered water; the initial biofilm of *Pseudomonas aeruginosa* was similar for both treatments (8.6 log cfu/cm²). After the CIP regime, 0.99 and 1.09 log cfu/cm² were detected for potable water and recovered water, respectively. The SEM images (Figure 4) taken before cleaning (A, C) show uniform biofilms for both treatments; after cleaning (B, D) few inactive bacteria debris were observed on the stainless steel surfaces. The student's *t*-test results did not show significant differences between the cleaning efficiency reached with potable water and recovered water (*P*-value 0.87; α 0.05). Besides microbial quality, hardness, alkalinity, pH, total and free chlorine were tested on potable and recovered water used for the CIP experiments. Parametric values of APC, total and free chlorine were similar for both water types (< 1 log cfu/ml, <0.5 ppm, <0.5 ppm, respectively); pH showed minimum differences 7.72 and 7.23 for potable and recovered water, respectively. However, important differences on hardness and alkalinity were detected; presenting values of 250 and 25 ppm for potable water and 200 and 40 ppm for recovered water, respectively.

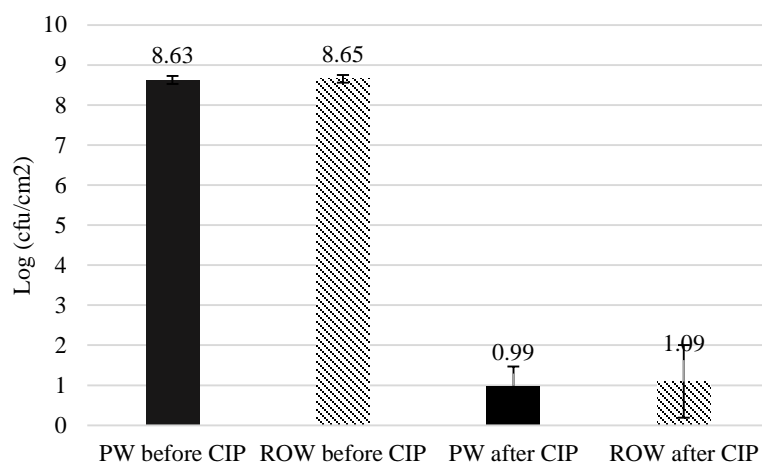


Figure 3. Mean values of *Pseudomonas aeruginosa* adhesion before and after CIP regime. PW: Potable water; ROW: Recovered water

These findings demonstrate that, if properly done, water recovered from whey can be reused in CIP operations without compromising the safety or quality of the final product; while protecting equipment from the negative effects of hard water.

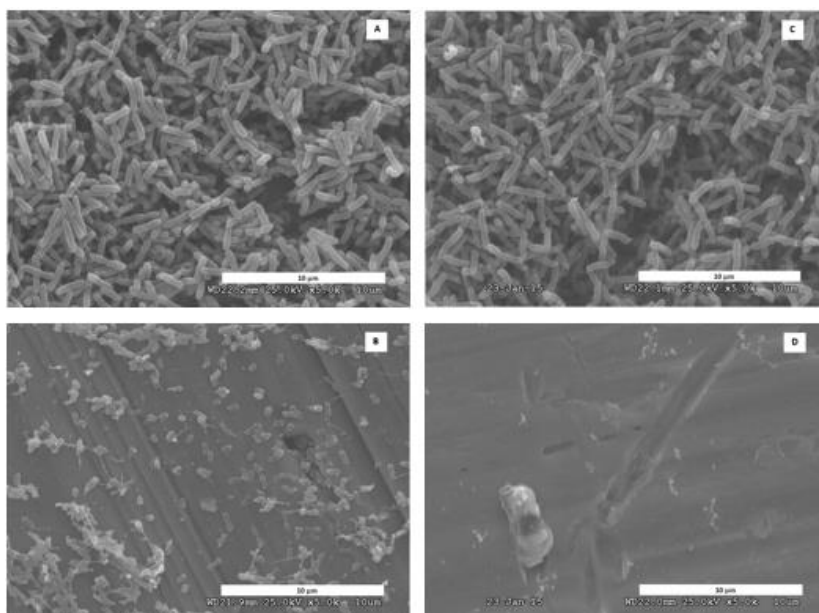


Figure 4. SEM images of *Pseudomonas aeruginosa* biofilm before and after CIP. (A and B) potable water, (C and D) recovered water. Resolution 5.0kCost Analysis Results

The diagram of the integrated UF/RO water recovery system, whey powder production and packing is represented in Figure 1. The cost analysis covers these processing operations, for three production levels (1, 20 and 225 million L of whey/year). Whey is the initial feed for the UF filtration, for which an opportunity cost of \$ 0.07/L was considered. UF permeate was fed to the RO membrane for lactose removal, generating the recovered water intended for reuse in CIP systems. As it can be observed in Figure 1, protein and lactose concentrates were separately spray dried, to obtain protein and lactose powders packed in 25 kg bags. The condensed water generated from the drying step (water class B and B₂) was accounted for the water recovery percentage on the entire system; no further treatment was considered for this water type. Still, depending on the desired reuse for this water type, additional chemical (e.g. chlorination), or physical treatment (RO polishing step) could be added. Mass balances for the modeled cheese production levels and the contributions of each product to the total revenue are presented in table 3.

Table 3. Modeled performance and revenue contributions for small, medium and large cheese manufacturing plants

Streams	Small	Medium	Large	Revenue price (\$)	Contribution to Revenue (%)
	(kg/batch)				
Whey	3,512	62,482	683,771	-	-
Protein concentrate	920	16,370	179,148	-	-
UF permeate	2,592	46,111	504,623	-	-
Lactose concentrate	912	16,231	177,627	-	-
Recovered water (permeate RO)	1,679	29,880	326,996	1.90/ 1000 gal	0.16
Protein powder	104	1,866	20,425	87.2/entity ¹	70.0
Lactose powder	140	249	27,262	27.7/ entity ¹	29.7
Water class B and B ₂ (condensed water)	1,470	26,160	286,289	0.95/1000 gal	0.07

¹ Entity: one 25 kg bag of protein or lactose powder

The results shown in table 4 indicate that a total of \$ 2.04, 6.72 and 56.3 million should be invested in order to process the total cheese whey generated by the small, medium and large cheese production scales, respectively. The payback time estimated for the different investments are 10.9, 5.17 and 4.36 years, with an IRR after taxes from 2.42 to 19.5 % for the simulated scenarios. The operating cost resulted in \$ 0.17, 1.87 and 20.1 million/year. Finally, the selling of protein powder, lactose protein and recovered water would generate an annual revenue of \$ 0.18, 3.05, and 33.4 million, for small, medium and large scale plants; respectively. For future studies, it is necessary to include the associated costs to membrane cleaning, thus, allowing for a better estimate of the total investment and annual operating cost. These costs were not included herein.

Table 4. Economic results of the whey recovered water system for different cheese production levels

Whey (Million L/year)	Investment (Million \$)	Revenue (Million \$/year)	Operating cost (Million \$/year)	IRR ¹ (%)	PBT ² (years)
1	2.04	0.18	0.17	2.42	10.9
20	6.72	3.05	1.87	15.9	5.17
225	56.3	33.4	20.1	19.5	4.36

¹IRR: Internal Revenue Rate, ² Payback time

When the cheese whey is not treated for by-product recovery, it is disposed in the municipal wastewater stream, in which case a fee should be paid by the dairy plant. Based on the rate for the state of Nebraska and the COD level on the whey samples, the fee was calculated to be \$ 40 per unit disposed (1 unit =780 gal). The savings for not

paying the municipal fee were not included in the cost analysis discussed earlier; but their impact on the total investment during the payback time is described in Figure 5.

The horizontal axis on Figure 5 represents 10% increments over the actual wastewater treatment price (\$ 40/unit disposed); while the vertical axis indicates the percentage of the total investment covered by the savings generated during the corresponding payback time, for the three described production scales. At point 0 %, the water treatment fee is the current cost calculated for Nebraska; using this cost 23.7 % of the total investment, for large scale production, could be covered by the savings generated during the payback time.

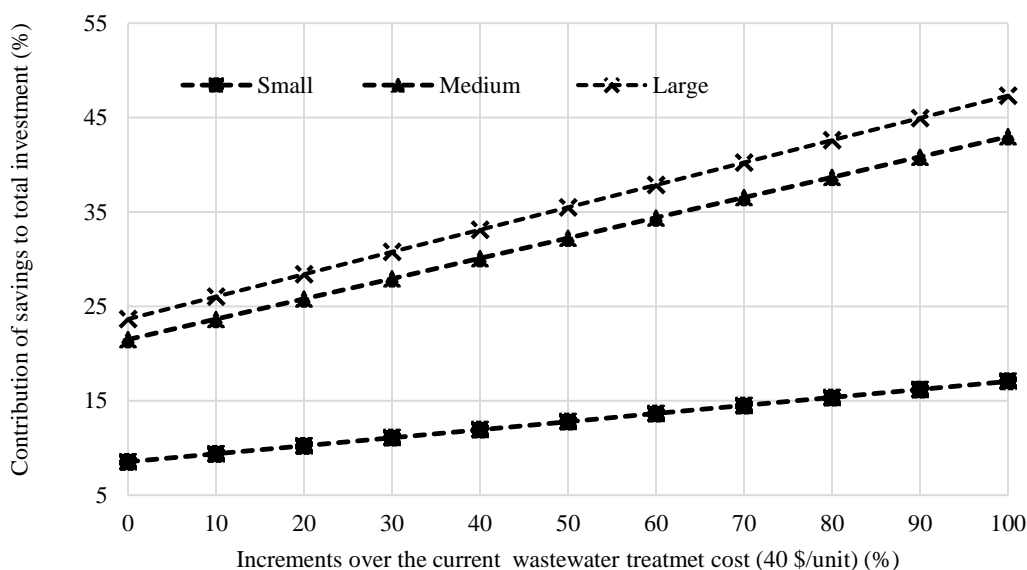


Figure 5. Contribution of savings to the total investment during the payback period, and the impact of wastewater treatment price increments over the current price; for three cheese production scales

Wastewater treatment fees are subject to increase due to location, wastewater specific characteristics, and changes in environmental regulations. If this fee doubles the actual price (100 % increase), the savings would cover as high as 47.3 % of the large scale investment (Figure 5). The same analogy was followed for small and medium scale production.

The contribution that the yearly savings would have over the annual operating cost, after the payback time, were also evaluated. Likewise the previous graph, Figure 6 shows the increments on the wastewater treatment fee versus the operating cost percentage that could be covered by the annual savings, for the three different scales after the payback time. For medium and large scale, savings contribution were similar, representing 14.9 % and 15.2 % of the annual operating cost, respectively using the current fee price. When the fee increases by 100 %, savings contribution increases to 29.8 % and 30.5 % for each scale production respectively. Savings contribution was lower for the small cheese production scale, reaching 9.51 % at the actual fee cost and 19.0 % when the wastewater treatment fee doubles.

Water reconditioning and recycle initiatives in food processing plants reduce water usage and wastewater volumes; consequently environmental impacts are reduced and more water is available for the community. Assuming the scenario of a highly efficient large scale plant, where 1 L of water is used for every 1 L of milk processed, then 80 % of its water demand could be supplied by the water conservation initiative proposed herein.

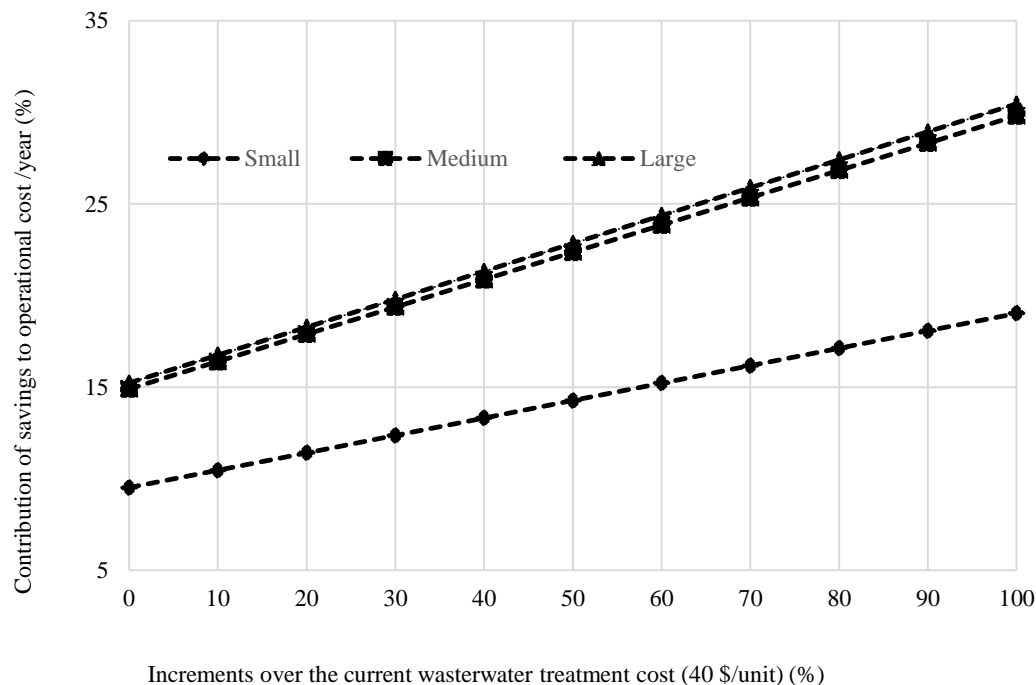


Figure 6. Contribution of savings to the annual operating cost, and the impact of wastewater treatment price increments over the current price; for three cheese production scales

Furthermore, the annual domestic water demand of up to 1,540 people in Nebraska (per capita water use 95 L/day) could be satisfied by the potable water that is not consumed in food processing operations.

Conclusions

The feasibility of water recovery from whey and reuse in CIP operations has been explored from different perspectives including technological efficiency, safety and financial needs. The most relevant findings from this study are:

1. A water recovery of 47.03% was obtained from the UF/RO filtration, whereas 85.65% was reached including the spray drying step as part of the integrated system.
2. The cleaning efficiency of the water recovered from the UF/RO filtration system is comparable to potable water, when used in CIP systems.
3. Good quality lactose and protein powders with commercial value were obtained from the concentrated streams.
4. The proposed intervention is economically feasible for different cheese production scales.
5. Water conservation initiatives in food industries have potential for sustainable production, without competing with the community for the use of natural resources.

The results obtained represent an important contribution to fill the gap of information related to the implications of water reuse and recycling for inside-plant applications. In this respect, complementary future work will focus on describing possible post-reconditioning treatment contamination of the recovered water based on a risk assessment approach, and a broader evaluation of the environmental impacts based on the life cycle assessment methodology.

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**CHAPTER 3. LIFE CYCLE ASSESSMENT OF AN ALTERNATIVE WATER
RECOVERY SYSTEM, FOR WATER CONSERVATION IN THE DAIRY
INDUSTRY**

Abstract

In order to comply with current environmental regulations, the food industry continuously seeks opportunities to reduce waste, water and energy usage; practices that promote more efficient and sustainable operations. Water reconditioning and reuse have been proposed as an alternative for food companies to reduce their water usage and wastewater footprint, however implementation of such initiatives could produce additional environmental impacts. Therefore, the objective of the present study was to conduct a comparative life cycle assessment of the water recovery system (WRS), proposed for the treatment of cheese whey and the recovery of water, versus wastewater (WWT) and water production processes (WPP). The study revealed that proposed system, offers reductions ranging from 19 % - 62 % in some environmental impacts including: photochemical oxidant formation, water eutrophication, human toxicity and ecotoxicity, in comparison to current practices of sending whey to a wastewater treatment plant to process a unit (780 gal) of wastewater with an organic load similar to whey. Whereas, for the second system comparison (WRS vs. WPP) 18% lower environmental impacts than the water production plant were observed for the water recovery system to produce one unit of water. Among the different inputs required for the operation of the water recovery system, electricity was identified as the one responsible for the majority of the environmental burdens.

Key words: water-energy nexus, food waste, water reconditioning, membranes.

