SIMULATING THE EFFECTS OF CROSS-CONTAMINATION OF *ESCHERICHIA COLI* O157:H7 ON FRESH-CUT LETTUCE DURING POST-HARVEST PROCESSING FROM AN AGENT BASED PERSPECTIVE

A Thesis

by

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ABSTRACT

Fresh-cut leafy greens are potential vehicles for foodborne pathogens such as *Escherichia coli O157:H7* and are at high risk of causing foodborne illnesses. Cross-contamination during post-harvesting processing of leafy greens is of great concern as it has been linked to many outbreaks in the US.

An agent-based simulation was developed to represent the spatial and temporal *E. coli* O157:H7 cross-contamination dynamics in a processing facility for fresh-cut romaine and iceberg lettuces using NetLogo. The model was designed to (1) track *E. coli* O157:H7 and lettuce movements in time, (2) evaluate microbial contamination in different equipment/surface and calculate the probability events of cross-contamination between lettuces and equipment, and (3) determine the number of fresh-cut contaminate processed bags and their level of contamination at the end of the processing line. An extension was also added to the main model to model *E. coli* O157:H7 growth due to temperature abuses in a cold storage facility. A user-friendly interface was created to follow spatial and temporal variations in model outputs. The number of contaminated bags, the lettuce contamination levels, were computed, and visualized on plots and diagrams. Diagrams representing equipment variables were also produced to track changes in these variables.

Experimental data of cross contamination from literature was used to describe the facility and validated the model. Sensitivity analysis of different factors influencing cross-contamination was tested.

The key factor affecting cross-contamination is the chlorination concentration dose rate. The number of contaminated bags is affected significantly by the initial level of contamination of

the incoming lettuce heads and the probability of contamination in the incoming produce. The level of contamination as well as probability of contamination in the facility environment (equipment) affect the number of bags contaminated. Batch size affects the number of contaminated bags when the first income lettuce batch is contaminated.

Storage room temperature fluctuations showed the importance of real-time monitoring to avoid microorganism growth and thus prevent an increase in the number of contaminated bags.

This work provides insights on applications of real-time cross-contamination data in fresh-cut leafy green processing operations. It analyzes the knowledge of cross-contamination information and its impact on processing performance by studying the effect of mitigation strategies.

DEDICATION

I dedicate my work to my family, especially my parents Rosalia and Blazio who were always by my side in both good and tough times. Even though you are thousands of miles away, it always felt like you were always by my shoulder, your comforting words and motivation pushed me through the finish line. To my younger siblings who inspire me to work hard, persevere, and lead by example, I thank you both. Knowing that you both look up to me has always helped me to be at my best. Chioniso and Tariro, you guys might not realize it but, casting a good shadow on you both has always been key to me completing this journey and all my accomplishments.

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CONTRIBUTORS AND FUNDING SOURCES

NOMENCLATURE

ABM	Agent-Based Modeling
AgMRC	Agriculture Marketing Resource Center
ANOVA	Analysis of Variance
AOP	Advanced oxidation Process systems
CDC	Centers for Disease Control and Prevention
CDF	Cumulative distribution function
CFU	Colony-forming unit(s)
COD	Chemical Oxygen Demand
CPU	Central Processing Unit
DBPs	Disinfection By-Products
DES	Discrete-time Event Simulation
FAO	Food and Agriculture Organization
FC	Free Chlorine
FDA	Food and Drug Administration
FS-ABS	Food-Safety Agent-Based Simulator
GMP	Good Manufacture Practices
HACCP	Hazard Analysis Critical Control Point
IDE	Integrated Development Environment
IID	Independent and Identically Distributed
ln	natural logarithm base e (2.71828…)
log	logarithm base 10

MSE	Mean Squared Error
ORP	Oxidation Reduction Potential
PMF	Probability Density Function
ppm	Parts per million
QMRA	Quantitative microbial risk assessments
RBPC	Risk Based Preventive Controls for Human Food
RMSE	Root Means Squared Error
RTE	Ready-to-eat
SD	System Dynamics
TAMU	Texas A&M University
TDS	Total Dissolved Solids
TIC	Theil's Inequality Coefficient
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION

Lettuce is a great source of vitamins, minerals, antioxidants, and other health-promoting compounds (Jung et al., 2014). In the U.S, lettuce is generally consumed raw in many forms including in salad, lettuces wrap, tacos, burrito bowl, and burgers. In the last two decades, the U.S. have seen a surge in the consumption of Ready-to-Eat (RTE) vegetable and leafy greens due to their convenience and nutrition value (USDA, 2019). Today, the salad industry is a multibillion dollar business that consolidates raw ingredients from many big producers (Mir et al., 2018). In 2016, store sales of packaged salad in the U.S. averaged 4,017 U.S. dollars per week (Statista, 2021).

It was not long ago that the vegetable industry was just local farmers supplying to the community. Today, the industry has grown into a massive centralized industry that supplies fresh produce to multiple states and even other countries. About 73 and 23 percent of all lettuce production in the US comes from California and Arizona, respectively (AGMRC, 2021). Centralization has many benefits, the main one being that it makes value-added processing much more efficient, which is very important because the shelf life of leafy greens is very short (3-7 days in refrigerated storage). One major drawback of centralization is that small incidences in one facility are turning into multi-state catastrophes, as what is happening with the current pandemic. Decentralization meant contamination incidences were isolated and could be contained (Scalco et al., 2020). After the current pandemic upended national supply chains, the sales of locally and sustainably grown lettuces started to surge and this trend is expected to grow in the future.

In the U.S., fresh produce remains the leading cause of foodborne illness outbreaks compared to meat, dairy, and seafood (CDC, 2020). From 2008 to 2018, the number of outbreaks related to leafy greens have increased sharply from 69 in 2008 to 2953 in 2018. During that same period, 28,217 outbreaks of foodborne illness were linked to the consumption of leafy greens resulting in approximately 853,000 illness, 16,00 hospitalizations, and 1,174 deaths (CDC, 2020). Consequently, this increased consumption of leafy greens worldwide and the surge in the number of produce-related disease outbreaks can have a serious socioeconomic impact.

Many factors affect the contamination of fresh produce with pathogens leading to foodborne illness outbreaks. These factors include worker health and hygiene, agricultural water quality, animal manure and other materials of animal origin as fertilizer, growing and harvesting operations, equipment and building sanitation, temperature abuse during processing, transportation, at the retail, and even the recontamination by microorganisms aerosolization in the facility's environment (den Aantrekker et al., 2003; FDA, 2018; Murray et al., 2017).