Introduction

As with any other businesses, the food industry looks to maximize profit margins. However, food processing operations are affected by a dynamic set of factors that could become real constraints to fulfill that objective. Water scarcity and wastewater management are among those factors, since they could potentially affect normal plant operations and force communities and the food industry to compete for resources. Therefore, sustainable management of water and wastewater are must-have factors, for food companies to stay in business and expand their activities (Peter Schulte, 2014).

As demonstrated in our previous work (Meneses and Flores, 2016), food processing operations have potentials for water reconditioning and reuse. The water recovery system proposed in this study used a combination of ultrafiltration (UF) and reverse osmosis (RO) membrane systems to separate water and by-products of concentrated protein and lactose, which were later spray-dried to obtain protein and lactose powders. Membrane replacement, power and chemical usage for membrane cleaning have been frequently mentioned to be important constraints for the implementation of this technology (Owen et al., 1995, Shannon et al., 2008). In our published study, system performance, water and by-product quality and utilization, as well as the cost associated with the intervention were evaluated. The published results reflect the potential of water reconditioning and reuse to reduce the water footprint of dairy products; however, the overall environmental impacts were not analyzed therein.

In the past, energy and water systems have been assessed independently. Nevertheless, today the efforts of public and private sectors are focused towards the integration of energy use in water management strategies to address the challenges and opportunities of the water-energy nexus (Rothausen and Conway, 2011, U.S. Department of energy, 2014).

Life cycle assessment (LCA) has been proposed as a metric tool to examine energy and water scenarios (Dale, 2013). LCA is a methodology used to evaluate from a complete perspective the environmental impacts associated with a product, process or service for human consumption or use (ISO 14040, 2006). LCA, applied as a tool to evaluate water conservation alternatives, provides the key information to improve the system; therefore effectively directing the efforts to specific hot spots. In addition, LCA can help on decision making processes and assist on the selection of relevant environmental indicators (ISO 14040, 2006). According to the ISO standard (ISO14044, 2006), four phases could be comprised in a LCA study a) goal and scope, b) inventory analysis phase, c) impact assessment and d) interpretation. All these phases have been considered in this study with the aspiration to offer, along with our previous work, meaningful information for an all-inclusive alternative towards water conservation in cheese production in the dairy sector.

One of the main objectives of this research was to perform an LCA of the water recovery system proposed in our published study (Meneses and Flores, 2016), which has a double effect in both treating wastewater (whey) and producing reclaimed water for

reuse in other dairy processing operations. The other objective of this research was to compare the environmental burdens of this proposed water recovery system against those produced by the current practice in treating organic matters in wastewater and producing industrial water. Moreover, the LCA conducted for the water recovery system allowed to explore opportunities for further improvement on the environmental efficiency of the system.

LCA has been applied to determine the environmental impacts of the production system of different food products such as: milk and beef (Cederberg and Stadig, 2003), cheese and whey production (Kim et al., 2013), and fisheries (Ayer et al., 2007). It has also been applied to evaluate wastewater treatment plants (Corominas et al., 2013) and potable water production (Friedrich, 2002, Bonton et al., 2012). However, to our knowledge, limited documented studies have reported to use the LCA approach to assess water reconditioning and reuse in the food industry. Therefore, it is our belief that the findings from this study will not only uncover opportunities for improvement in the water recovery alternatives, but also provide some guide to incorporate LCA in the assessment of sustainable strategies for a more efficient food production chain.

Methodology

Goal and scope of the study

The main goal of this work is to provide relevant and complementary environmental information to support the implementation of whey–recovered water for reuse in CIP systems, as proposed in our previous study (Meneses and Flores, 2016). The LCA study

provides insights to cheese manufacturers about the impacts on different environmental indicators. Besides the water footprint reduction, the overall sustainable performance of the water conservation initiative was incorporated into the decision-making process.

The scope of the project includes a gate-to-gate analysis, with particular emphasis on whey treatment after cheese manufacture. The process operations included in the analysis are those required for water recovery from whey (membrane filtration system) and the subsequent production and packaging of protein and lactose powders (produced by spray drying). Total annual consumables for the large-scale cheese plant producing 225 million L of whey/year, simulated in the refereed study (Meneses and Flores, 2016); were considered in the analysis. Such data was obtained from the itemized breakdown report generated in SuperPro designer (not disclosed in the study but presented here), including membrane replacement, total energy consumption, water and chemical inputs for cleaning-in- place (CIP) operations, steam and chilling water for drying and condensation during protein and lactose production.

By implementing the proposed water conservation alternative, the dairy plant evades wastewater treatment of whey and consumption of new fresh water for cleaning operations. Therefore results from life cycle inventory (LCI) from the water recovery system (WRS) were compared to the wastewater treatment (WWT) of whey and production of potable water. The input data on a yearly basis for wastewater treatment and production of potable water were obtained from local plants.

The findings from the present study expose the components of the evaluated systems that generate the highest impact, on which further improvement is required. Results are intended to be shared with the scientific community and other public and private sectors associated to the food industry, to contribute in the evaluation and implementation of water conservation initiatives.

Functional Unit

The ISO standard indicates that the functional unit provides reference to relate the inputs and outputs of each operation (in this case) and that the functional unit is necessary to ensure comparability of results among systems on a common basis. This is particularly complex for the systems evaluated in the study, since they engage different raw materials and generate different outputs. In general terms, the proposed whey-recovery system uses cheese-whey as raw material and generates water, protein and lactose as main outputs. The wastewater treatment plant receives the whey as general industrial wastewater and separates the solid portion from water. Solids are then sent to agricultural land, while water is returned to the environment. Potable water production takes water from the environment and the complexity of the treatment depends on the initial water quality; while the outflows generated from the operation depends on the selected treatment. Consequently, choosing a single functional unit to compare the three systems (WRS, WWT and WPP) at the same time was not possible. Instead, the evaluation of the treatments was performed based on a more general unit, **780 gal** of water and wastewater, which is commonly used by the water service and the wastewater treatment plant to

charge for their services. The same unit was selected in our published study to determine the contribution to the savings that the cheese processing plant could potentially obtain by implementing the proposed water conservation alternative. Therefore, choosing this unit is consistent with the collective evaluation of the proposed system.

System boundaries

Given that the objective was to evaluate and compare the environmental impacts associated with the operations included in the water recovery system (WRS), wastewater treatment (WWT) and water production (WPP) plant, a gate-to-gate approach was selected for this study. Nevertheless, differences among the systems will be observed on the treatment options to manage whey (WRS vs. WWT) and the recovery of water from whey (WRS vs. WPP).

The system boundaries for the water recovery system comprised the following unit operations; membrane filtration (including membrane cleaning), spray drying and packaging of protein and lactose powder. Treatment of the aqueous waste from the membrane cleaning and final membrane disposal were also considered. Environmental impacts associated to milk production, cheese manufacturing and transportation are not altered by implementation of the water recovery system and therefore not included in the evaluation.

The system boundaries for the wastewater treatment and production of fresh water, were also set around the unit operations required for treatment of wastewater and

production of water, respectively. Inputs were provided for each system as a whole, thus impacts for individual operations were not determined.

In order to conduct a fair comparison among the systems, only annual consumables were included. Equipment manufacturing and plant infrastructure were not considered in any of the systems due to the lack of detailed information and the fact learned from previous LCA studies that environmental impacts associated with the construction are negligible in comparison to the operating phase (Hancock et al., 2012). Figure 1a and 1b outlines the boundaries for the systems mentioned above.

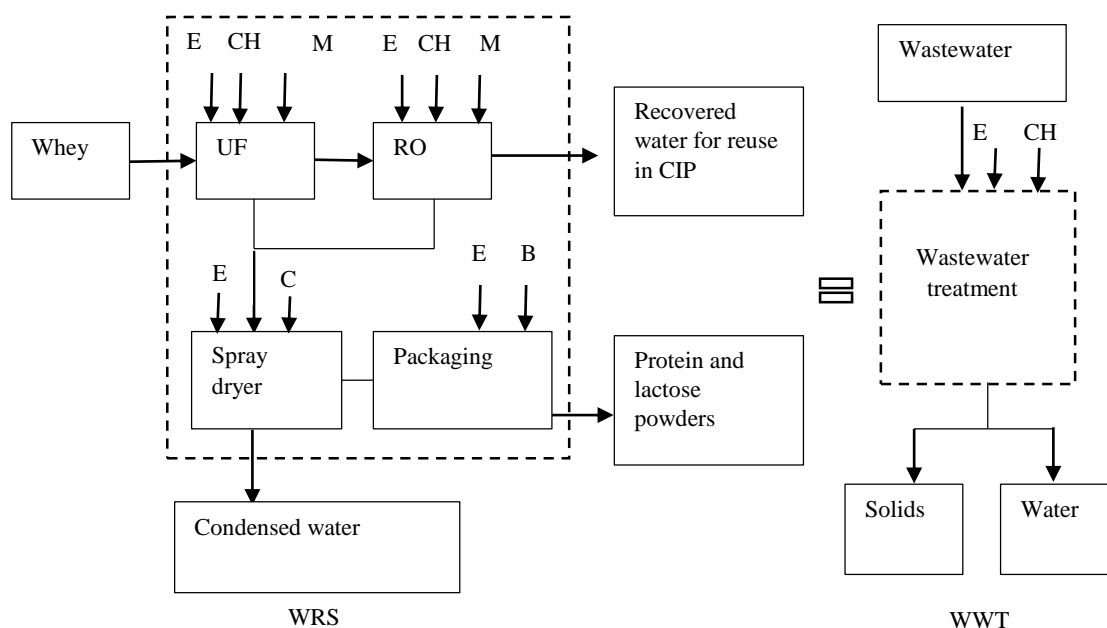


Figure 1a. Schematic representation of the water recovery system (WRS), wastewater treatment (WWT), and water production plant (WPP). E: Energy; B: bags; C: concentrates; CH Chemicals; G: gas; M: Membrane

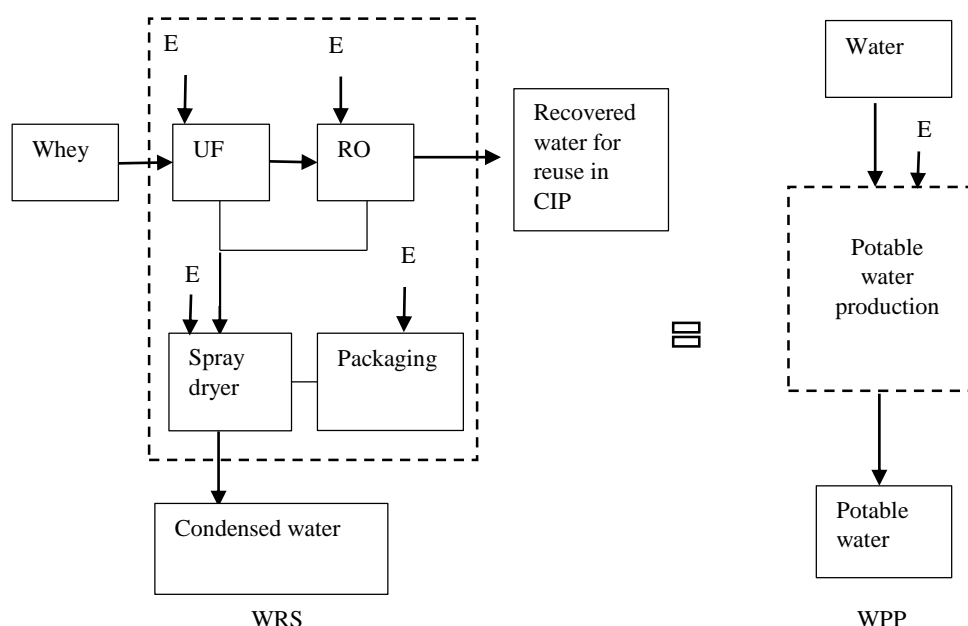


Figure 2b. Schematic representation of the water recovery system (WRS) and water production plant (WPP). Only energy (E) inputs were considered in this system comparison.

Allocation Procedures

Food production systems are complex scenarios for LCA, since several by-products and outputs can be generated. Consequently, allocation is especially necessary in these systems to assign the environmental burdens associated to each product or process of interest and to determine individual contributions of the inputs to the overall environmental impact category (Ayer et al., 2007). Allocation refers to the partitioning of input and output flows of a process between the product system under study and one or more other product systems (ISO 14044, 2006). The ISO standard (ISO 14044, 2006) specifies three steps to handle co-product allocation: i) avoid allocation by dividing the system into sub processes or expanding the system to include additional co-products; ii) when avoiding allocation is not possible, inputs and outputs should be separated between

its product and functions to reflect the underlying relationships between them; iii) when the physical relationship cannot be established, other associations could be used, such as economic value.

For the first system comparison (WRS vs. WWT) allocation was not necessary, given that inputs were normalized to the treatment of one unit of whey or wastewater, respectively, and not to the production of a particular output. In the wastewater treatment, dosing of chemicals and duration of the operation are associated with the initial organic load measured in the wastewater. Thus, total organic carbon (TOC) measured in the feed material (whey or wastewater) was considered as well in the normalization of inputs to avoid biased estimations of the burdens.

In the second system comparison (WRS vs. WPP) allocation was required to determine the proportion of burdens from the WRS associated to the production of water only. Within the recovery system, filtration and spray drying require allocation since both generate more than one output, those are protein, lactose and water. Filtration and spray drying operations are already sub-processes of the system; inputs from these operations cannot be divided for each output because protein and lactose are simultaneously separated from water. Consequently, the first two ISO principles cannot be implemented. Frequently, allocation in dairy products has been based on the solid content, nutritional value and economic revenue of the final product (Flysjö, 2011, Aguirre-Villegas et al., 2012, Kim et al., 2013). Water content is not particularly considered when deciding allocation methods since it acts as a carrier. In this study, mass-weighted economic co-

product allocation was applied for membrane filtration and spray drying systems to be able to assign some burdens to water; which will not be possible through methods based on the solid content. Mass recoveries and market prices were obtained from the economic simulation generated for the large cheese production scale in the baseline study for the WRS (Meneses and Flores, 2016). Protein and lactose concentrates are intermediate by-products of the filtration system, a price of 0.32 \$/kg was considered for each stream type; based on a whey market price of 0.64 \$/kg. For protein and lactose powder, market prices of 3.48 \$/kg and 1.1 \$/kg were assigned, respectively. Tables 1 and 2 show the calculated mass-weighted economic allocation for the membrane filtration system and spray drying. Water represents 0.14% of the environmental burdens while protein and lactose are responsible for 99.86%, for both operations

Allocation by mass was also calculated, resulting in 85% of the environmental burdens attributed to water, however mass-weighted allocation was a more appropriate allocation of environmental burdens given that revenue from the sale of protein and lactose are the primary motivation for dairy processors to provide treatment to cheese-whey.

Table 1. Allocation for the membrane filtration process

Product	Mass Recovery (Million kg/year) ^(a)	Market Price (\$/kg) ^(b)	Mass –weighted Economic Allocation (%)
Protein concentrate	58.7	0.32	50.1
Lactose concentrate	58.3	0.32	49.7
Recovered water	107	0.0005	0.14

^(a) Data obtained from the SuperPro simulation for the water recovery system for a large scale cheese plant (Meneses and Flores, 2016)

^(b) Values calculated based on a whey market price of 0.64 \$/kg and mass recovery.

Table 2. Allocation for the spray drying operation

Product	Mass Recovery (Million kg/year) ^(a)	Market Price (\$/kg) ^(b)	Mass –weighted Economic Allocation (%)
Protein Powder	6.70	3.48	70.0
Lactose Powder	8.94	1.11	29.8
Condensed water	93.9	0.0005	0.14

^{(a), (b)} Data obtained from the SuperPro simulation for the water recovery system for a large scale cheese plant (Meneses and Flores, 2016)

For the wastewater treatment and potable water production system, 100% of the inputs are attributed to wastewater and water; respectively; since these are the primary functions of each system.

Impact categories and methods

Several methods have been developed to evaluate diverse impact categories, thus results obtained in a particular category are directly associated to the method applied. In the present study, impact categories and methods frequently found in publications related to LCAs for dairy products were chosen (Eide, 2002, González-García et al., 2013, Kim et al., 2013), these are: The Intergovernmental Panel on Climate Change (IPCC) for climate change, Cumulative Energy Demand (CED) for energy, USEtox for human toxicity and freshwater ecotoxicity, ReCiPe midpoint and ReCiPe endpoint for eutrophication, photochemical oxidant formation, fresh water depletion and ecosystems.

Methodology for the life cycle inventory

SuperPro Designer® v8.05.13 software (Intelligen, Inc., Scotch Plains, NJ) was used to carry out the LCA. This software was selected to model the life cycle because it consists of several database and assessment methods and allows uncertainty evaluation

on the data. Its graphical interface is user-friendly and shows in detail the factors among the system having the biggest contribution to the environmental impacts. The Ecoinvent 3-allocation, default-unit database from SimaPro was referred to for the inventory of individual inputs such as membrane, energy, steam, water, chemicals, packaging materials and waste management for the three systems evaluated here.

Life cycle inventory (LCI) for the water recovery system

Ultrafiltration and Reverse osmosis membrane system. The initial operational performance of the system was obtained from pilot scale filtrations and spray drying systems. Results were then used to scale up the system for three processing levels. The large scale plant was selected to conduct the LCA study, since it showed to have better financial advantages (a revenue of 33.4 million/year, internal rate of revenue of 19.5 % and payback time of 4.36 years) (Meneses and Flores, 2016), and therefore more likely to undertake the proposed water conservation alternative. The annual consumables from each unit process were normalized with respect to the functional unit described before.

SuperPro ® designer makes available several reports breaking down materials, costs, consumables, environmental impacts and emissions from each operation in the system. These reports provided the data related to annual consumption of energy, chilled water, steam, total membrane area, chemicals for CIP and packaging bags used to build the LCI for the water recovery system.

Spiral-wound membranes were the main components for ultrafiltration and reverse osmosis. Data related to their structure was obtained from publications reporting the

composition of the different membrane elements (Prince et al., 2011, Lawler et al., 2012). Three predominant components can be distinguished in a membrane system; fiberglass, membranes sheets, permeate tubes and ends. However, in this study similar composition was assumed for UF and RO membranes. Impacts related to the manufacturing process of the spiral-wound membranes were not considered due to the lack of available data. Table 3 contains detailed information about the materials and the specific amounts calculated for the LCI.

Membrane CIP regime. The cleaning regime applied in a membrane system depends on the product being filtered, the type of membrane and the configuration of the system (D'Souza and Mawson, 2005). In our published work (Meneses and Flores, 2016), which is the baseline model for this study, membrane-cleaning cycles were not considered in the simulation. However, a membrane cleaning regime is described in the material and method section, which includes standard steps for membrane cleaning such as alkaline wash, acidic wash and water rinses at the beginning to remove product left in the system, in between detergent washes and at the end to remove the remaining cleaning agents.

Table 3. Annual consumables break down for the water recovery system

Inputs	Component	Percentage (%)	Amount per year	Normalization per unit
Cheese whey (million kg/year)			1087.8	367,966
Membrane element ^(a)			7,080.96	0.0192
outer casting (kg/year)	fiberglass	30	2,124.3	0.0058
feed spacer (kg/year)	polypropylene	5	354	0.0010
permeate spacer (kg/year)	polyester	5	354	0.0010
membrane sheet (kg/year)	aromatic polyamide	19	448	0.0012
	microporous polysulfone		448	0.0012
	polyester support		448	0.0012
permeate tube and end caps (kg/year)	acrylonitrile butadiene styrene (ABS)	27	1,912	0.0052
Glues (kg/year)	epoxy resin	9	637	0.0017
rubber ring (kg/year)	EPDM	5	354	0.0010
electricity (million kw-h)			10.05	27.31
chilled water (m3)			172,163	0.468
membrane disposal (kg/year)	to landfill		7,081	0.019
Membrane cleaning ^(b)				
sodium hydroxide (kg/year)			2,172	0.006
nitric acid (kg/year)			866	0.002
electricity (kw-h)			256,008	0.696
water (million m ³)			1.68	4.567
aqueous waste (million m ³)	to treatment plant		1.68	4.575
Spray Drying and Packaging ^(c)				
electricity (million kw-h)			2.34	6.360
chilled water (million m3)			1.44	3.915
steam (ton)			202,763	0.551
bags (entities/year)			625,641	0.173
electricity for loading and packing (million kw-h)			3.10	8.417

^(a) Values calculated from published literature on membrane composition (Prince et al., 2011, Lawler et al., 2012)

^(b) Values obtained from a simulation performed in SuperPro ® for membrane cleaning, based on the cleaning cycle published by D'Souza and Mawson, 2005.

^(c) Values obtained for the large scale cheese production simulation performed for the economic analysis (Meneses and Flores, 2016). Appendix A.

In order to include the environmental impacts associated to membrane cleaning, for the present study, SuperPro® designer was used to create a simulation for the CIP cycles for a large- scale production plant. Additional information required for the simulation related to the duration of each step in the cleaning regime, and the concentration of cleaning agents were obtained from published literature (D'Souza and Mawson, 2005); while water temperature of the solutions was based on the maximum levels membranes

can tolerate, according to manufactures recommendations (25 °C for UF and RO). The volume of water per cycle was determined from (Eide et al., 2003) (1,000 L of water per cleaning period), this volume was normalized to the large scale production model. Once the simulation was performed, the annual amount of water, cleaning agents and energy were collected from the consumable break down report from SuperPro®. Wastewater generated from the cleaning cycles was assumed to be disposed for treatment. Table 3 shows the input data used for the LCI of membrane cleaning cycles.

Spray drying and packaging. Spray drying was considered for protein and lactose powder production in the simulation model. Therefore, data on annual consumable for both processes were available in the SuperPro® reports. For spray drying, electricity, steam and chilling water were considered. Whereas for the packaging operation the total number of low density polyethylene (LDPE) bags consumed in a year were included. Input data used in the LCI of these two processing steps are included in table 3.

LCI for the wastewater treatment plant

Data for the wastewater treatment plant was obtained from a local facility that treat an average flow of 23.8 million gal/day (11.08 million units/year). The treatment process in this plant encompasses eight operations including: screening, primary clarification, biological treatment, secondary clarification, disinfection, solids digestion, solids dewatering and land application. Similar to the WRS, only annual direct inputs from the WWT plant related to chemicals for odor control, electricity, natural gas, water usage and biosolids disposal were considered for the LCI, as detailed in table 4. The annual

consumables for this system were first normalized to the functional unit (1 unit of wastewater), then the average total organic carbon (TOC) reported by the local plant was considered to calculate the final input quantity used for the treatment of wastewater with organic load similar to whey treated in the WRS.

Table 4. Annual consumables break down for the wastewater treatment operations

Inputs	Annual Consumption ^(a)	Amount/unit (TOC 120 ppm)	Normalization (TOC 23,637 ppm)	Units
Wastewater (million gal)	8,646	11.08		Million unit/year
Sodium hypochlorite (gal)	52,019	0.02	3.49	kg/unit
Liquid Caustic Soda (gal)	20,674	0.007	1.39	kg/unit
Ferrous Chloride (gal)	245,624	0.08	16.50	kg/unit
Hydrogen Peroxide (gal)	574,109	0.19	38.56	kg/unit
Polymer (gal)	147,249	0.05	9.89	kg/unit
Electricity (million kw-h)	15.09	1.36	268.1	kw-h/unit
Natural gas use (therms)	176,522	0.002	0.31	Million Btu/unit
Water use (ccf)	89,718	6.05	1,192	gal/unit
Biosolids –landfarming (tons)	4,837	0.0004	0.08	tons/unit

^(a) Source: Lincoln wastewater treatment plant. Appendix B.

LCI for the water production plant

LCI for potable water production was assemble from data reported in a previous work focused on the analysis of greenhouse gas production from a water production plant (Gakuria, 2013). Combination of annual electricity usage (based on 2009-2011 energy use) for well pumps, water treatment plant, operational energy and pumping for distribution to the city was considered from the cited study.

Table 5. Electricity break down for water production from the water plant and the water recovery system

Water production plant^(a)			
Input	Consumption	Units	Kw-h/unit of water
Electricity	12.7	Million kw-h/year	0.787
Water production	12,600	Million gal/year	
Units of water/year	16,13	Million units/year	
Water recovery system^(b)			
Electricity	15,74	Million Kw-h/year	
Water recovered	201.2	Million kg/year	
Units of water/year	68,046		0.648

^(a) Total electricity usage for well pumps, water treatment plant, operational energy, and distribution (Gakuria, 2013) .

^(b) Total electricity usage for membrane filtration, membrane cleaning operation, spray drying, loading and packaging (Appendix A).

To calculate the amount of energy consumed per unit of water produced, total water consumption in the area supplied by the WPP in the same period was used. All the data was normalized to one unit of water produced by the WPP. Similar normalization was applied for the WRS, following the water allocation procedures described before, in order to make them comparable. Table 5 shows the input data used for both systems.

Results and discussion

Implementing the water recovery system supposes two main benefits for the dairy industry in terms of environmental burdens. First the elimination of wastewater and second the recovery of water. In order to evaluate the real impacts of these advantages the water recovery system was compared with traditional operations for wastewater management and water production. The results from the gate-to gate comparison of these systems are presented in figure 2 and 3, while the quantitative results are indicated in tables 6 and 7.

Life cycle impact assessment (LCIA) comparison for the treatment of whey by the water recovery system and by the wastewater treatment plant

As described earlier only annual consumables were accounted in the present study for the evaluation and comparison of the environmental impacts related to the treatment of one unit of whey and wastewater (with TOC similar to whey) by the water recovery system and the wastewater treatment plant, respectively. Results shown in figure 2 indicate that the WWT plant causes higher environmental impacts than the alternative process (WRS), per unit of whey treated in some specific environmental impacts including: climate change, cumulative energy demand, water depletion and ecosystems with values ranging from 77 % to 94 %. In the other hand, the WRS presented higher environmental burdens than the WWT plant in the photochemical oxidant formation, fresh and marine water eutrophication, human toxicity (non-cancer) and ecotoxicity categories with values ranging from 38% to 81 %.

Climate changes including increased precipitation, extreme heat waves, draughts and rising sea level are consequences of global warming (Jeppesen et al., 2015). Increased temperatures caused by global warming result from the release of vapor and carbon dioxide (CO₂) from combustion processes, and the production of methane from livestock farming, landfills, and nitrous oxide produced from fertilizers application in agriculture (Hepperly and Setboonsarng, 2015). Climate change is capable of modifying ecosystems, causes natural disasters, supports spread of diseases and threatens economy and food security (by impacting agricultural yields) (Jeppesen et al., 2015).

The global warming potential of a substance represents its contribution to the greenhouse effect. Some substances gradually decompose and become inactive in the long run (Baumann, 2004); in the method selected to evaluate climate change (IPPC) a 100 year time horizon was used for the CO₂ characterization factor . Quantitative results presented in table 6 indicate that 250 kg CO₂ equivalents (eq) are emitted in the WRS operation versus 2,140 kg CO₂ eq calculated for the WWT plant.

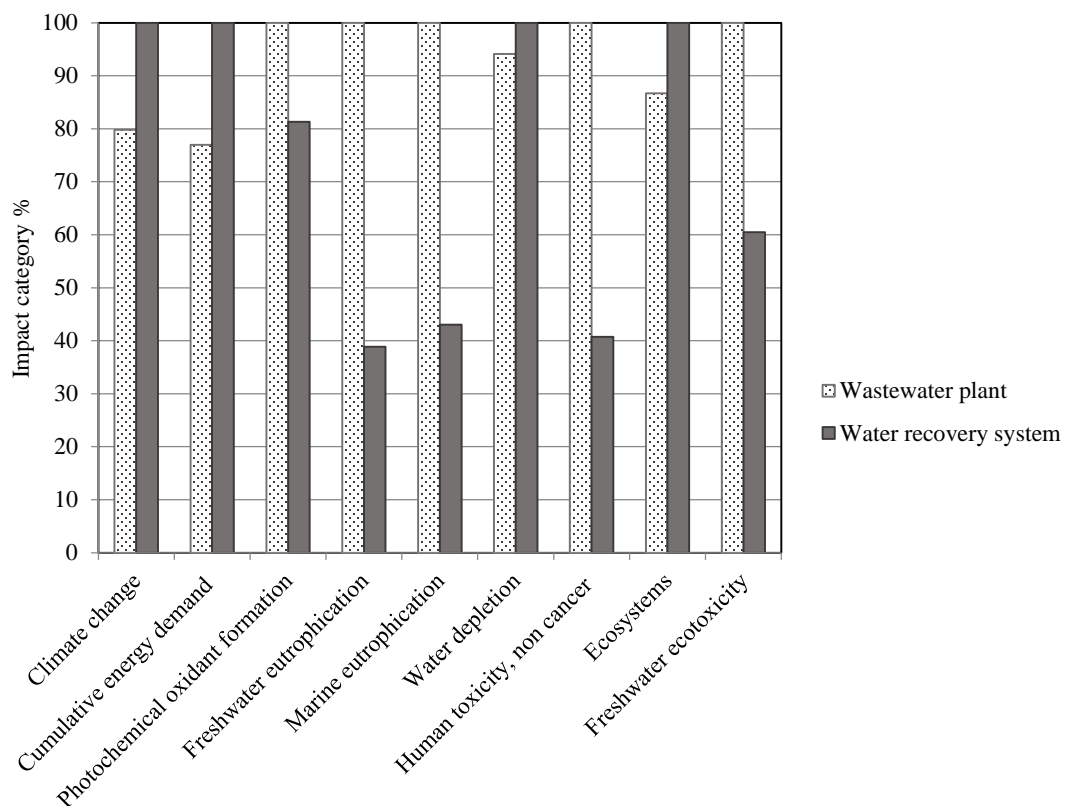


Figure.3. Comparison of the treatment of 1 unit of whey by the water recovery system and with the wastewater treatment plant

The cumulative energy demand category assesses the total energy required to operate a system or manufacture a product (PRé Consultants, 2015). Results in table 6 indicate that 3,720 MJ of energy are consumed by the WRS, while the WWT uses 2,860 MJ to treat a unit of wastewater with an organic load similar to whey.

The photochemical oxidant formation category refers to the formation of secondary photochemical pollutants (mainly ozone O_3), which are detrimental for human health (Jenkin and Clemitshaw, 2000). Secondary pollutants result from the sunlight-triggered oxidation of pollutant gases such as nitrogen oxides (NO_x) and volatile organic compounds (VOCs) that have been released into the troposphere. The characterization factor used for this impact category is defined as the change in ozone concentration due to the change in emission of certain substance (PRéConsultants, 2015), the units are reported in kg of Non Methane Volatile Organic Carbon (kg NMVOC) (Mark Goedkoop, 2009). For the compared systems, the photochemical oxidant formation resulted in 0.5 kg NMVOC and 0.63 kg NMVOC for the WRS and the WWT, respectively.

Eutrophication of aquatic systems occurs due to nutrient enrichment of these environments, causing excessive algae growth (Baumann, 2004). Consequently, light penetration is limited affecting photosynthesis in plants and making it difficult for predatory fish to catch their prey. Algae decomposition promotes complete oxygen depletion, driving extinction of animal species. Phosphorus (P) and Nitrogen (N) have been identified as the predominant macro-nutrients for algae growth in fresh water and marine ecosystems, respectively (Baumann, 2004). Wastewater treatment facilities,

fertilizer use in agriculture, manure, and other emissions can be sources for P and N containing compounds. Operation of the WRS produces 0.03 kg P eq and 0.07 kg N eq per unit for freshwater and marine eutrophication, respectively. In the same categories, operation of the WWT plant generates 0.08 kg P eq and 0.16 kg N eq per unit, respectively.