Investigation of the causes of these outbreaks is critical in developing targeted mitigation strategies. The process requires globally inclusive analyses looking for pathogens related to the production and processing of leafy greens. However, the complexity in reliable trace-back data make it difficult to identify the contamination sources; there is lack of information in the literature on the setting in which outbreaks occurred; and relevant outbreak data (type of microorganism, pathogen location and pathway) for risk management analysis is not available (Machado-Moreira, 2019). In addition to proper information and reliable data, new approaches are needed to accurately detect microorganisms and track contamination sources in real-time so new methods can be developed to minimize pathogen contamination in leafy greens during pre- and post-harvesting settings.

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Food safety of leafy green vegetable processing is miles behind compared to other industries. Most of the commercial sanitizers available to treat fresh produce are ineffective in reducing the microbial load, being only capable of 1 to 2 log CFU/g reductions. The post-harvest wash process is considered as a potential contamination point, which has resulted in large numbers of contaminated lettuce bags containing a significant number of pathogens (Barrera et al., 2012). There is not a single point of entry for pathogens in a processing line, so it is assumed that any surface that comes into contact with fresh produce is a potential contaminating source. Although the subject of cross-contamination dynamics in leafy greens processing line is still not fully understood, through a series of experiments, it has been proven that the transfer of *E. coli* microbial load between leafy greens and processing equipment surfaces is bi-directional, contaminated surfaces can contaminate fresh produce, and it is reciprocal (Buchholz et al., 2014).

In the absence of reliable pathogen cross-contamination detection systems in fresh produce, computer models and systems have become important information tools (Mishra et al., 2017; Mokhtari et al., 2018; Mokhtari and Van Doren, 2019; Perez-Rodríguez et al., 2011; Zoellner et al., 2019). Computer models can help in analyzing the spread of contamination in a produce processing facility, with temporal and spatial features (Mokhtari et al., 2018). An accurate model can help establish which guidelines and actions will have the greatest impact on reducing cross-contamination, or how to best prevent contamination from starting and spreading.

In recent years, machine learning has been used to predict contamination in other fields, such as in soil and crop science to predict bioaccumulation of heavy metals; in hydrology for mapping groundwater contamination of aquifers, and in civil engineering to predict building contamination (Barzegar et al., 2018; Hu et al., 2020; Martin and McKenna, 2007). Machine

learning has not yet been applied to cross-contamination in lettuce processing mainly due to the lack of reliable data. Mishra et al. (2018) stated that the lack of adequate data was the main limitation to their model in predicting the survival, growth, and death of enteric pathogens in leafy green processing.

Recently, researchers in food engineering have gravitated towards simulation, specifically agent-based modeling (ABM), to counter for this data deficit, and to apply other statistical methods to analyze microbial cross-contamination in fresh-cut lettuce/leafy greens processing (Mokhtari et al., 2018; Rodríguez et al., 2011; Zoellner et al., 2019).

CHAPTER II

OBJECTIVES

In this work, we simulated the cross-contamination of a common pathogen, *E. coli* O157:H7, in a fresh-cut lettuce processing facility using agent-based model (ABM) approach. This approach can capture the complex interactions between factors and emergent results based on agents' (lettuce and equipment) interactions within the model that other types of models cannot. These are essential in understanding the dynamics of cross-contamination during the post-harvest processing chain of fresh-cut leafy-greens. Most of the factors that affect cross-contamination in fresh-cut lettuce (equipment surfaces, lettuces, wash water) were considered in this study. Decontamination in the wash tank was evaluated in terms of levels of free chlorine and chemical oxygen in demand. Additionally, pathogenic growth due to temperature abuses during storage (post processing) were integrated into the model. Available literature data (Buchholz, et al., 2012) from a pilot size fresh-cut processing facility were used to create the model.

To better understand the role of processing facility patterns on *E. coli* O157:H7 cross-contamination at romaine/iceberg lettuce-processing equipment interface, we developed an agent based model for a pilot plant processing facility for fresh-cut romaine/iceberg lettuce (Buchhlolz et al., 2012a) using the software NetLogo (Wilensky, 1999). NetLogo, a programming language and integrated development environment (IDE) for agent-based modeling, it is a simulation tool representing interactions between multiple agents in a spatially explicit environment. Our model was designed to (1) track *E. coli* O157:H7 and lettuce movements in time, (2) evaluate microbial contamination in different piece of equipment (spatially explicit) and calculate the probability events of cross-contamination between agents (lettuces) and patches (equipment), and (3)

determine the number of fresh-cut contaminate processed bags and their level of contamination at the end of the processing line. An extension model was also added to the main model to determine the temperature pattern in a cold storage facility.

Our agent-based model represents interactions between the facility equipment and lettuce in a bottom-up approach. Lettuces are agents that move and interact with the facility environment, which is represented by the leafy greens' facility layout and attributes. There are decision rules that specify behavior of the lettuces at a micro level. Therefore, in our model the basic units (lower level components) are the lettuces and equipment patches. The higher-level components, such as *E. coli* O157:H7 outbreak, result from interactions between the agents and patches.

This work represents the development of a food-safety agent-based simulator (FS-ABS) to address the effect of facility patterns on the cross-contamination of *E. coli* O157:H7 outbreak at a leafy green processing facility and cold storage. The main objective of this study was to develop a simulation model to virtually represent a leafy green processing facility over time and allow testing of hypothesis based on various scenarios related to the cross-contamination of pathogens in fresh-cut produce. Visualization could be used not only as a validation tool but also to provide an aid to the leafy green producer who is unfamiliar with simulation and modeling. The developed simulation and visualization tools (NetLogo software) were used to analyze cross-contamination of fresh-cut produce in a standard leafy green processing facility line.

The specific objectives of this study were:

a) To model cross-contamination in a fresh-cut lettuce processing facility line using discrete event agent-based modeling approach.

b) To validate the developed model characteristics using experimental results from literature.

c) To run sensitivity analysis to analyze the effects of variations and uncertainty in input on the resulting output

d) To evaluate cross-contamination in the processing facility using different scenarios to determine the impact of different input conditions on the number of contaminated fresh-cut lettuce bags.

e) To model the growth of *E. coli* O157:H7 in fresh-cut lettuces due to temperature abuse in storage.

f) This work will help the fresh-cut processing operations by investigating benefits of facilitating real-time product data along processing and storage.