Table 6. Results for different impact categories for the treatment of 1 unit of wastewater by the water recovery system and the wastewater treatment plant

Category	Unit	Water Recovery System	Wastewater plant
Climate change	kg CO2 eq	250	200
Cumulative energy demand	MJ	3.72E+03	2.86E+03
Photochemical oxidant formation	kg NMVOC	0.50	0.63
Freshwater eutrophication	kg P eq	0.03	0.09
Marine eutrophication	kg N eq	0.07	0.16
Water depletion	m3	10.2	9.62
Human toxicity	CTUh	9.76E-10	2.39E-09
Ecosystems	Species/year	2.41E-06	2.09E-06
Fresh water ecotoxicity	CTUe	0.52	0.46

Human toxicity and freshwater ecotoxicity results from the exposure to excessive amounts of toxic pollutants. USEtox, the method used for these categories, calculates carcinogenic, non-carcinogenic, and total impacts for chemical emissions to air, water, and soil; considering the environmental fate, exposure and potential damage of the emitted substance (PRé, 2015). The results are expressed in Comparative Toxic Units (CTU) (GaBi, 2016). Results for human toxicity refer to non-cancer related impacts only, the 9.76E-10 CTUh reported for the WRS represents 40.7 % of the value calculated for the WWT plant (2.39E-09 CTUh). For freshwater ecotoxicity 0.52 CTUe was reported for the WRS against 0.46 CTUe calculated for the WWT plant in the same category.

However, the uncertainty analysis discussed later (figure 4) showed significant levels of uncertainty for freshwater ecotoxicity and water depletion, the last one defined as the water consumed during production (other than process or cooling water) and not returned to the same source (Kim et al., 2013).

The natural heterogeneity of ecosystems has made them difficult to monitor. ReCiPE endpoint method bases ecosystem assessment on the assumption that diversity of species is directly correlated to the ecosystems' quality (Mark Goedkoop, 2009). The unit used to express the result from the ecosystem impact category (species/year) indicates the loss of species in a certain region during certain time (Mark Goedkoop, 2009). Results in table 6 report 13% higher loss of species caused by operation of the WRS plant ($2.41\text{E-}06$ species/year) than the WWT ($2.09\text{E-}06$ species/year).

Life cycle impact assessment (LCIA) for the production of water from the water recovery system and the water production plant

The system on which the present LCA analysis is based, recovered water from whey to be reused in cleaning applications, providing an additional benefit besides whey management itself. For that reason, a supplementary analysis of the environmental burdens associated to the proposed system for water recovery and the traditional water production process was necessary. Energy usage associated to the production of one unit of water from the WRS and the WPP was the only input considered in the assessment of these systems. The LCIA results reported in figure 3 indicate that recovering 1 unit of

water from cheese whey using the WRS produces 18% lower overall impacts than the traditional water production system evaluated presented herein.

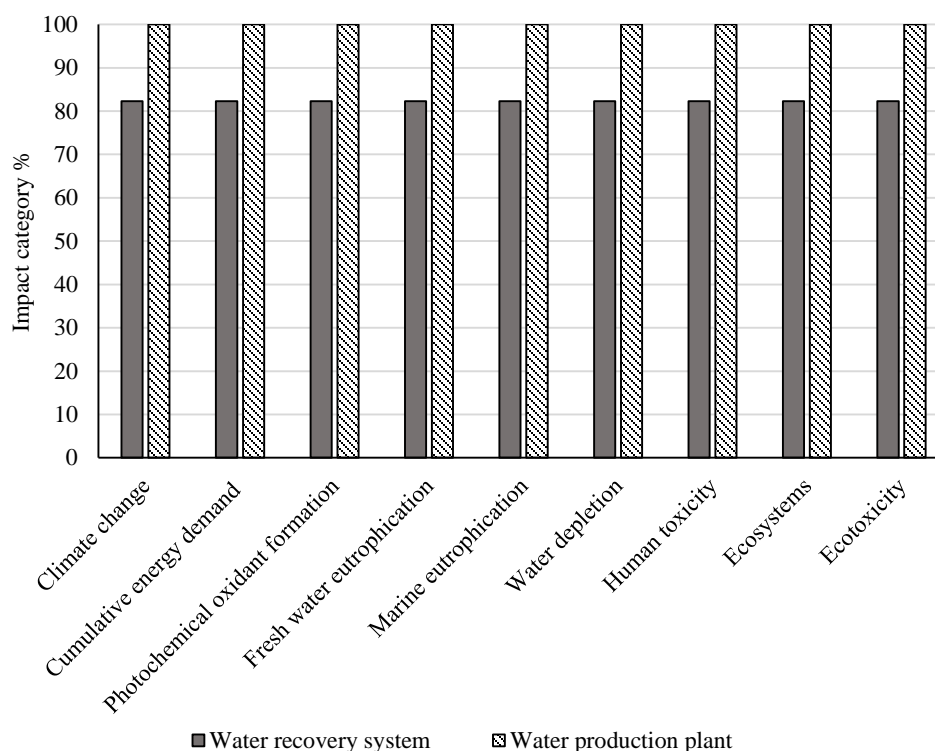


Figure 4. Comparing 1 unit water produced by the water recovery system and by the water production plant

Quantitative results for the comparison of the systems are presented in table 7. As could be expected from the inventory data, cumulative energy demand is the impact category directly affected by electricity usage. The operations under study reported a consumption of 4.1 and 5 MJ per unit of water produced from the WRS and the WPP, respectively. As effect of the energy consumption in the WRS, 0.3 kg CO₂ eq are emitted to the environment, with a 0.0006 kg MNVOC reported for the photochemical oxidant formation category, 4.68E-5 kg P eq and 8.51 E-4 kg N eq for freshwater eutrophication

and marine eutrophication, respectively. The effects in human toxicity were in the level of $2.18 \text{ E-}11 \text{ CTUh}$, while species loss for the ecosystems category was reported to be $2.9\text{E-}9 \text{ species/year}$. All these environmental impacts, calculated for the WRS represented 82 % of the total burdens generated by the WPP to produce 1 unit of water. Water depletion and freshwater ecotoxicity categories are not discussed here, due to the considerable uncertainty detected on the results associated to both impact categories (Figure 5).

Table 7. Comparison of the LCIA results per unit of water produced by the water recovery system and the water production plant

Impact category	Unit	Water recovery system	Water production plant
Climate change	kg CO ₂ eq	0.274	0.333
Cumulative energy demand	MJ	4.11	5
Photochemical oxidant formation	kg NMVOC	0.00059	0.00716
Fresh water eutrophication	kg P eq	$4.68\text{E-}05$	$6.02\text{E-}08$
Marine eutrophication	kg N eq	$8.51\text{E-}05$	0.000103
Water depletion	m ³	0.00309	0.00375
Human toxicity	CTUh	$2.18\text{E-}11$	$2.65\text{E-}11$
Ecosystems	species/year	$2.90\text{E-}09$	$3.53\text{E-}09$
Fresh water ecotoxicity	CTUe	$3.71\text{E-}04$	0.0045

Uncertainty analysis

Data quality analysis is an optional element in the LCIA (ISO14044, 2006), but it is useful to determine the overall uncertainty of the results. A Monte Carlo analysis with 1,000 iterations was performed using SimaPro. Results from the WRS and WWT comparison (figure 4), indicate that for all impact categories, except water depletion and fresh water ecotoxicity, the environmental impacts produced by the WRS system (A) were lower than the WWT plant (B) 100% of the time. Nevertheless, 15.7 % of the time

the impacts in freshwater ecotoxicity caused by the WRS could be higher than those produced by the WWT plant, in a 95% confidence interval (CI) ($A \geq B$). The same scenario was observed for water depletion, in which 55% of the time the results were consistent with the general observation for the other categories evaluated ($A \leq B$), but 45% time the impacts caused by the WRS can be higher than WWT.

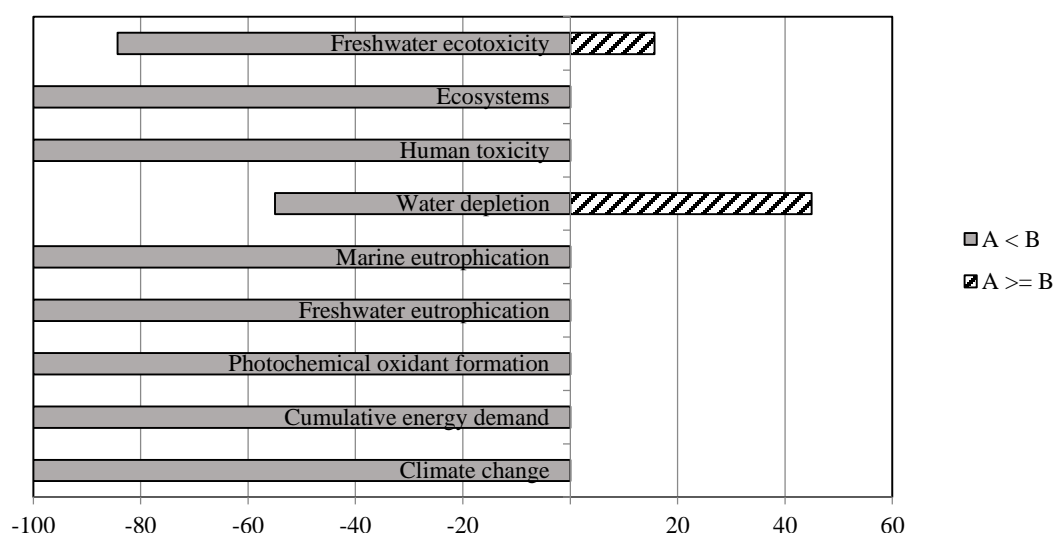


Figure 5. Uncertainty analysis of 1 unit of whey treated by the water recovery system (A) and by the wastewater treatment plant (B), confidence interval: 95%. Dual bars for freshwater ecotoxicity and water depletion indicate that the differences between the two systems are not statistically significant.

For the second system comparison presented herein (WRS versus WPP), results for the uncertainty analysis (figure 5) were similar to the ones described before. The uncertainty of the results for freshwater ecotoxicity and water depletion were 21.9 % and 44.8 %, respectively.

Due to the significant uncertainty detected in both system comparisons, conclusive observations about the environmental impacts in freshwater ecotoxicity and water

depletion cannot be made in the present study and therefore these categories are not further included in the discussion.

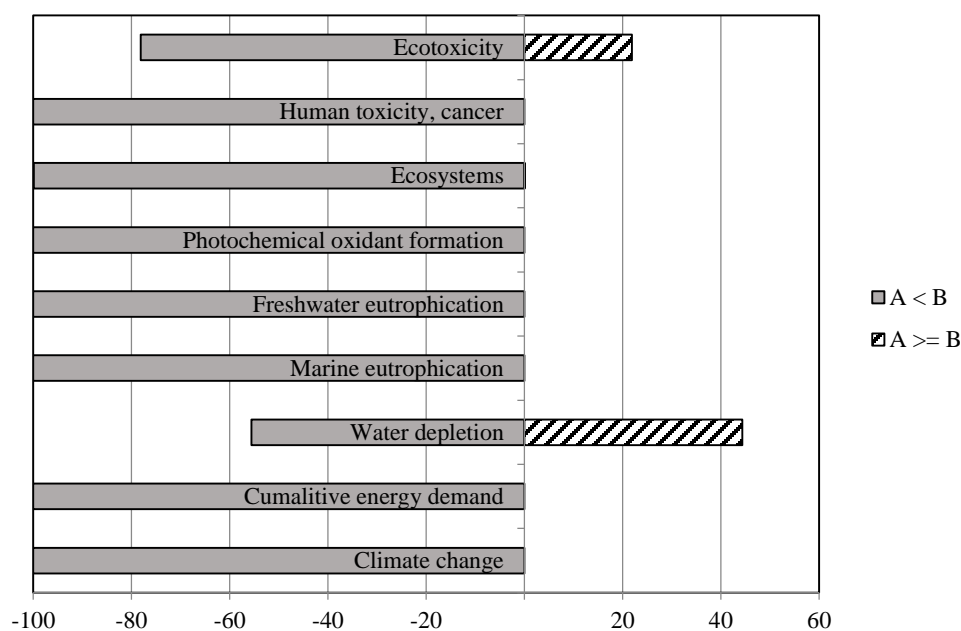


Figure 6. Uncertainty analysis for the production of 1 unit of water by the water recovery system (A) and a water production plant. Confidence interval: 95 %. Dual bars for freshwater ecotoxicity and water depletion indicate that the differences between the two systems are not statistically significant.

Relative contribution of inputs to the environmental impacts of the gate-to-gate analyses

From the previous discussion, it was observed that the environmental impacts associated with the treatment of whey and the production of water by the water recovery system are lower than those environmental burdens generated from the traditional systems (wastewater and water production plants, respectively). Yet, the individual contributions of the processing inputs to the total impacts reported for each impact category have not been analyzed to this point.

Thus, relative contributions for the WRS and WWT were calculated, results are presented in figures 6 and 8, respectively. Given that the electricity consumption was the only input considered for the WPP, the relative contribution analysis was not necessary for that system, all environmental impacts are attributed to that particular input. The relative contribution analysis makes possible to identify potential opportunities for environmental impacts reduction, by denoting those processing inputs with the highest

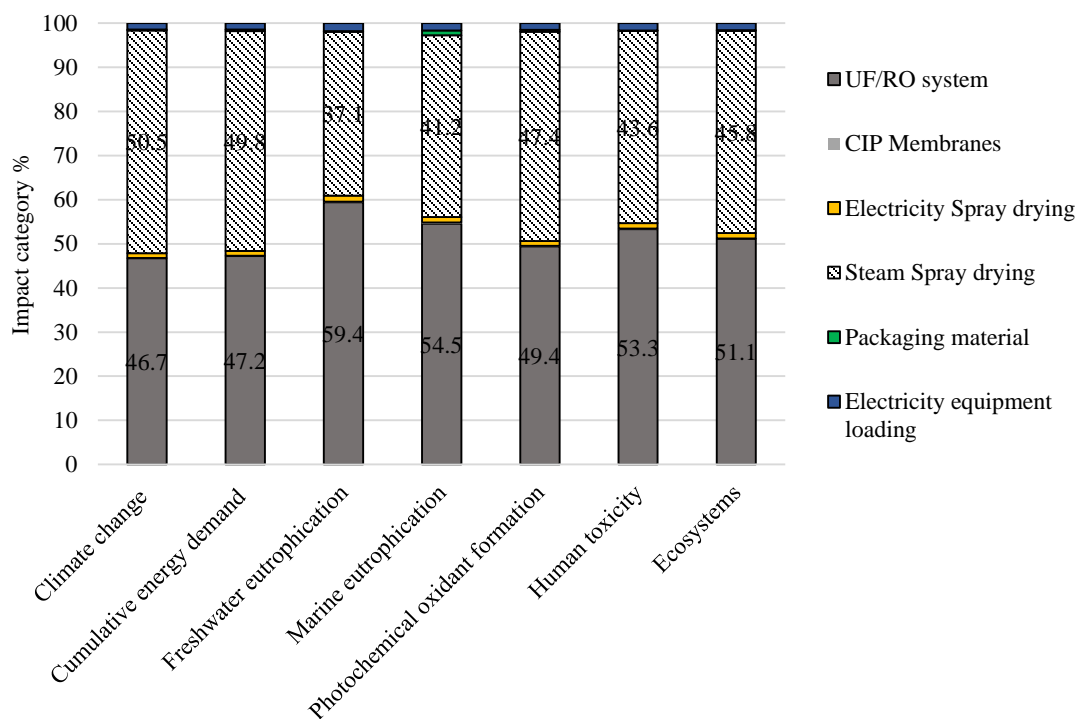


Figure 7. Relative contribution of processing inputs to the environmental impacts from the gate-to-gate analysis for the water recovery system

influence on the overall burdens of the system. Results presented in figure 6 indicate that among the different inputs consumed by the WRS, those used to run the UF/RO system and to generate the steam applied for the spray drying step are the ones responsible for 46-59 % and 37-50 % of the total impacts, respectively, throughout the environmental categories evaluated. Consumables for the UF/RO system included membranes,

electricity, water, and membrane disposal to landfill. The relative contribution analysis for this operation (figure 7) demonstrates that electricity is responsible for more than 98% of the impacts for all categories.