CHAPTER III

LITERATURE REVIEW

3.1 Food Safety

In recent years, consumer demand for fresh, healthy, and convenient foods has resulted in a dramatic expansion of the market for fresh-cut produce (Duff and Phelps, 2017). Nevertheless, there has also been an increased number of foodborne illness outbreaks associated with a variety of fresh produce. This is mainly because many types of fresh-cut produce are ready-to-eat (RTE) foods that are eaten without cooking, a step which would aid in killing pathogens before consumption.

All types of fresh produce can be contaminated with foodborne pathogens. Several produce that were never associated with outbreaks have now been contaminated with *Salmonella* including peaches and onions in 2020 (CDC, 2020), papayas in 2017 (CDC, 2020), and cucumbers, the latter been frequently contaminated since 2012 (Sharma et al., 2017). On the other hand, studies have shown that close attention to both pre- and post-harvest food safety practices improved the safety of tomatoes, showing very few outbreaks of *Salmonella* since 2011 (Ilic et al., 2017). Fresh produce types implicated in outbreaks are potentially linked to several factors including globalization of the fresh produce supply, aging population, increased consumption, and possibly climate change (Murray et al., 2017).

Coulombe et al. (2020) revealed that from 2008 to 2018, 11 outbreaks of *E. coli* O157:H7 infection in Canada were linked to leafy greens, including 7 (63.6%) linked to romaine lettuce, 2 (18.2%) linked to iceberg lettuce. The reported indicated that the commercial distribution, travel distances between California and the eastern states of Canada, and the storage practices used for lettuce may be important factors for these outbreaks.

As the lettuce/leafy greens supply chain industry grew, so did the focus of the food safety efforts. Initially, it used to be an on-farm only operation that included initial cooling and distribution points and value-added processing. The food safety program was largely centered on current Good Manufacture Practices (GMPs) and the principles of Hazard Analysis Critical Control Point (HACCP) programs. Although produce can become contaminated before it reaches a fresh-cut processing facility, practices or conditions at a fresh-cut processing establishment can also lead to contamination of fresh-cut produce (Gaul et al., 2013).

The process of fresh-cut leafy-greens consists of a series of steps including harvesting, cold storage, trimming, shredding, washing/rinsing, dewatering, packaging, cold storage, and distribution (Buchholz et al., 2012a). The washing is done to remove dirt, foreign materials, tissue fluids from cut surfaces, and microorganisms. Because fresh-cut produce does not undergo intensive inactivation treatments during processing, washing is the only processing step that reduces the microbial load on leafy-greens (Van Haute et al., 2013).

The current common packinghouse practices (water washing and liquid sanitization treatments using chlorine) are not effective to ensure the safety of the produce when initial contamination loads are high (3-log CFU/g could be shed in 1-log CFU/g) or when a substantial amount of pathogenic bacteria gets into the processed produce by cross-contamination (Puerta-Gomez et al., 2013). Washing with water or chlorine only reduce at most 2-log of the surface microbial population and the organic load concentration in the water can reduce even more the efficacy of the sanitizers (Luo et al., 2018). Consequently, producers of leafy greens should control their washing water sanitation procedures by monitoring microbial counts in bagged products, water turbidity, water temperature, and Oxidation Reduction Potential (ORP) (Puerta-Gomez et al., 2013). Additionally, the use of high chlorine concentrations in fresh-cut produce

may cause the generation of chlorine gas in the processing facilities and may lead to the production of excessive amounts of harmful disinfection by-products (DBPs) in the water (Van Haute et al., 2013).

Cross-contamination is a major issue in fresh produce safety during processing (Buchholz et al., 2012a) with post-harvest wash (using chlorine) being considered the high-risk cross-contamination point. Alternative decontamination methods have been suggested such as irradiation, ozone, chlorine dioxide, and Advanced oxidation Process systems (AOP) with few commercial adoptions because of costs and limited applications of these technologies to leafy greens (Murray et al., 2017).

In the meantime, sampling procedures in critical control points and bagged product, including sampling frequency and size (number of bagged products tested) and rapid detection methods must be established for possible pathogen contamination at the most effective cost-benefit interest.

A schematic of a general supply chain for lettuce/leafy greens is depicted in Figure 3.1. The process is complex and involves many parties along the whole operation, which includes harvesting, processing, packing, transportation, distribution, and handling. Lettuce/leafy greens may be harvested mechanically or by hand and are almost always consumed uncooked or raw. Consequently, there are many opportunities for cross-contamination as the produce is handled by a worker or contacts the surface of an equipment.

Pang et al. (2017) developed a QMRA (Quantitative microbial risk assessments) model describing the fresh-cut lettuce production and supply chain from field production -- with both irrigation water and soil as initial contamination sources -- to consumption at home, and

concluded that that retail and home storage temperature were the most important factors affecting the predicted number of illness cases.
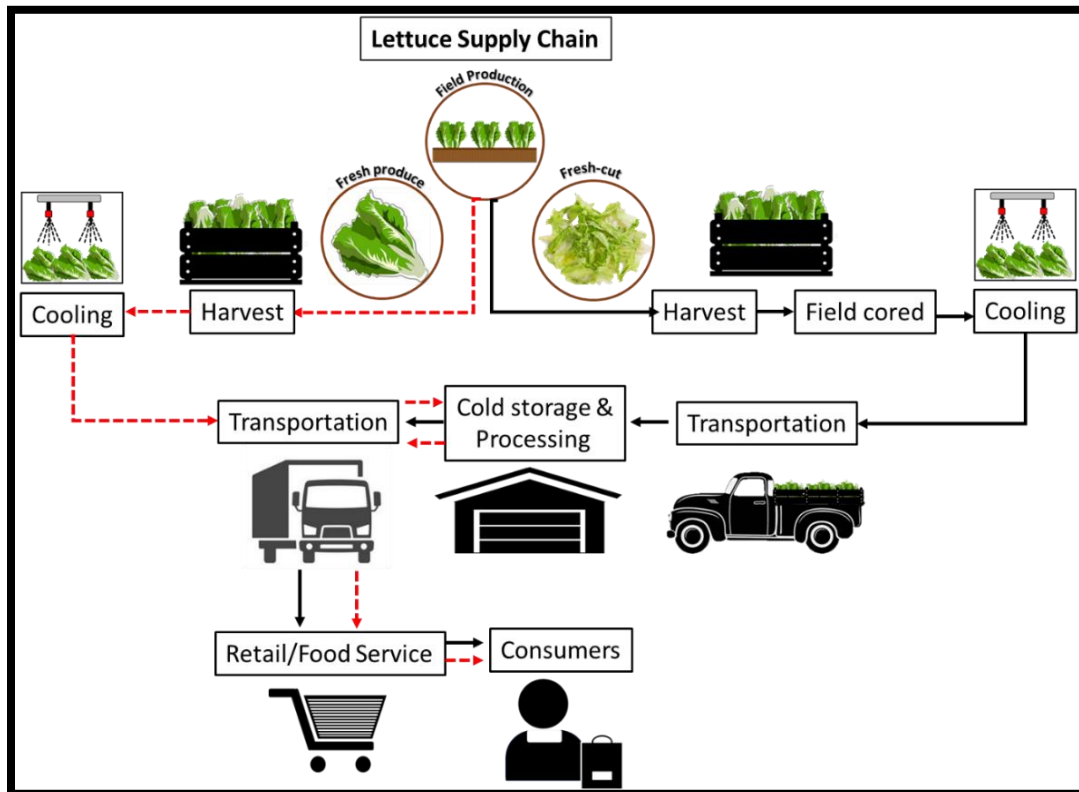
Figure 3.1: Lettuce supply chain