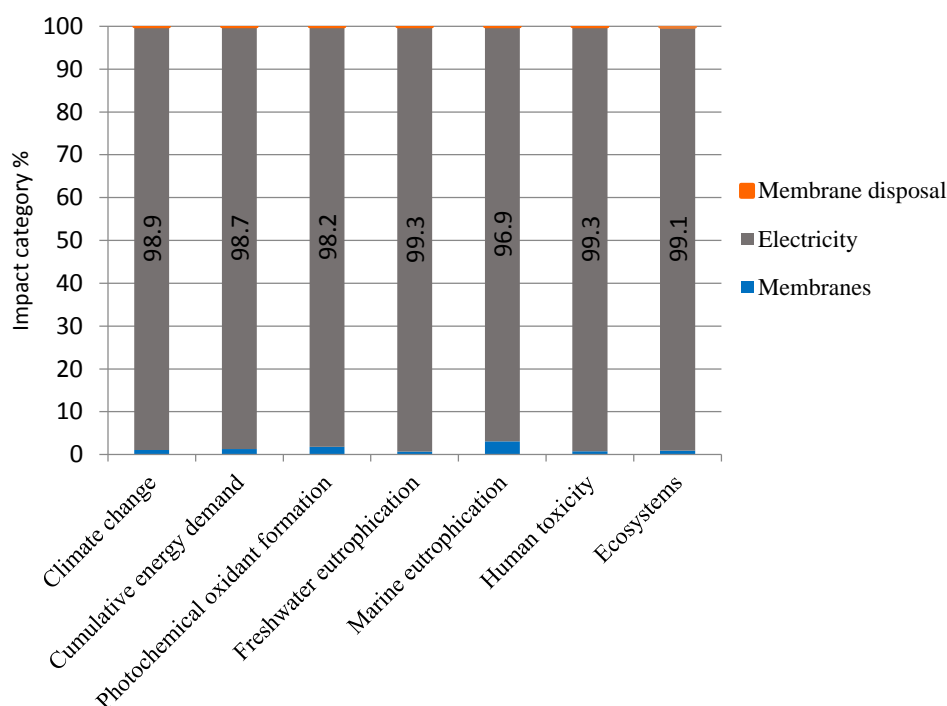


Figure 8. Relative contribution of processing inputs to the environmental impacts of the Ultrafiltration (UF)/ Reverse Osmosis (RO) system

The relative contribution analysis presented in figure 8, points out electricity, hydrogen peroxide, gas consumption and polymers used for odor control (in some categories) as the inputs collectively responsible for at least 80% of the environmental burdens in the WWT system. Electricity alone contributes to more than 50% of the emissions affecting climate change, cumulative energy demand, human toxicity and ecosystems; while electricity usage contributes to 21-36 % of the impacts, for photochemical oxidant demand, freshwater and marine eutrophication. Hydrogen

peroxide is used in the WWT for odor control and is the second input adding emissions in a range of 21-36 % to most of the categories, except for freshwater eutrophication, in which landfarming of solids takes the second place producing 44.8 % of the emissions. Gas consumption and polymer usage for odor control are responsible for less than 20% of the burdens in different categories, still they are among the inputs causing 80% of the overall impacts associated to the treatment of 1 unit of wastewater by the WWT.

According to the findings from the present study and under the conditions specified herein, implementing the water recovery system to remove organic components (protein and lactose) and to remove water from cheese whey as proposed by Meneses and Flores (2016) produces 19 % - 62% lower environmental impacts than the wastewater treatment plant in five out the nine categories evaluated and 18% lower environmental impacts than the water production plant; systems that otherwise will have to be operated to achieve the same results.

Besides the comparison among different systems, the LCA conducted for WRS was aimed to provide perspective about opportunities for further improvement on the environmental performance of the system. Electricity usage for filtration operations and for steam production in the spray-drying step, was identified as the input with the highest contribution to the total environmental impacts associated to the system. Pressure demand and high latent heat of water vaporization (2257 kJ kg^{-1} at atmospheric pressure) are responsible for the significant energy demand for filtration (Al-Karaghoul and Kazmerski, 2013) and spray drying operations (Rad and Lewis, 2014), respectively.

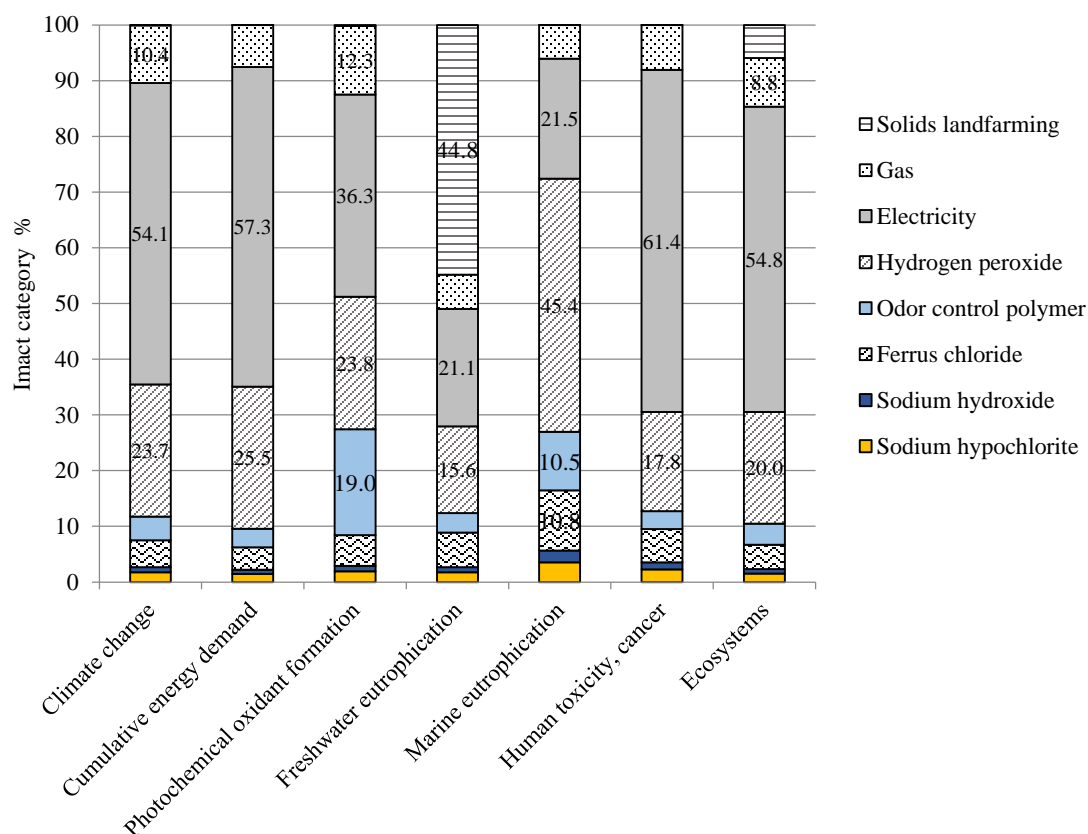


Figure 9. Relative contribution of processing inputs to the environmental impacts from the gate-to-gate analysis for the wastewater treatment plant

Compared to the production of several dairy products, dry whey has been reported to be the most energy demanding process with a consumption of 11 GJ/ton of product (UNIDO, 2010). Interesting enough, application of membrane filtration (UF and RO) for suitable products has been suggested as an approach for energy reduction (Rad and Lewis, 2014).

The water recovery system, as assessed for the LCA presented in this study, did not consider any energy conservation strategies, although opportunities exist. Several tactics previously proposed for the dairy industry are applicable to the system including, process

optimization, energy-efficient equipment, co-generation of electricity and heat, controlling steam leak and reusing heat from vapor (Rad and Lewis, 2014).

Membrane science continues to make advances towards more efficient membranes not only in terms of energy usage but also for higher water flux rates, resistance to fouling and easier cleaning. Membrane distillation (MD) and forward osmosis (FO) are promising alternative technologies to the traditional pressure-driven membrane systems used in the food industry (UF and RO). The biggest advantage of MD is the small temperature difference required to operate, however this system is not available in an industrial scale due to cost limitations (Al-Karaghoul and Kazmerski, 2013). In the case of FO, higher recovery with less energy input, due to the low pressure required, are among the advantages that FO conveys. Water permeation in this membrane is driven by osmotic pressure gradient from the low osmotic pressure (feed solution) to high the osmotic pressure (draw solution) side (Zhao et al., 2012).

Electricity production in the U.S. is mainly based on fossil fuels (67%) including coal, natural gas and petroleum (USEI, 2015). Consequently, using renewable-energy-powered technologies for the operations included in the water recovery system will reduce the environmental footprint of the proposed system. This approach has already been evaluated in membrane filtration systems for water desalination (Peñate and García-Rodríguez, 2012, Al-Karaghoul and Kazmerski, 2013, Richards et al., 2014), which provides the opportunity to extend application of this technology to the food industry. Solar and wind powered technologies are well evaluated sources for renewable energy, the latter being the most cost-effective option (Peñate and García-Rodríguez, 2012).

Another alternative of growing interest worldwide is the production of biofuels (biogas) from food waste through anaerobic digestion, which provides the additional benefits of organic by-product recycling and reduction of food waste (Pham et al., 2015), according to Murphy et.al. 20.4 kw-h of electricity could be generated from 1 m³ of biogas (assuming a 35% generation efficiency) (Murphy et al., 2004).

Even though the biggest opportunities for reduction of environmental burdens caused by the WRS are related energy conservation in the filtration and spray drying operations, additional improvements can be achieved from other components in the system. In cleaning-in-place operations, for example, water and chemical usage could be limited to the optimum level so that hygienic and environmental requirements are satisfied (Eide et al., 2003). Another option was proposed by Lawler and colleagues, related to the reuse, recycling and alternative disposal of used membranes to reduce the impacts associated to current membrane landfill (Lawler et al., 2012).

The inextricable relationship of water and energy has been demonstrated in the present study, thus looking into opportunities for water reduction and energy conservation on individual operations will contribute to the reduction of impacts in more complex systems. The evaluated initiative makes it possible for the dairy industry to demonstrate its commitment to the reduction of greenhouse emissions negotiated by U.S. and other nations in 2015 in the global agreement on climate change.

Limitations

The inventory used in the water recovery system LCA came from a simulation based on experimental data. While the general conclusion, of electricity being the input responsible for most of the environmental impacts, is in line with other LCA studies on dry whey production; data from operating plants will help to strengthen data quality. Another limitation was observed in the lack of information related to membrane manufacture; only membrane materials were included in the analysis. Finally statements on the environmental burdens of wastewater treatment and water production should be made cautiously, since the complexity of treatments for both systems can vary depending on local regulation and the initial quality of the wastewater and water, respectively. Results presented herein are valid within the context and conditions established for the present study.

Conclusions

The environmental impacts associated with the operation of a water recovery system, proposed as a water conservation initiative for the dairy sector, were assessed in this study. The most relevant findings indicate that the proposed system, offers reductions ranging from 19 % - 62 % in some environmental impacts including: photochemical oxidant formation, water eutrophication, human toxicity and ecotoxicity, in comparison to current practices of sending whey to be treated in a wastewater treatment plant. While up to 82% lower emission were detected for the production of water from the proposed water recovery system in comparison to the water production plant. The life cycle

assessment performed in the water recovery system alone, scored electricity usage as the main input responsible for the environmental burdens of the system, throughout several impact categories included in the assessment. Membrane filtration and spray drying are the hot spot operations, where efforts should be focused to reduce consumption of fossil fuel-generated electricity.

The results reported here provide important information to evaluate water conservation initiatives from a holistic perspective that goes beyond operational performance and economic feasibility. The analysis concluded that water reconditioning could reduce some environmental impacts associated to the dairy industry, while reducing waste and promoting water conservation; all important constraints that if properly addressed will serve as solid platform for food companies to stay in and/or improve their business.

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**CHAPTER 4. MICROBIOLOGICAL RISK ASSESSMENT FOR THE SAFE
REUSE OF WHEY-RECOVERED WATER IN CLEANING OPERATIONS: A
CONTAMINATION SCENARIO WITH *LISTERIA MONOCYTOGENES* IN
DAIRY PROCESSING**

Abstract

The risk of microbial contamination has been suggested as an important limitation for the implementation of water reconditioning and reuse in food processing operations. The objective of the present study was to assess a possible route of contamination in pasteurized fluid milk, when reconditioned contaminated water, recovered from whey, is used in cleaning operations. *Listeria monocytogenes*, a pathogenic bacteria associated with dairy processing environments, was used to simulate post-reconditioning treatment contamination of the recovered water. A quantitative microbial risk assessment approach was applied to model fluid milk processing from the receiving tank, at the plant level, to the packaged product. Experimental data and published literature were used to populate the model. Results showed that the attachment levels of bacteria from the contaminated reconditioned water to the equipment, during cleaning operations, are low. Such levels do not impact significantly the probability of detecting *Listeria monocytogenes* in the final product. A sensitivity analysis of the system indicated that the concentration of *Listeria monocytogenes* in the final product is governed by the initial contamination detected in raw milk. A reference HACCP plan for the reuse of reconditioned water is provided, this model integrated results obtained from the research framework developed for the effective evaluation of a water conservation strategy in the dairy sector.

Key words: Risk assessment, *Listeria monocytogenes*, water reconditioning, HACCP

Introduction

Water reconditioning and reuse in food industry processes offer the opportunity to conserve this limited natural resource and contribute to assure water supply for food production. A case study in water conservation for the dairy industry evaluated the feasibility of recovering good quality water from cheese-whey to be reused in cleaning in place operations, assessing the economic aspects of the recovery system as well as the safety implications of water reuse (Meneses and Flores, 2016). This and other studies (Balannec et al., 2002, Aydiner et al., 2013, Aydiner et al., 2014) have supported the fact that combination of properly monitored membrane filtration systems can yield water of high quality, however the negative perception has been identified as a barrier to the implementation of water reuse in the food industry (Casani et al., 2005). The development of risk assessment and HACCP plans have been proposed as potential solutions to overcome this limitation (Casani et al., 2005).

Microbial risk assessment (MRA) is an organized process applied to estimate the probability of becoming ill after exposure to a microbial pathogen (Parkin, 2007). Conducting MRA provides scientifically-based information to support decision making processes in regulatory standards, develop guidelines, expose different risk management alternatives, help to prioritize risk and research needs (Parkin, 2007). The U.S. Environmental Protection Agency (EPA) has applied MRA to evaluate water purification treatments and to establish quality standards (EPA, 2014), while in the food industry this tool has been extensively used to determine the survival of pathogenic microorganism to

different food processing treatments (Nauta, 2005, Mataragas et al., 2010) and the likelihood of contamination throughout the food supply chain (Bouwknegt et al., 2015). To the best of our knowledge microbial risk assessment has never been applied to evaluate water reconditioning and reuse in the food industry from the food safety perspective.

Listeria monocytogenes is a gram positive pathogenic bacteria, able to grow at refrigeration temperatures, in a pH ranging from 4.6 to 9.5 and water activity of 9.2 (Carpentier and Cerf, 2011). A national survey conducted by the USDA reported a 7.1% prevalence of *L. monocytogenes* on bulk tank milk among the dairy operations under study (Van Kessel et al., 2011), but it has also been commonly isolated from floors, drains and equipment in dairy premises. This bacteria is of importance to the dairy industry and regulatory agencies due to several outbreaks resulting in food-borne illness that have been associated to the consumption of contaminated dairy products (Antognoli et al., 2009). Proper pasteurization is effective in the controlling of *L. monocytogenes*; nevertheless post-processing environments have repeatedly been proven to be a major source of contamination (Kabuki et al., 2004). The prevalence of *L. monocytogenes* in the dairy processing environment represents a food safety risk for food manufacturing operations occurring in the plant, including whey-recovered water; therefore, this pathogenic bacteria was selected to conduct a microbial risk assessment.

The risk assessment presented in this study simulates a scenario where the recovered water, intended to be used in equipment cleaning operations, becomes contaminated with *L. monocytogenes* after the recovery treatment. This is an infrequent event, however, if it

did occur the impacts could be significant. Thus results from the present MRA will provide perspectives about how big the consequence could be, in order to propose precautionary measures and improve quality and safety control protocols. The present risk assessment aims to be the first step towards the development of robust HACCP plans and guidelines for the successful implementation of water reconditioning and reuse in the food industry.

Materials and methods

Risk assessment model development

The conceptual model, upon which the mathematical model was established, is shown in Figure 1. The quantitative microbial risk assessment model describes the supply pathway starting from raw milk in commingled silos at the processing plant level, followed by pasteurization and storage in milk tanks, until the final packaging of products right after the filler. The model describes the changes in *Listeria monocytogenes* concentration through the pathway, with a final output of the probability distribution of pathogen load per package of milk produced. During the process, the efficacy of cleaning-in-place (CIP) in the receiving tanks and pasteurized tanks using contaminated-reconditioned water was incorporated into the analysis, to determine the pathogen level present in the final milk product.

For the initial concentration of bacteria of interest in the silo at the food processing plant ($C_{rawtank.milk}$), data from a nationwide survey conducted in 2012 by the USDA to provide information for risk assessment (Jackson et al., 2012) was used. Before milk

from trucks is added to the silos in the dairy plant, the storage tank is supposed to be cleaned by the contaminated-recovered water following an standardized cleaning regime with cleaning efficiency measured as efficiency factor (EF) and efficiency ratio (ER) (obtained from this project). When milk is added to the commingling silo, a cross contamination event is modeled as a transferring of the residual pathogen from the silo inner surface to the raw milk with a transfer coefficient (TC).

Washing efficiency

The washing efficiency was evaluated by two measures, efficiency ratio (ER) and efficiency factor (EF), using equation 1 and 2. The data was obtained from our published work (Meneses and Flores, 2016) assuming that the washing efficiency for *Pseudomonas aeruginosa*, described in the referred study, is similar for *Listeria monocytogenes*.

$$ER = \frac{p_{aft}(1-p_{bf})}{p_{bf}(1-p_{aft})} \quad \text{Eq. 1}$$

$$EF = \log_{10} c_{aft} - (\log_{10} c_{bf} + \log_{10} c_{att}) \quad \text{Eq. 2}$$

Where p_{bf} and p_{aft} are the probability of a randomly selected coupon being positive for *Listeria monocytogenes* calculated as the ratio of number of positive coupons out of the total coupons sampled, before and after CIP washing, respectively. c_{bf} and c_{aft} are average concentrations among all the positive coupons before and after CIP washing, c_{att} represents the attachment of bacteria from the contaminated water to the surface of the equipment.

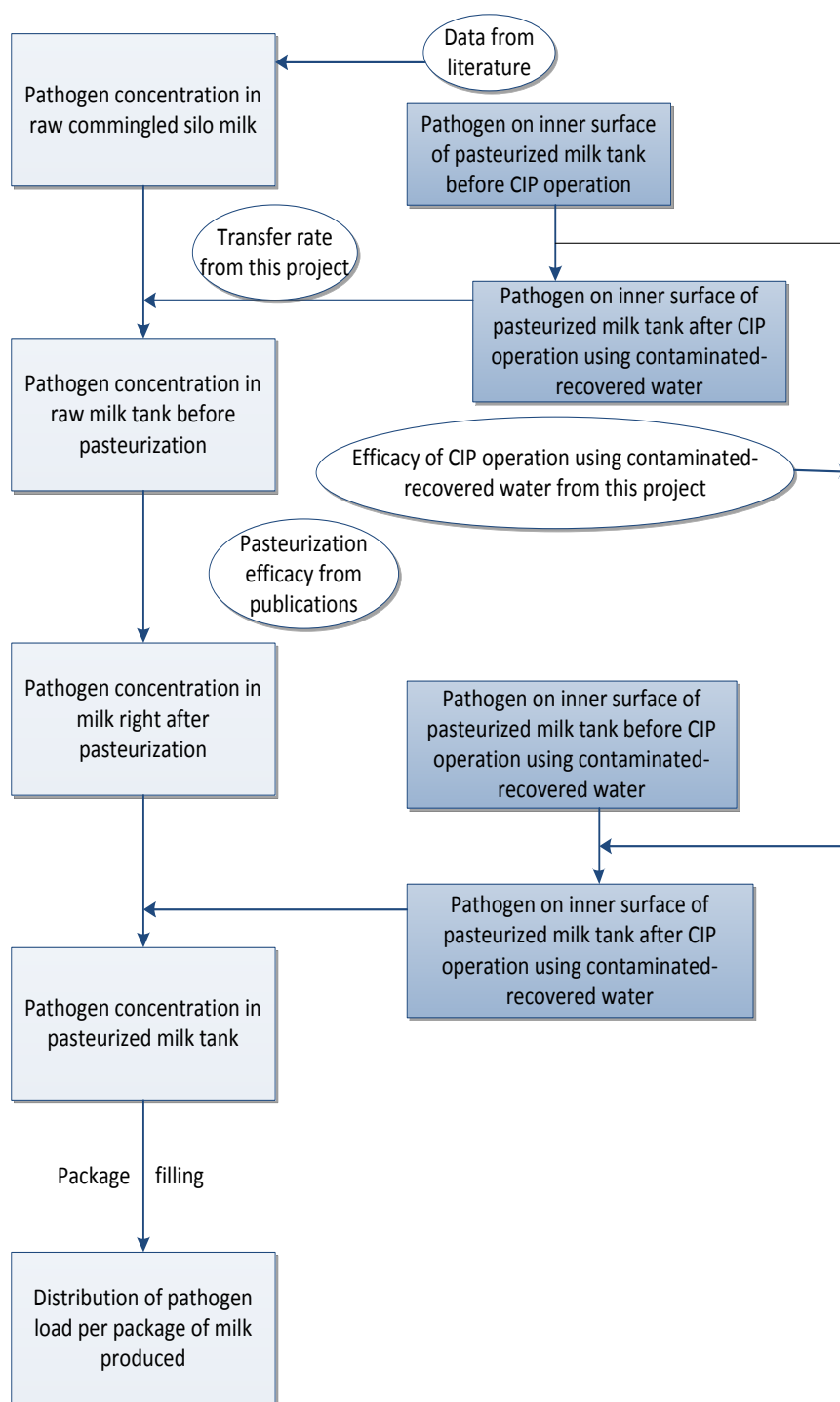


Figure 1. Flow diagram of the risk assessment conceptual model of *Listeria monocytogenes* contamination in packaged fluid milk when contaminated recovered water is used in CIP operations

Transfer rate of *Listeria monocytogenes* from contaminated recovered water to equipment surface

Experimental trials were carried out in lab-scale equipment to mimic CIP operations using recovered water that has been contaminated, in low levels (10^3 cfu/ml), after reconditioning treatment. Low level contamination was considered given that in practice, controls to monitor the microbial quality of the recovered water and efficacy of cleaning operations with good quality water are continuously being performed, keeping microbial loads at low levels. Therefore, the scenario being modeled is a system failure situation that allows contamination of the recovered water post-treatment.

For these experiments, pasteurized milk (absence of *Listeria* was confirmed) was placed in the bioreactor (Biosurface Technologies Corp, Bozeman, MT) containing 24 stainless steel coupons that had previously been autoclaved to assure bacterial attachment came only from the contaminated-recovered water. The operating conditions for the bioreactor were followed from a standardized procedure (International, 2012). The bioreactor containing milk was stored for 24 h at 7°C. After 24 h, the standard CIP regime (described later) was applied using contaminated-recovered water containing 10^3 cfu/ml of *Listeria monocytogenes*. Then, a set of eight coupons were taken from the bioreactor, sonicated for 4 minutes and tested using the Most Probable Number (MPN) method; these counts provided the data for the initial bacteria attachment from the contaminated water to the equipment surface, after the CIP (Eq. 2). Eight additional coupons were taken from the bioreactor and placed directly in 9 ml of milk, these

coupons were immediately tested (time 0). While the remaining eight coupons were placed in 9 ml of pasteurized milk and stored for 24 h (time 24 h) at refrigeration temperatures (7°C) before testing, to simulate storage conditions during processing. Results from time 0 and time 24 h allowed to determine the transfer coefficient using equation 3. These experiments were performed three times.

Transfer coefficient estimation

$$TC = \frac{\sum_1^{Binomial(\frac{a_{tank}}{a_{coupon}}, p_{24h})} a_{coupon} * c_{24h}}{\sum_1^{Binomial(\frac{a_{tank}}{a_{coupon}}, p_0)} a_{coupon} * c_0} \quad \text{Eq. 3}$$

Where a_{coupon} is the total surface area of the coupons used in the experiment; a_{tank} is the total contact surface of the equipment during processing procedure, which varies depending on the supply need and capacity; p_0 and p_{24h} are the probability of a randomly selected coupon being *Listeria* positive at time 0 and 24 h; and c_0 and c_{24h} are average concentrations among all the positive coupons at time 0 and 24 h.

Standardized CIP Regime. The CIP regime consisted of an initial 5 min water rinse (25 °C), a 10 min wash with caustic cleaner (30 g/L, 65 ± 1 °C) (Spartan ®), followed by a 5 min water rinse (25 °C), a 10 min wash with an acidic solution (6 g/L, 65 ± 1 °C) (Spartan ®), and a final 5 min water rinse (25 °C).

Three-tube most probable number method. Samples were serially diluted in peptone water (10⁰, 10¹, 10²), and 1 ml of each dilution was transferred to three tubes

containing UVM Modified Listeria Enrichment Broth (Neogen, Lansing, MI); tubes were then incubated at 30 °C for 24 h. After incubation 0.1 ml were transferred from the UVM tubes to three additional tubes containing Fraser broth (Neogen, Lansing, MI), tubes were then incubated at 35°C for 48 h. Positive tubes (blackening of the medium) were confirmed by streaking one loopful of the secondary enrichment culture onto Modified Oxford (MOX) plates (Neogen, Lansing, MI). Plates were incubated at 35 °C for 48 hours. The number of confirmed positive tubes were recorded and the MPN determined using the statistical tables available for the method (FDA, 2015).

The concentration of pathogen of interest in the raw milk tank before pasteurization was modeled as equation 4.

$$C_{\text{rawtank.milk.bfpast}} = \frac{\sum_1^{\text{Binomial}\left(\frac{a_{\text{rawtank}}}{a_{\text{swab.sample}}}, \frac{p_{\text{swab.sample}}^{*ER}}{1 - p_{\text{swab.sample}}^{*ER} + p_{\text{swab.sample}}}\right)} (a_{\text{swab.sample}} * c_{\text{swab.sample}} * 10^{EF}) * TC) + C_{\text{rawtank.milk}} * v_{\text{rawtank}}}{v_{\text{rawtank}}}$$

Eq. 4

Where a_{rawtank} is the inner surface of the raw milk tank that has direct contact with the raw milk. The naturally occurring contamination of pathogens of interests on the tank inner surface was quantified by collecting and synthesizing findings from existing publications with the information of the area of swabbing ($a_{\text{swab.sample}}$), the prevalence of being pathogen positive among the swabbed samples ($p_{\text{swab.sample}}$), and the average concentration of pathogens among the positive swabs ($c_{\text{swab.sample}}$).

Milk pasteurization conditions are specified in state and local regulations. For high-temperature-short-time (HTST) pasteurization, milk must be heated to at least 71.7 °C for 15 seconds. Under such conditions, *Listeria monocytogenes* is not able to survive pasteurization treatment (CDC, 2001). Therefore, the 5-log reduction pathogen standard for dairy products was used for the model (FDA, 2007). During the storage of pasteurized milk, the pathogen concentration ($c_{pasttank.milk}$) was modelled with a cross-contamination event following a similar logic as during the storage of raw milk in the receiving tank. With an assumption of no post-contamination event occurring during the package filling procedure, the final number of pathogenic organisms in a random selected pasteurized milk package was modeled as equation 5.

$$n_{pathogen} = Poisson(c_{pasttank.milk} * v_{pack}) \quad \text{Eq. 5}$$

The risk assessment model with Monte Carlo simulation using Latin Hypercube sampling was constructed in Microsoft Excel 2013 with the add-on package @Risk (Version 6.2, Palisade Corporation, New York, USA). For the data that needed to be retrieved from existing publications, U.S. data was used to populate the model where possible. The simulation was run with 10,000 iterations to generate the predicted distribution of pathogen contamination level per package of fluid milk.

Alternative Scenarios

Chlorination is a chemical control widely applied to inactivate bacteria and some viruses in drinking water and wastewater processing (Virto et al., 2005). According to the

World Health Organization (WHO), drinking water typically has free chlorine levels ranging from 0.2 to 1 mg/L (WHO, 2003). Free chlorine is defined as the remaining chlorine concentration after the chlorine demand has been satisfied, and free chlorine offers protection from recontamination during storage (CDC, 2016).

Table 1. Summary of the input data used in the model simulation

Parameter	Parameters			Distribution	Reference
Efficiency ratio raw milk tank					
Probability Before	s+1= 19	N-s+1=1		Beta	(Meneses and Flores, 2016)
Probability after	0.47				(Meneses and Flores, 2016) ^(a)
Efficiency factor raw milk tank					
Concentration after CIP (log ₁₀ CFU/m ²)	5.48				(Meneses and Flores, 2016)
Concentration before CIP (log ₁₀ CFU/m ²)	12.66				(Meneses and FLores, 2016)
Concentration _{att} after CIP (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.91		Uniform	Experimental data
Transfer coefficient raw milk tank					
Tank area (m ²)	Min=22.8	Mode= 201.1	Max=203.2	Triangle	^(b)
Coupon area (m ²)	0.006				CDC Bioreactor
Probability at 0h	s+1= 2	N-s+1=23		Beta	Experimental data
Probability at 24h	s+1= 3	N-s+1=22		Beta	Experimental data
Concentration at 0 h milk storage (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.11		Uniform	Experimental data
Concentration at 24 h milk storage (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.42		Uniform	Experimental data
Pathogen concentration before pasteurization					
Receiving tank inner area (m ²)	Min= 22.8	Mode= 201.1	Max=203.2	Triangle	^(b)
Area of swabbing (m ²)	0.005				(Doijad et al., 2011)
Prevalence among swabbed samples	s+1= 5	N-s+1=22		Beta	(Doijad et al., 2011)
Concentration in swabbed samples (Log ₁₀ CFU/m ²)	5.48				(Meneses Yulie E., 2016)
Concentration in raw milk (Log ₁₀ CFU/m ³)	Min=3.74	Mode= 5.08	Max=7.46	Triangle	(Jackson et al., 2012)
Probability of positive silo	N=1	P=0.6028		Binomial	(Jackson et al., 2012)

Receiving tank volume (m ³)	Min=2.27	Mode= 151.4	Max=154.4	Triangle	(Jackson et al., 2012)
Pasteurization effect (Log)	5				(CDC, 2001)
Pathogen concentration after pasteurization					
Prevalence among swabbed samples	s+1= 1	N-s+1=34		Beta	(Doijad et al., 2011)
Concentration in swabbed samples (Log ₁₀ CFU/m ²)	0				^(c)
Volume pack (gal)	1				
Efficiency ratio pasteurized milk tank					
Probability Before	s+1= 19	N-s+1=1		Beta	(Meneses and Flores, 2016)
Probability after	0.47				(Meneses and Flores, 2016) ^(a)
Efficiency factor pasteurized milk tank					
Concentration after CIP (log ₁₀ CFU/m ²)	5.48				(Meneses and Flores, 2016)
Concentration before CIP (log ₁₀ CFU/m ²)	12.66				(Meneses and Flores., 2016)
Concentration _{att} after CIP (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.91		Uniform	Experimental data
Transfer coefficient pasteurized milk tank					
Tank area (m ²)	Min=22.8	Mode= 201.1	Max=203.2	Triangle	^(b)
Coupon area (m ²)	0.006				CDC Bioreactor
Probability at 0h milk storage	s+1= 2	N-s+1=23		Beta	Experimental data
Probability at 24h milk storage	s+1= 3	N-s+1=22		Beta	Experimental data
Concentration at 0 h milk storage (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.11		Uniform	Experimental data
Concentration at 24 h milk storage (log ₁₀ CFU/m ²)	Min= 5.03	Max= 5.42		Uniform	Experimental data

^(a) Deterministic value calculated using combined single proportion R package

^(b) Area calculated based on the tank volume and considering radio of 1.66 m and height of 17.8 m

^(c) Positive samples were not detected, therefore microbial concentration is not determined

In the baseline model, recovered water used in the experiments was tested to verify zero free chlorine concentration to assure that inoculated bacteria was not affected by the residual chlorine. Later, scenarios of recovered water containing free chlorine (0.2 and 1 mg/L) were modeled, in order to determine its effect on the reduction of the initial contamination. These scenarios were modeled using the equation provided by (Ndiongue et al., 2005), considering a storage temperature for the water of 18 °C and presence of

biodegradable organic matter in a concentration of 250 µg C/L. Only the minimum initial bacteria attachment from the water to the equipment, was calculated from the equation. For the bacterial transfer at 0 h and 24 h, the transferring proportions observed in the *L. monocytogenes* experiments were applied to calculate these values. Table 2 presents the data used to model the two free chlorine scenarios.