Gehringer et al. (2017) discussed the importance of understanding the individual unit operations in fresh produce processing to design better alternative systems. They mentioned that for development of effective sensors, each step in the food supply chain needs to be identified to find the highest safety risk. Figure 3.2 shows the processing steps and the duration of each step from harvest to consumption in the leafy green processing chain.

To avoid risk of spoilage and therefore contamination during transportation, trucks/trailers must be refrigerated (1 - 3ºC), cleaned often, and the products must be placed within palletized crates and should not directly contact the trailers floor (Sargent et al., 2000).

Once the product is cooled down, it is transported to the processing plant, which can take up to three days (from California to the Midwest).

From harvesting to processing

Harvesting	Chlorination 20-200 ppm	Transport to cooler	Cooling to 2-3°C	Transport to packing
In field 10-60 min	On site < 1-2 min	Roadway 10-60 min	Cooler 0.5-4.0 h	Roadway 1-84 h

From processing to consumer

Cutting, washing, packaging	Transport to retail	Retailer	Consumer
Facility ~ 1 hour	Roadway 1-10 h	5-10 days	5-10 days
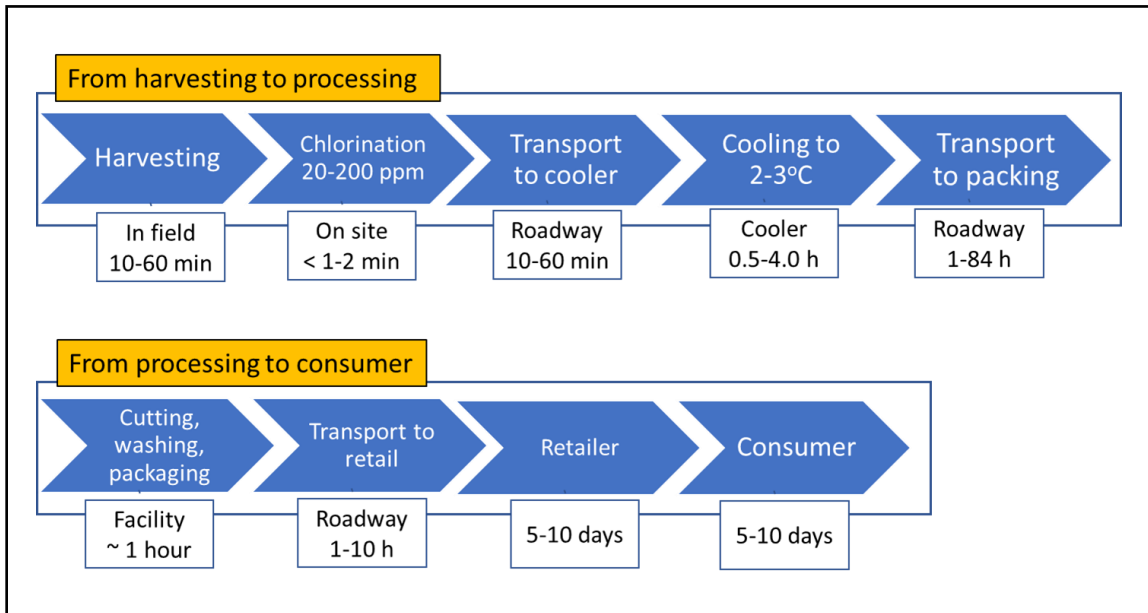
Figure 3.2: Processing steps for fresh green leafy produce and the approximate time elapsed at each unit operation (Gehringer et al., 2017).

Leafy green vegetables should be held at 0-1°C temperature throughout the shelf life. After harvest, these produces need to be rapidly cooled down to remove field heat, to reduce respiration and water loss, and to limit microorganism growth. In the processing plant, the product is cut, washed, centrifuged, and packaged (Artes et al., 2009).

The product storage conditions during distribution must be well maintained and controlled not only by the retailer but also by consumers to extend the shelf life of leafy green vegetables. The time it takes to get from the refrigerated truck into the coolers must be minimized. The refrigeration systems should keep the fresh produce at a temperature of 0-1°C until the product is purchased (Robinson et al., 1975). Temperature fluctuations during storage may accelerate moisture loss, thus reducing the shelf life of fresh produce.

To improve the safety of the lettuce/leafy greens food supply, the FDA (2006) issued guidelines by providing suggested potential actions to reduce, control or eliminate microbial contamination of lettuce/leafy greens in the field to fork distribution supply chain. Most of these guidelines were focused on whole produce, with little information on fresh-cut produce.

Several factors can lead to the contamination of fresh-cut produce.

1. On-farm produce production is susceptible to contamination from multiple sources such as soil, water, biological amendments, and activity of wild animals (Murray et al., 2017). Pathogens can survive for lengthy periods within the environment (Yang et al., 2012).

2. Processing produce into fresh-cut products increases the risk of bacterial growth and contamination by breaking the natural exterior barrier of the produce (FAO, 2008). During chopping or shredding, the release of plant cellular fluids provides a nutritive medium in which pathogens, can survive, grow, and contamination may spread (FAO, 2008).

3. Handling practices that are very common at fresh-cut processing facilities (mixing large batches of fresh-cut produce), can potentially cross-contaminate a larger volume of product (Harris et al., 2003).