Table 2. Input data for the effect of chlorine residue on bacteria survival in recovered water

Free chlorine (mg/L)	Parameter	Initial attachment	Transfer at 0 h ^(a)	Transfer at 24 h ^(b)	Distribution
		Log (cfu/m ²)			
0.2	Min	2.45	2.45	2.45	Uniform
	Max	3.34	2.53	2.84	
1	Min	0.28	0.28	0.28	Uniform
	Max	1.16	0.36	0.67	

^(a) Values calculated using 1 and 0.16 as the transfer proportion from the initial attachment for minimum and maximum parameters, respectively. Chlorine reduction 2.58 log

^(b) Values calculated using 1 and 0.32 as the transfer proportion from the initial attachment for minimum and maximum parameters, respectively. Chlorine reduction 4.75 log

Assumptions

- For the calculation of the efficiency ratio (ER) and efficiency factor (EF), it was assumed that the washing efficiency for *Listeria monocytogenes* was similar for *Pseudomonas aeruginosa*, so that prevalence and concentration determined for *Pseudomonas aeruginosa* in the baseline study (Meneses and Flores, 2016) could be used in the simulation
- The volume and inner surface of the storage tank for pasteurized milk was assumed to be the same as the volume and inner surface of the commingling silo reported in the USDA survey study (Jackson et al., 2012)

- Bacteria concentration in swabbed samples before pasteurization was considered to be equal to the concentration of *Pseudomonas aeruginosa* after CIP reported in the baseline study (Meneses and Flores, 2016)
- For the scenarios modeling different free chlorine levels, the probability of detecting *Listeria* positive coupons among samples, was considered to be the same as the baseline mode
- The effect of chlorine residual on the initial bacteria survival for heterotrophic bacteria reported in the referred study (Ndiongue et al., 2005) was assumed to be similar to *Listeria monocytogenes*

Results and discussion

The conceptual model simulated in this study evaluated the safety implications of the use of reconditioned contaminated water in cleaning operations, in the final product. The pasteurization process is an effective killing step that controls not only naturally occurring contamination from raw milk, but also takes care of bacterial load transferred from the contaminated water to the equipment surface and from there to the milk. Bacteria concentration in the tank before pasteurization resulted in a value ranging from 0 to 6.48 log (cfu/m³), HTST pasteurization can reduce 5 log of the bacteria present in the milk. After pasteurization, contamination transferred from the surface of the storage tank contributes to the final bacterial concentration in the pasteurized milk tank, which for the model resulted in a value ranging from 9.4 E-7 to 28.8 cfu/m³. Figure 2 shows the predicted distribution of *Listeria monocytogenes* per package of product. Under the

conditions and assumptions applied to the model, 98 % of the total number of 1-gal packages produced, will test negative for *Listeria monocytogenes*, while concentrations between 0 and 1 cfu/gal could be found in 2% of the packages.

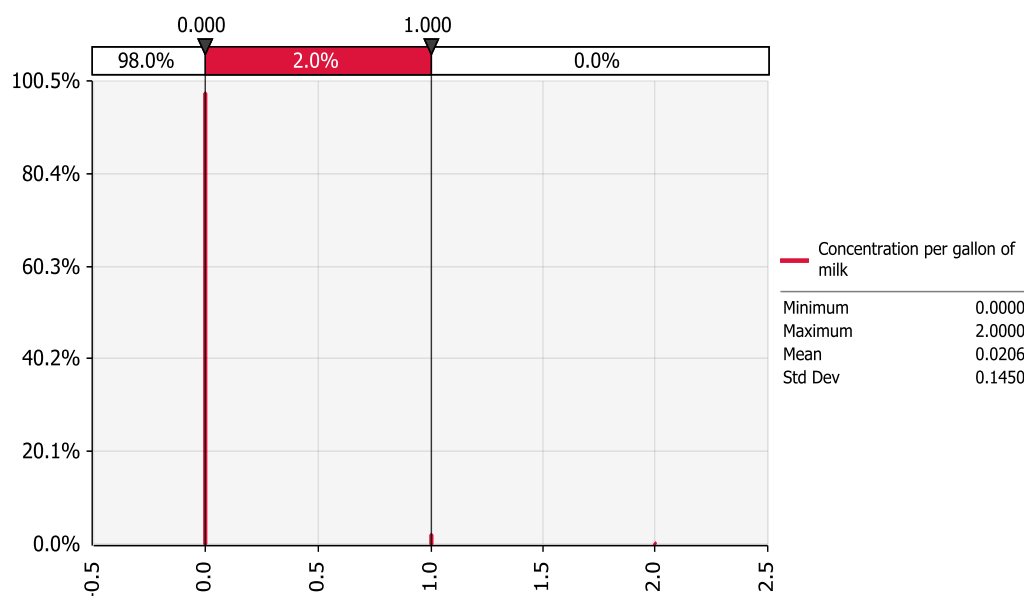


Figure 2. Predicted distribution for the final concentration of *Listeria monocytogenes* per gallon of milk.

Studies reporting the prevalence of *L. monocytogenes* in pasteurized fluid milk in the U.S. are limited. The only report available in the U.S., was a survey conducted in 2005 reporting a prevalence of *L. monocytogenes* of 0.018 % in pasteurized milk samples, with a contamination level lower than 0.3 MPN/g (Frye and Donnelly, 2005). The same author reported a prevalence around the world ranging from 0 to 21.4%. Results from our risk assessment study reported higher prevalence of *L. monocytogenes* in pasteurized milk samples in the U.S than the cited study. Differences might be explained by the locations where milk samples were collected. Data on the prevalence and concentration of *L.*

monocytogenes in raw milk came from samples collected during 2009-2010, from 32 states in all five regions (Northeast, Southeast, Central, Southwest and Pacific) (Jackson et al., 2012); while for the survey on pasteurized milk only four sites were included and samples were collected during year 2000. Given the time and state representation in the data source used in our study, results reported here might be a closer estimation of the national prevalence.

Sensitivity analysis

A sensitivity analysis was conducted in order to determine the components included in the simulation that affected the most to the final output. Figure 3 indicates that microbial contamination in raw milk positive silos, was the major factor influencing the final concentration in the pasteurized milk tank.

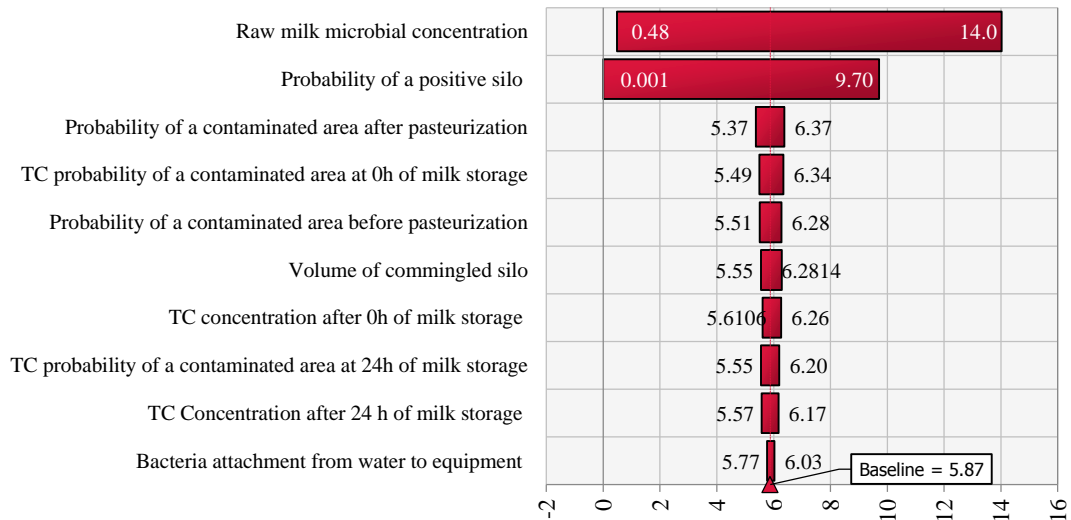


Figure 3. Sensitivity analysis of the bacteria concentration in the pasteurized milk tank, inputs ranked by effect on output mean (cfu/m3). TC: transfer coefficient

The spider graph presented in figure 3 shows how changes across a range of input values affect the final bacteria concentration in the pasteurized milk tank. Changes ranging from 0% to 100% of the input values related to the probability of a positive *L. monocytogenes* area before pasteurization, the probability of a positive area after 24 h of milk storage calculated for the transfer coefficient, and the volume of the tank caused negligible change in the final output. However, when initial concentration in raw milk form *L. monocytogenes* positive silos ranges from 4.90 Log (cfu/m³), 5 % input percentile, the bacteria concentration in pasteurized tank results in 0.48 cfu/m³. When bacteria in raw milk reaches 6.35 Log (cfu/m³), a 95% input percentile, the concentration in tank is 13.2 cfu/m³.

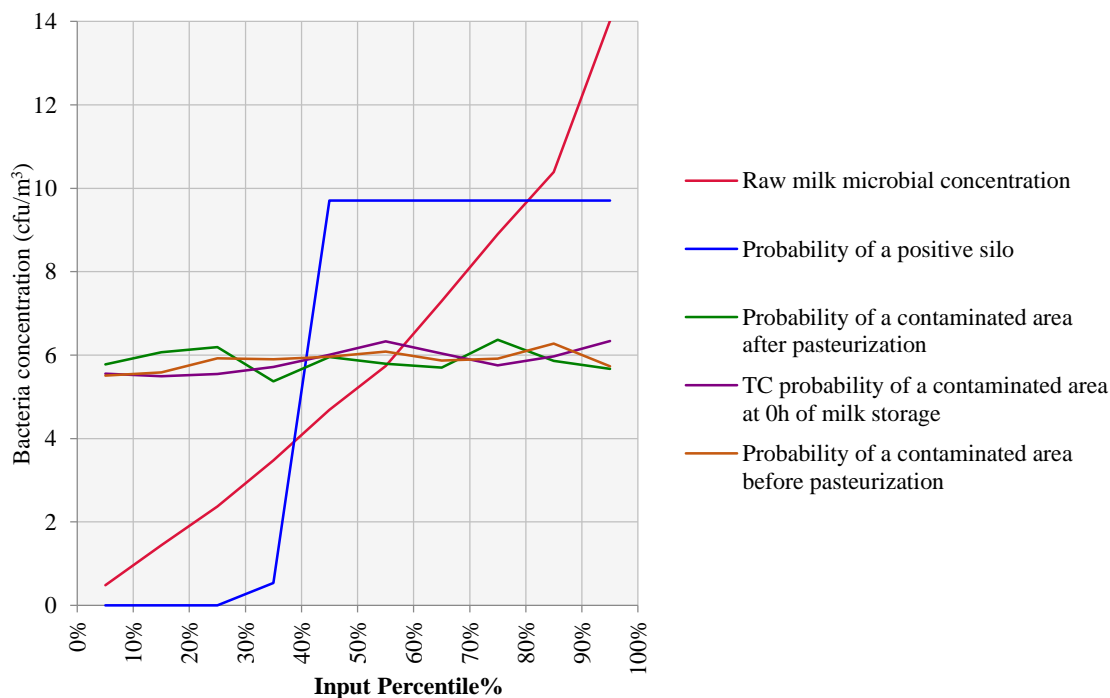


Figure 4. Spider graph of the bacteria concentration in the pasteurized milk tank (cfu/m³), change in output means across a range of input values

Scenario Analysis

It was previously discussed that the initial bacteria concentration in raw milk is the major input affecting the final contamination in the pasteurized milk tank. Therefore, a percentile matrix is provided in table 3 showing the effect of bacteria reduction, caused by different free chlorine concentrations, in the percentile values of the major input. Results presented in table 3 indicate that bacteria transferred from contaminated recovered water to the equipment, is minimal. Although free chlorine significantly reduced the initial contamination in reconditioned water (see table 2), such reduction did not change the concentration in raw milk. Consequently, the final concentration in the pasteurized milk tank is not affected, neither the predicted distribution of *Listeria monocytogenes* per package of milk, final output of the model assessed in this study.

Table 3. Changes in raw milk bacteria concentration due to changes in free chlorine levels in recovered water

Free Chlorine (mg/L)	Percentiles from the bacteria concentration in raw milk				
	0%	25%	50%	75%	100%
0	3.30	5.60	5.93	6.16	6.46
0.2	2.88	5.60	5.93	6.16	6.46
1	3.14	5.60	5.93	6.16	6.46

In this study, the impacts of a possible contamination scenario in the reconditioned water used in cleaning operations was assessed. The quantitative results discussed above, indicated that when a silo tested positive for *L. monocytogenes*, the contamination in raw milk was the major factor contributing to the final concentration in pasteurized milk tank. In comparison with the contamination coming from raw milk, the contribution of bacteria

transferred from the contaminated reconditioned water to the equipment surface and from there to the milk, is minimal and even lower when free chlorine is present. However, regulations require that when reconditioned water is intended to be used in food contact surfaces, such as for cleaning applications, controls should be established to assure the safety of the final product. Free chlorine is an effective disinfection strategy that has been applied in different sectors including, drinking water production and food industry (Bremer et al., 2002, Virto et al., 2005). Consequently, there are several studies showing the effect of free chlorine in the reduction of organisms, their resistance, and effective doses. Virto et al. (2005) demonstrated that gram negative organism are more resistant to free chlorine action than gram positive, when organic matter is present in the water. Another study focused in gram negative bacteria reported a 3 log reduction in *Pseudomonas fluorescens* with a free chlorine dosing of 0.05 mg*min/L, while for a reduction of 2-3 log in *E.coli* a dosing of 0.3 mg*min/L was required (Wojcicka et al., 2007).

An alternative approach for the treatment of drinking water is the use of UV disinfection, which does not contribute to the formation of undersirable disinfectant by-products (DBPs) and treatment costs do not differ significantly from those associated with chlorine disinfection (Wolfe, 1990). The combined application of both treatment options has been shown to have synergistic effect in the reduction of bacteria load. According to (Wang et al., 2012) a reduction of 3.46 and 4.5 log units in total bacteria

count and heterotrophic plate count, respectively, was achieved in wastewater with a UV treatment (40 ml/cm²) followed by chlorine treatment (2 mg/L).

The majority of the published literature assessing the effectiveness of free chlorine and UV have been developed for drinking water quality, thus indicator bacteria for that scenario (heterotrophic bacteria, viruses, coliforms and *E.coli*, *Pseudomonas*, *Legionella*, *Vibrio cholerae*, etc.) have been targeted. However, reconditioning of water from food processing wastewater streams do not necessarily contain those microbiological hazards, such as the case presented here, *Listeria monocytogenes* in the dairy processing scenario. This fact confirms the need to develop studies associated with microorganisms likely to be present in food processing environments in order to implement water reconditioning and reuse alternatives.

Risk assessment and HACCP plans for the reuse of reconditioned water

Microbial risk assessment is a tool that allows identification and evaluation of potential risks in a defined scenario. However, it also offers key information to develop robust hazard analysis and critical control point (HACCP) system, which is methodical tool widely applied in different food sectors, but never introduced in the reuse of reconditioned water. Casani and Knøchel (2002) proposed a HACCP based generic model for the reuse of water in the food industry. Our published work (Meneses and Flores, 2016) together with results from this study, provided information to design a reference HACCP plan for the reuse of whey-recovered water in cleaning operations

following the generic model. Figure 5 contains a schematic representation of the HACCP plan for the reuse of reconditioned water in a dairy processing facility.

Microbial quality of whey and possible water reuse have been established, both are preliminary steps suggested in the generic HACCP model. Water was recovered from pasteurized cheese-whey, total plate counts, coliforms and *E.coli* were tested in the whey for microbial quality; only the starter culture bacteria used for protein coagulation remained in the whey in a concentration of 7.2 Log₁₀ (cfu/ml) (Meneses and Flores, 2016). Cleaning-in place applications were defined as the intended reuse of the recovered water. Once the prerequisites have been fulfilled, the principles of the HACCP system should be applied, which have been described in the generic model (Casani and Knøchel, 2002) as well.