4. Post-harvest wash process, which serves to remove not only soils and debris but also field-acquired contamination (Barrera et al., 2012), may contribute to disseminating pathogens if the tanks are not properly sanitized within wash tanks.

5. Fresh-cut produce can potentially be cross-contaminated from equipment surfaces (Buchholz et al., 2012a) or be re-contaminated via air as microorganisms present on the facility (floor, pipes, light, cables, human skin) can be transferred to the air (e.g., spraying during cleaning) thus causing aerosol formation (den Aantrekker et al., 2003).

6. High moisture and nutrient content of fresh-cut produce and temperature abuse during processing, storage, transportation, retail have the potential for pathogens to survive or grow (FAO, 2008).

Puerta-Gomez et al. (2013) showed that cross-contamination is responsible for prevalence of contamination on an entire lot of baby spinach on a daily production. The authors indicated that for low cross-contamination levels (1-log CFU/g), either on the field or after the washing treatments, the percentage of samples over the safety limit for *Salmonella* (1.33 cells) increased by 17% and by 84% when cross contamination was high (3 log CFU/g).

Arienzo et al. (2020) showed a high prevalence of *Salmonella* spp. (67%) on RTE leafy green salads samples. European Regulation (EC) indicates that the absence of *Salmonella* spp. and concentrations of *L. monocytogenes* lower than 100 CFU/g are considered essential criteria to define the safety of RTESs placed on the market during their shelf-life. *E. coli* contamination should be less than 10 CFU/g to be considered acceptable for consumption.

3.2 Modeling cross-contamination in a produce facility

Perez-Rodríguez et al. (2011) were the first to design a probabilistic mathematical model to quantify the number of contaminated of bags of lettuce with Escherichia coli. The model was based on experimental data by Buchholz et al. (2008). Three scenarios were observed separately of the contamination levels of 0.01, 1, and 100 CFU/g. The simulation was based on processing 22 batches of lettuces with 1 batch being randomly selected to be contaminated. The number of contamination of bags was then calculated at the end of the processing line. A probabilistic approach was used to describe the transfer of E. coli from surface to surface (cross-contamination) and removal of the microorganism during the washing process. The free chlorine (FC) depletion during flume tank due to organic materials was not considered in their model.

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The washing phase has been postulated to be a major source of bacterial cross-contamination due to the decrease in the efficacy of chlorine-based sanitizer as the organic load in the wash water increases (Chen and Hung, 2017).

Free chlorine (FC) is the free available chlorine in the wash water that is free to disinfect pathogens. FC is erroneously referred to all forms of chlorine present in water, such as OCl^-(aq), HOCl (aq), and Cl_2 (g). However, it is the aqueous forms of hypochlorous acid (HOCl) and hypochlorite ion (OCl) that are usually measured in free chlorine amounts. This amount is different from total chlorine added to the wash water, as some portion of that chlorine might have reacted with the chemical oxygen demand (COD). Having large concentration of organic load in the wash water will reduce FC levels and promote the survival of pathogens by shielding them (Luo et al., 2018).

Leafy greens entering the washing tank introduces a significant amount of organic material, increasing the chemical oxygen demand (COD) in the water. The COD increased linearly with the amount of lettuce entering the tank (Luo et al., 2012). Maintaining a high enough chlorine concentration is crucial to ensure that no bacteria survives in the wash water solution. Having a free chlorine concentration less than 10 ppm allows survival of bacteria (Luo et al., 2018). On the other hand, high concentrations of chorine can affect the quality of leafy greens such as appearance, texture, flavor, nutritional value, and safety (Francis et al., 2012). Additionally, using too much chlorine to sanitize produce propagates the production of chlorinated organic compounds such as chlorinated trihalomethanes and chloramines1, and diminish the quality of the produce (Francis et al., 2012).

Shredding/cutting produce leads to leakage of nutrients via the exposed surface, thus making the produce more susceptible to microbial attack (Qadri et al., 2015). It is therefore

critical to have quick and accurate techniques to monitor and control chlorine levels in water to ensure that proper sanitation is accomplished while simultaneously preserving the quality of the produce.

Munther et al. (2015) built a mathematical model that described the dynamics of water chemistry and pathogen cross-contamination during the wash procedure based on the experimental data of Luo et al. (2012). The model considered time as an independent variable while COD and FC increased and decreased, respectively, with time. The model can accurately predict free chlorine levels in a pilot plant scale washing process and considers chlorine dosages and the natural time decay of chlorine.

The model was built fundamentally on two key equations:

(1) The rate at which organic exudates are deposited into the wash water

$$\frac{dO}{dt} = k_o \qquad \text{[3.1]}$$

(2) The rate of depletion of free chlorine, which depends on the rate of organic load depositing, chlorine dosages, and the natural time decay of chlorine

$$\frac{dC}{dt} = -\lambda_c C - \beta_c CO + D \qquad \text{[3.2]}$$

where O (mg/L) is the COD in the wash water, k_o is a constant with units (mg/(L min), C is the concentration of FC available in the wash water (mg/L), λ_c is the natural decay rate of chlorine (1/min), β_c is the rate at which organic materials react with free chlorine, and D the chlorine dosage to account for the addition of FC to the process water, and was described as:

$$D = \sum_{k=1}^{N} r_k X \qquad \text{[3.3]}$$

where *X* is the indicator function, taking the value 1 on time interval $[k_t, k_t + t_o]$ for some

small time increment t_o and value zero elsewhere, *N* is the number of doses added, and $r_k > 0$

reflects the rate increase of FC from each dose.

Buchholz et al. (2012 a,b; 2014) built on research by performing a series of experiments

designed to quantify cross-contamination in fresh-cut lettuce for the first time. Figure 3.3 shows

a schematic of the small-scale commercial leafy-green processing line capable of processing

approximately 3,500 kg/h of fresh-cut lettuce with the different unit operations: step conveyer,

flume tank, shaker table, and dewatering centrifuge. Based on their studies, a series of three

experimental results, described below, were used in this study to validate the agent-based model

described in this work.
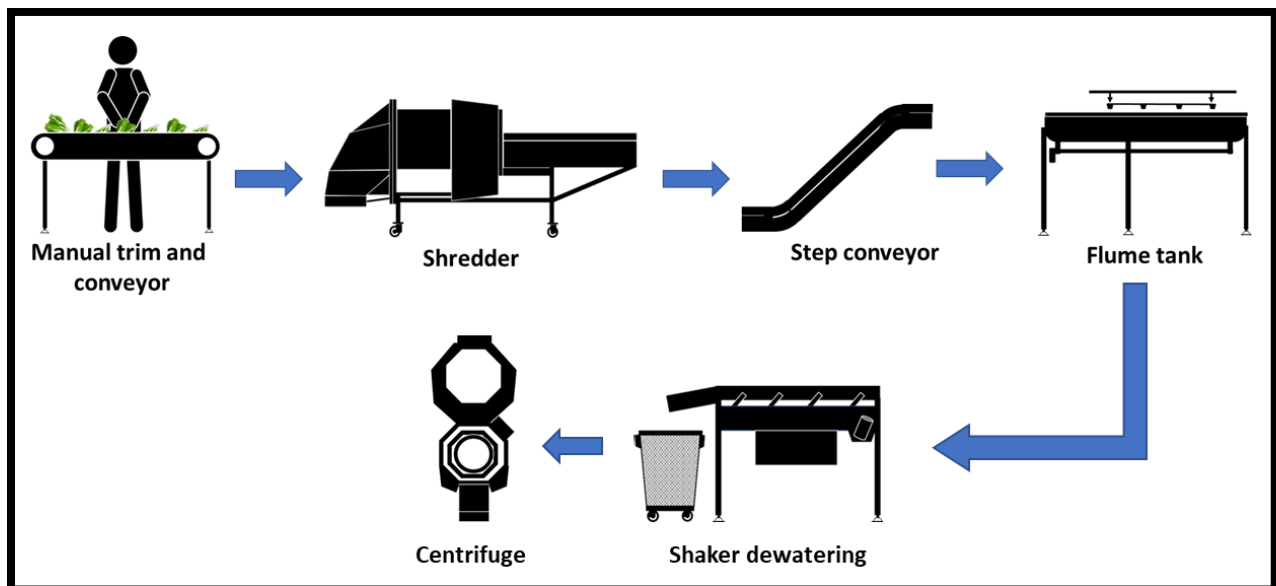
Figure 3.3: Typical equipment pieces used during leafy green postharvest processing including a shredder, conveyor belt, flume tank, shaker table, and dewatering centrifugal dryer.

Experiment # 1: In their study, the transfer of E. coli O157:H7 from leafy greens to equipment

surfaces by processing 22.7 kg of baby spinach, iceberg and Romaine lettuce was analyzed

(Buchholz, et al. 2012a). The results (Figure 3.4) showed that 86.6/83.1 and 48.5% of the

original E. coli O157:H7 inoculum was shed, respectively, from iceberg lettuce/shredded, Romaine lettuce into the 890 liters of processing water used for fluming. Approximately 90% of the E. coli O157:H7 inoculum was shed in the sanitizer-free water. After processing, E. coli O157:H7 populations were highest on the conveyor and shredder followed by the centrifugal dryer, flume tank, and shaker table, with 29% of the remaining product inoculum lost during centrifugal drying.
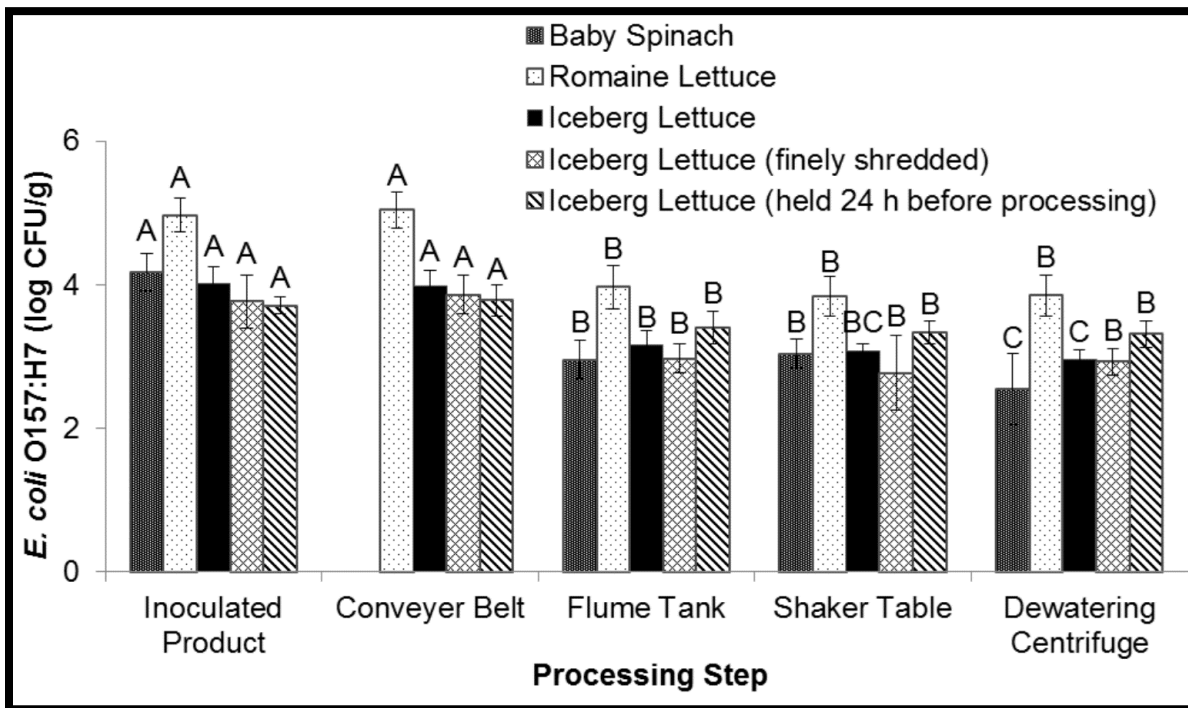
Figure 3.4: *E. coli* O157:H7 populations (mean ± SD) a on the product during processing of leafy greens inoculated at ~4 log CFU/g (n = 3) (Buchholz et al., 2012a).