1. **Hazard identification.** The generic model indicates that hazardous microorganisms that can be potentially present in the water prior to reuse should be listed, but leaves out those that are destroyed during processing. For our HACCP model, pasteurization of milk used in cheese manufacturing reduces pathogenic bacteria to safe levels. Membrane filtration removes starter culture bacteria present in whey, while chlorination of the recovered water provides some additional protection for recontamination (as the case of *L. monocytogenes* presented in the risk assessment model).

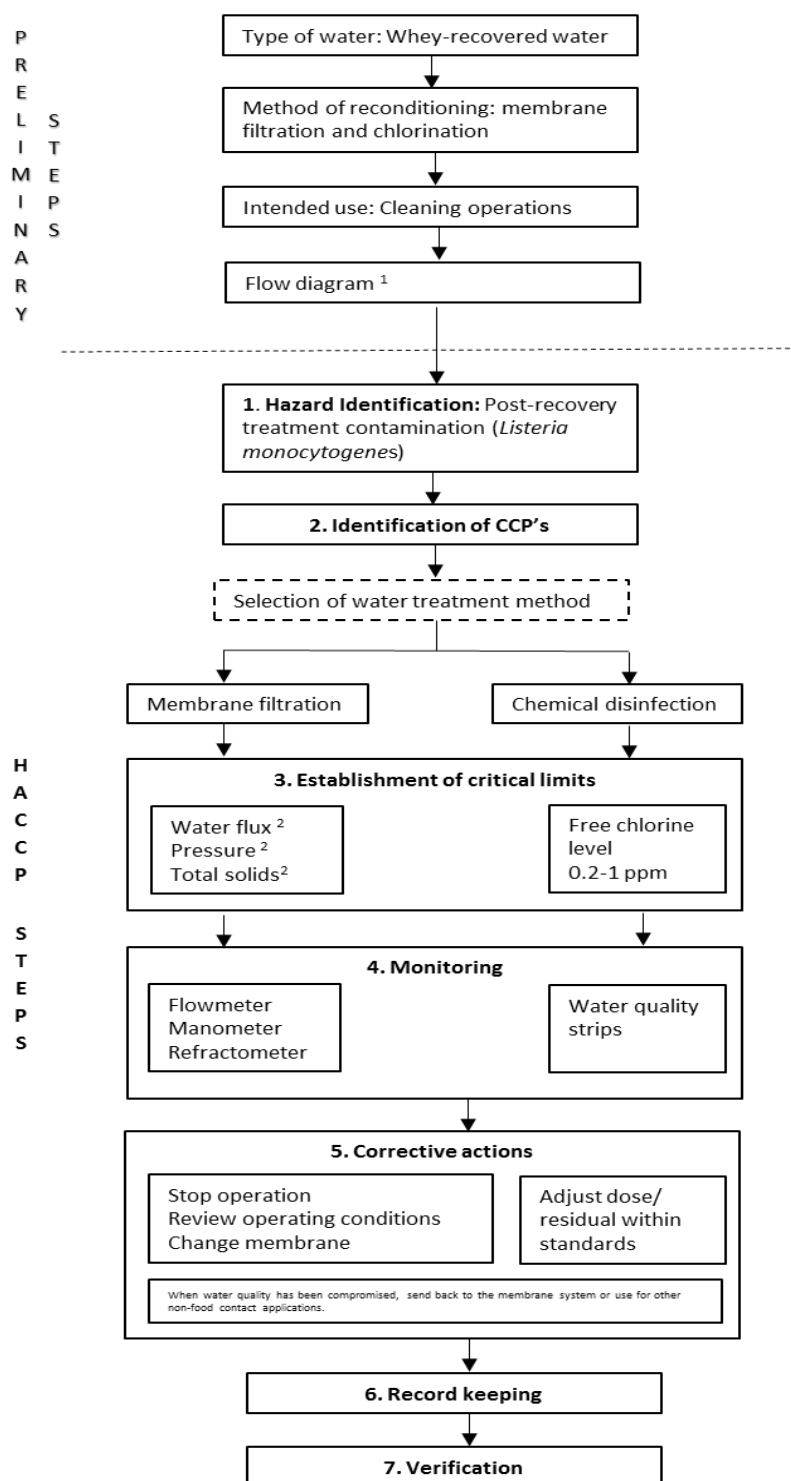


Figure 5. Schematic representation of a reference HACCP plan for reconditioning of whey-recovered water
^{1, 2}. Flow diagram and parameter values presented in our published paper (Meneses and Flores, 2016)

2. **Identification of CCP's.** According to the model presented by Casani and Knøchel (2002) an identified critical control point is the reconditioning treatment, since this step is intended to bring the microbial hazard to acceptable limits. Membrane filtration followed by chemical treatment in the recovered water are the reconditioning steps proposed in our published work (Meneses and Flores, 2016). Water flux, pressure, total solids and water quality were monitored to evaluate process efficiency in membrane filtration; the same parameters could be used to monitor possible treatment failures such as membrane leakage. For the chemical disinfection step (chlorination), dosing within safe established parameters (WHO, 2003, CDC, 2015) for potable water were followed in our baseline study. However, from the risk assessment study we learned that acceptable levels of free chlorine provide protection from recontamination. Consequently, the critical control points for the water recovery system are membrane operational conditions and free chlorine levels in the recovered water before reuse.
3. **Establishment of CCP critical limits.** This HACCP step indicates that one or more measures should be put in place to control the established critical control points. For each measure, critical limits should also be defined. Herein, we are providing the lower and upper values obtained in the water recovery system. However, it is imperative to mention that these values were established from a pilot scale system and are being presented as a reference. Extensive validation on an industrial scale is required to determine the final critical limits for the

membrane and chemical disinfection steps. The values obtained from the membrane filtration system were, 41 ± 2.05 and 24.7 ± 2.85 L/m²h at the beginning of the filtration, decreasing to 4.80 ± 1.85 and 5.71 ± 0.82 L/m²h for UF and RO, respectively. Pressure levels started at 0.3 and 3 MPa and reached 0.5 and 4 MPa at the end of the filtration for UF and RO, respectively. Solid content in the concentrate increased from 6.87 ± 0.02 % and 5.83 ± 0.02 % to 10.11 ± 0.17 % and 16.57 ± 1.42 %, for UF and RO, respectively.

Results for the water quality analyses in the recovered water are reported in our published work (Meneses and Flores, 2016). The chlorine demand for the recovered water was 1 mg/L, for total disinfection of the recovered water (< 1 cfu/ml), residual chlorine was not detected in the water. The risk assessment study reported that a free chlorine level ranging from 0.2 to 1 mg/L is frequently found in drinking water and provides protection for possible post-recovery- treatment contamination.

4. **Monitoring.** This step requires specific procedures to monitor the control points as well as the frequency. For the water flux and pressure, flowmeter and manometers devices were available in the membrane system, while for a solid content a refractometer provides immediate information during the filtration. All these parameters were monitored in our study in 10-minute intervals throughout the duration of the filtration. Microbial testing of the recovered water was performed 30 minutes after dosing to allow enough contact time. For free chlorine levels the 5 in 1 Aquacheck water quality test strips were used; these strips

measure total and free chlorine, as well as hardness, alkalinity and pH. Several tests were applied to evaluate the final quality of the recovered water in our study, including conductivity, nitrate, nitrite, ammonia, total organic carbon (TOC) and chemical oxygen demand (COD), specific methodologies are available in the material and methods section (Meneses and Flores, 2016). In a processing applications, conductivity and/or TOC and COD provide a good references for the effective removal of pollutants from the wastewater.

5. **Corrective actions.** Deviations from the established critical limits could happen, thus corrective actions should be defined as part of the HACCP plan. In the membrane filtration system, deviations in the water flux and pressure could affect the quality of the recovered water, for such cases the operation should be suspended to review that initial operation conditions are set to optimal levels and to check membrane for damages. In either case, total volumetric permeation collected at that point should be send back to the feed tank for recirculation in the membrane system, once operating conditions have been corrected or/and membrane has been replaced, depending on the identified cause of deviation. For the chemical disinfection, when erroneous calculations result in excessive dosing (total chlorine > than 5 ppm), water should not be used in cleaning operations. When free chlorine levels have not been reached, additional dosing should be performed without exceeding limits for potable water (WHO, 2003, CDC, 2015).
6. **Record keeping and Verification.** These two steps are crucial to demonstrate to regulatory agencies the robustness of the HACCP system. (Casani and Knøchel,

2002), described in detail the documentation required for these steps, all have been provided in the previous principles with exception of the flow diagram that was provided in our published work. For verification of the HACCP plan, three stages have been defined including, validation, on-going verification and annual reassessment. To accomplish this final principle, validation of the entire water recovery system in an industrial scale is still necessary.

In this final section, we have integrated results from a previous work focused on the operational performance of the proposed water recovery system and the microbiological aspects of water reuse in cleaning-in place operations, with the findings obtained in the risk assessment model. Results from both studies established the bases for a HACCP plan on the reuse of whey-recovered water in cleaning operations for the dairy industry, demonstrating that in order to propose efficient and applicable water conservation strategies for the food sector, specific case studies should be developed.

Limitations and further research needs

The risk assessment model presented here, was populated with data from published literature and obtained from experimental trials. One considerable limitation found while developing the model, is the lack of case studies in microbiological impacts of the reuse of reconditioned water in food processing operations. Thus, several assumptions had to be made. Experimental data was useful, nevertheless industrial scale validation is still

required, especially to define critical limits for the control points identified in the HACCP plan.

In order to strengthen the case study presented here, some additional knowledge might be obtained with further research on the effect of length of storage on the microbial quality of the reconditioned water. As part of the cleaning procedures, some industries apply a chemical sanitation step after the final water rinse, or allow equipment to dry after cleaning; any of these steps were considered in the present study. In addition, the contribution of the residual TOC in the recovered water to DBPs formation, should be assessed.

Conclusions

The safe reuse of reconditioned water in food processing operations is feasible. There are several tools developed for the evaluation of safety strategies in the food sector that can be applied in water reconditioning and reuse, as well, such as quantitative microbial risk assessment and HACCP plans. Findings from the present study suggest that in the event of contamination of the recovered water, bacteria transferred from the water to equipment and from the equipment to the manufactured product, is minimal. Under the simulated conditions for the risk assessment, it was determined that initial bacteria concentration in raw milk, remains as the major factor responsible for microbial quality of the final product. The HACCP plan, presented as a reference example, provides key information for the safe water recovery from whey and its subsequent reuse in cleaning operations. The framework developed for the evaluation of a water conservation strategy

in the dairy industry, set the grounds for the application of similar models in other food sectors.

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CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

This study demonstrated that water reconditioning and reuse would offer several benefits for the dairy industry, specifically for cheese manufacturing operations. The proposed water reconditioning as a conservation alternative has been evaluated from different perspectives including technical performance, economic feasibility, food safety and environmental impacts. All with the goal of providing scientifically sound information to overcome constraints that, in the past, prevented water reconditioning and reuse to become standard practices in food processing operations.

Efficiency of the system, quality of the recovered water and cost associated to the implementation, are among the most common questions when proposing a water conservation intervention. The main findings addressing these questions are detailed below.

- Good quality water can be recovered from cheese whey to be reused in CIP systems. The proposed system showed water recovery of 47.03% from the UF/RO filtration, whereas 85.65% was reached including the water recovered from the spray drying step as part of the integrated system.
- The cleaning efficiency of the water recovered from the UF/RO filtration system was comparable to potable water, when used in the CIP system.
- Spray drying of the concentrated streams produced protein and lactose powders satisfying commercial standards.
- The proposed intervention is economically feasible for different cheese production scales.

As a result of water reuse, a reduction in the total water consumption is expected. However, the next concern is related to other environmental impacts that implementing the water recovery system might cause. The life cycle assessment performed in this study reported the following findings.

- The water recovery system offers significant reductions of the environmental impacts related to current practices of sending whey to be treated in a wastewater treatment plant and/or to obtain water for industrial use from a water production plant.
- The water recovery system represents 12% of the total emissions calculated for a wastewater treatment plant and 82% of the ones related to the water production plant for the treatment of one unit of whey and the production of one unit of water, respectively.
- Electricity usage was identified as the main input responsible for the environmental burdens of the system, throughout several impact categories included in the assessment.
- Membrane filtration and spray drying are the hot spot operations, where efforts should be focused to reduce consumption of fossil fuel-generated electricity.

Microbial contamination is a significant aspect that contributes to the risk perception about reuse of reconditioned water for processing operations, as proposed in this study. The microbial risk assessment simulating a possible route of contamination of the recovered water with *Listeria monocytogenes*, revealed the following outcomes.

- In the event of contamination of the reconditioned water, bacteria transferred from the water to equipment and from the equipment to the manufactured product, was minimal.
- Under the simulated conditions for the risk assessment, it was determined that initial bacteria concentration in raw milk, remains as the major factor responsible for microbial quality of the finished product.
- The HACCP plan, presented as a reference example, provides key information for safe recovery of water from whey and its subsequent reuse in cleaning operations.

The findings reported herein have set the ground to implement water reconditioning and reuse practices in the food industry, yet they also open opportunities for further research. Some ideas are proposed below.

- The first step to identify possible sources of wastewater and water quality requirements for reuse of reconditioned water, is the quantification of current water consumption in different operations within a food processing plant. This detailed information is lacking for most food sectors in the U.S.
- Redesign of cleaning operations, where food safety continues to be the major priority, but water conservation and reduction of other environmental impacts are also considered. Use of carriers, other than water, such as air for initial rinses, use of biodegradable chemicals, application of sensor technologies to adjust the intensity of the cleaning operation to the initial organic load, and chemical recycling are some proposed alternatives.

- Determination of water quality requirements for specific operations within the food processing plant, that way potable water use is restricted to certain operations to assure safety and quality for the final products. For other, not so critical operations, reconditioned water could be used; thus, creating parallel water systems.
- Evaluation and application of emerging technologies on water reconditioning treatments to reduce cost, increase water recovery, reduce cleaning frequency, improve safety levels, and conserve energy.
- Risk assessment studies evaluating different aspects associated to water reconditioning and reuse in the food industry are lacking. Application of this important tool is essential for the development of specific HACCP plans and guidelines suitable for a particular food sector. Risk assessment could be applied to determine maximum organic residual in reconditioned water for safe chlorination (avoid formation of disinfection by-products), to establish safety levels among different reconditioning treatments to assure water quality and safety, to simulate possible routes of contamination in order to establish suitable processing control points.

Food industries offer suitable scenarios for the implementation of water reconditioning and reuse. The five components presented in this dissertation (technical, value-added of by-products, economical, safety and environmental), provide a strong framework for the evaluation of water conservation alternatives on specific food sectors from different angles. The findings, discussed earlier, provide key information to bring

water conservation initiatives to the forefront of the discussion as effective options to reach sustainable operations throughout the food supply chain; thus, a template for water optimization in food processing systems.

APPENDICES

Appendix A. Annual consumables break down for the application of the water recovery system in a large scale cheese plant. Data simulated in SuperPro® designer.

Annual consumables	Amount
Membrane system	
UF membranes (m ²)	10,525
RO membranes (m ²)	4,227
Total membrane area (m ²)	14,752
Membrane weight (0.48 kg)	0.48
Total membrane weight (kg)	7,080
Electricity (Million kw.h)	10.07
Chilled water (MT)	172,163
Membrane CIP	
Sodium hydroxide (kg)	2,172
Nitric acid (kg)	866
Water (Million kg)	1.68
Energy (kw-h)	256,008
Spray drying and packaging	
Energy (Million kw.h)	2.32
Energy for steam production (kw.h)	202,763
Energy for loading and condenser (Million kw.h)	3.09
Chilled water for condensation (Million MT)	1.44

Appendix B. Annual consumables break down from the Theresa wastewater treatment facility located in Lincoln-Nebraska. Year 2014.

Annual consumables	Amount
Treated flow (Million gal)	8,646.2
Sodium hypochlorite (gal)	52,019
Liquid caustic soda (gal)	20,674
Ferrous Chloride (gal)	245,624
Hydrogen peroxide (gal)	574,109
Polymer agent (gal)	147,249
Electricity (Million kw.h)	15.09
Natural gas (therms)	176,522
Water use (Hundred cubic feet)	89,718
Outputs	
Biosolids (dry tons)	4,837