Experiment # 2: The transfer of *E. coli* O157:H7 from equipment surfaces to iceberg and Romaine lettuce during process was investigated. A total of 22 kg of contaminated lettuce was processed, but this time there was an equal amount of uninoculated lettuce that was processed first to "prime the processing line" followed by 90.8 kg of lettuce. The amount of *E. coli*

O157:H7 on lettuce (log CFU/g) and the cumulative mass (kg) of lettuce were continuously

measured during processing. Experiment #2 measures the impact of cross-contamination on the

output, which correlates to how many bags are contaminated at the end of the process (Buchholz

et al., 2012b). The results are shown in Figure 3.5.
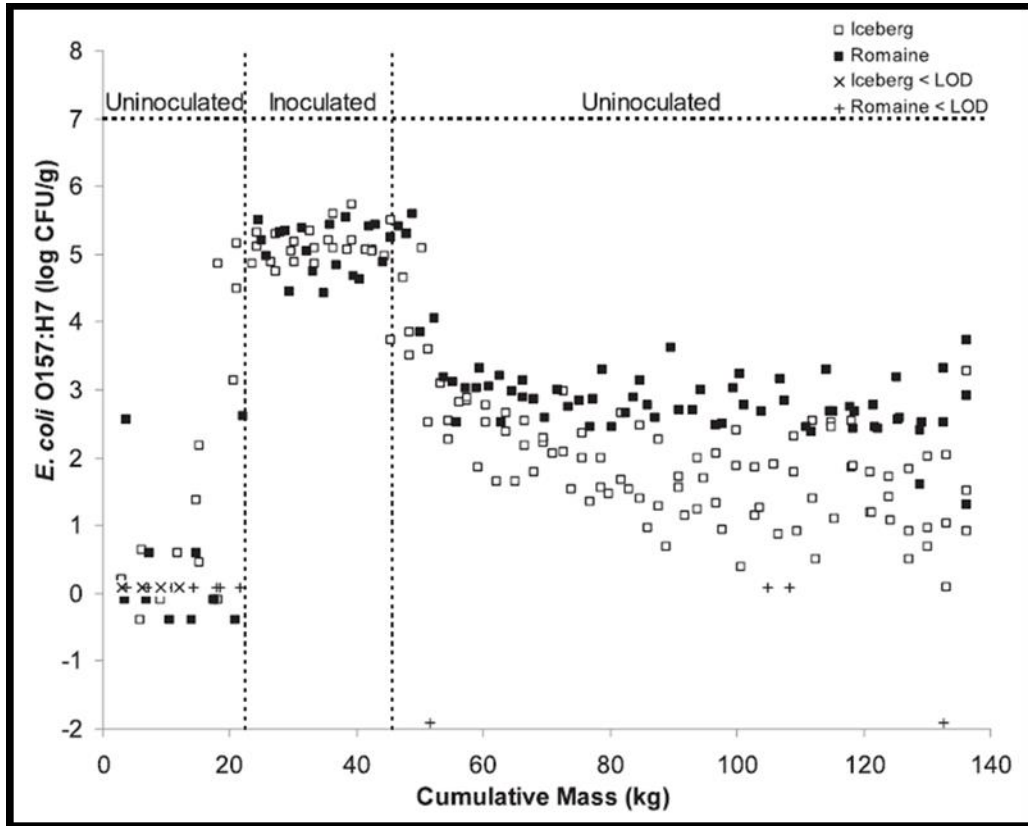
Figure 3.5: *E. coli* O157:H7 populations (mean ± SD) on the product during processing of iceberg
and Romaine lettuce inoculated at ~6 log CFU/g (n = 3) (Buchholz et al., 2012b).

Experiment # 3: This study was designed to track an *E. coli* O157:H7 contaminated batch of

leafy greens through a commercial processing line. A total of 9.1 kg of radicchio was inoculated,

processed first and immediately after, 45.4 kg of Iceberg lettuce was processed. The amount of

radicchio was measured in collected bags. The first bags of produce relative to cumulative mass

had 100% radicchio, which was expected since radicchio was processed first. As throughput

increased the percent radicchio decreased. Smaller pieces of radicchio were present in every

iceberg "bag", and only the last bag had no radicchio at all. This experiment shows the mixing

effect of lettuce pieces through the processing line (Buchholz et al., 2014) The results are shown

in Figure 3.6 using the Weibull model.
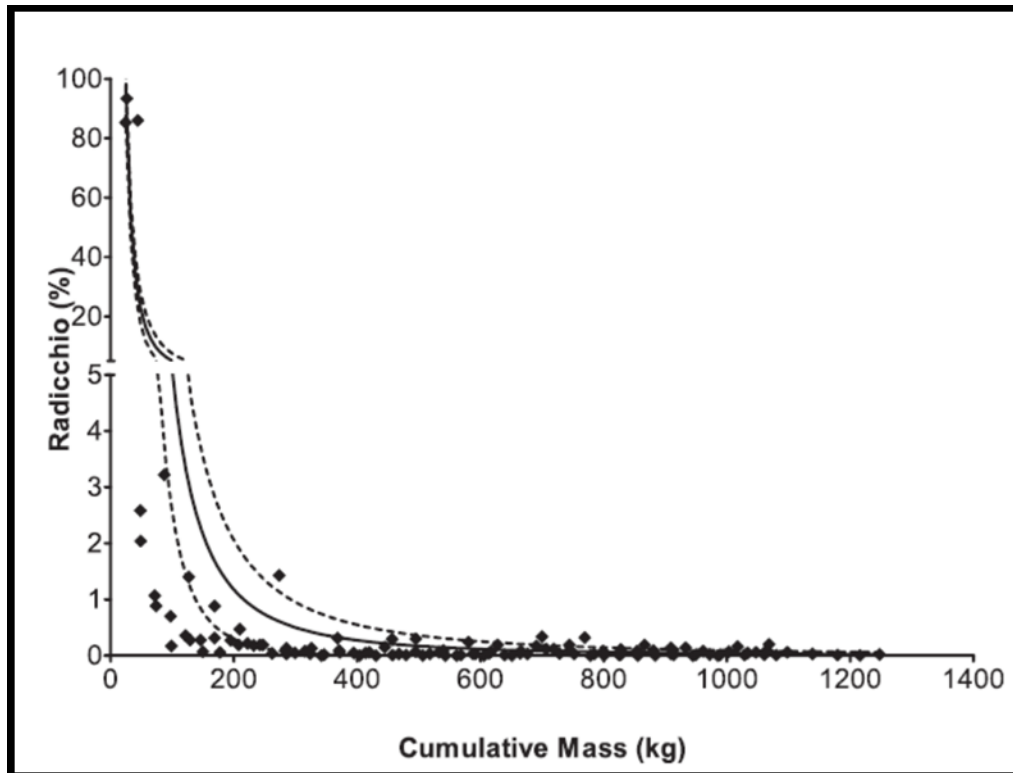
Figure 3.6: Weibull model fitted to percentage (± SD) a of Radicchio recovered from iceberg lettuce after leafy green processing (Buchholz et al., 2014).

Mokhtari et al. (2018) developed an agent-based model (ABM) simulation based on the

experimental results of Buchholz et al. (2012a). Their model was based on the initial

contamination of lettuces or contact surface. The authors assumed that inoculations happened at

the beginning of the process. The model processes batches separately, which is not what happens

in practice, because lettuce processing is a continuous process and there is no separation between

batches. Based on such model, it is not possible to determine how many lettuce pieces of batch #

1 are in batch # 2, similar to Experiment # 3 described above where there is a mixing effect

(Buchholz et al., 2014). Furthermore, this model is a black box which does not allow for

visualization of the entire process where different lettuce pieces are contained at the same time in

different locations of the same equipment.

Mokhtari et al. (2018) model did not consider the log reduction of pathogens in the wash

tank when FC was added to the tank. Additionally, the authors used the model developed by

Munther et al. (2015) to describe the FC concentration in the wash tank, which was designed for

a specific ratio of 1:1000 contaminated vs uncontaminated product in a different water system.

Another problem with this model is that the residence time in the wash tank was supposed to be

26 seconds according to Luo et al. (2018), and values between 0.5 to 1.5 min. were used instead.

Unlike Mukthari et al. (2018), whose developed ABM was based on mathematics and is a

rigid model, in our present study we used the sequences of events approach (discrete time event

simulation) that describes the entire lettuce process using global variables, counters, and statistic

tallies that trigger/execute events and measure a statistical property at a group/collective level.

Every function in the model is an action, an event that happens at a scheduled time. Bacteria is

introduced into the system as an occurrence (event) rather than an initial condition. The

parameters used by Luo et al. (2018) can be verified in real-time using plots that illustrate the

results during the simulation process.

Zoellner et al. (2019) developed a probabilistic ABM simulation that had two

agent types, facilities, and employees, with *Listeria* contamination being tracked and observed in

four different zones of a cold-smoked salmon facility. The zones were classified as Zone 1

surfaces which were in direct contact with the food; Zone 2 surfaces which were close to the

food but not in direct contact; Zone 3 surfaces which were farther from the food but inside the facility; and Zone 4 surfaces which were outside the facility. All zones affected the amount of *Listeria* on food product differently. The amount of *Listeria* was tracked among agents, but there was no cross-contamination transfer between the agents themselves. This approach was similar to Mokhtari and Van Doren, (2019), who used the same zoning approach, and added employee compliance.

3.3 Summary

From Mokhtari et al. (2018) we have learned the fundamentals of constructing an agent-based model, especially cross-contamination between two agents, which we used in designing our model, because their model fits what our perspective of what lettuce processing should look like. We have identified the key parameters we need to focus on such as contamination on surfaces (manual trim, shredder, wash tank, shaker dewatering, and centrifugation), and in addition COD and FC levels (Munther et al., 2015) as predictors. Our response variable is the concentration of *E. coli* after packaging.

3.4 Agent-based Modeling

Agent-based modeling (ABM) is a type of computer simulation composed of agents that can interact with each other and with an environment. Agents are autonomous entities/objects with behavior and properties. The behavior determines an agent's role in the environment and its interactions with other agents. An agent's behavior may change as the simulation progress through simulated time. The agent properties also change during the course of the simulation, usually as a result of certain trigger points, for example, changes in the simulation time (in ABM the simulation time increments in discrete time steps), changes triggered by an agent's internal state change, changes brought about as a result of messages being received from other agents,

etc. Since there are usually several self-governing agents in an environment, each with its characteristics, the overall system state is determined by the agents' dynamic interactions through time (Wilensky and Rand, 2015).

ABM is becoming popular in infectious disease epidemiology as the models can capture the dynamics of disease spread combined with the heterogeneous mixing and social networks of agents (Bobashev et al., 2007). Other applications include food supply chain simulation (Fikar, 2018), and many other fields including social science, economics, business, technology, network theory, and biology (Wilensky and Rand, 2015).

Traditional methods, like analytic models, classical operational research methods, continuous time differential equation models, and discrete time difference equation models, are not able to cope with the inherent complexity of food system operations, such as the high number interactions that take place between different unit operations, or the stochasticity and uncertainty present in most of food processes. For example, classical operational research methods are not always able to handle the inherent dynamic characteristics of food supply chain (Dominguez and Canella, 2020). Continuous time and discrete time difference equation models are not always suitable for analyzing complex food system structures, given the high order of differential equations which makes analytical analysis difficult (Dominguez and Canella, 2020). Thus, different modeling techniques are required.

Simulation modeling and visualization are useful tools in the field of food processing. It consists of developing a model that is the 'virtual representation of the real-world process over time' (Banks et al., 2010). Simulation modeling takes data from the simulator, as input, based on experimental data and assumptions. The output is then generated based on the interaction between the input data and the model. Visuals can be used to provide animation of the process

being simulated, as a validation tool (Sargent, 2011), as an aid for those who are unfamiliar with simulation to understand the modeled process.

A brief discretion of the main simulation approaches including system dynamics (SD), discrete-time event simulation (DES), and agent-based modeling (ABM) are presented to illustrate the context for the research study.

System dynamics (SD) is a continuous modelling technique (previously known as industrial dynamics) develop in 1958 (Forrester, 1961). In SD, variables are connected via flows. It has been used extensively in a wide range of application areas, for example economics, supply chain, ecology, and population dynamics. SD has a limitation in relation to spatial simulation, since the movement of individual entities cannot be illustrated (Greasley and Owen, 2015).

Discrete-time event simulation (DES) originated in the 1950s with the development of early computers (Tocher, 1963). DES represents individual entities as they move between different equipment and are processed or wait in queues. The main areas of application are manufacturing, supply chain and logistics, military, emergency logistics, and more recently, healthcare.

Agent-based modeling (ABM) has its origins in game theory (Axelrod, 1997). ABM differs from both SD and DES in the philosophy of application (Greasley and Owen, 2015). With ABM, the researcher is interested in studying the behavior of agents bottom up, i.e., agent behaviors are defined, agents are released into the environment of study, agents interact with the environment. The system behavior is an emergent property of the agent interactions. ABM has been applied in such fields as economics, human behavior, supply chain, emergency evacuation, transport, and healthcare (Axelrod, 1997).

There are differing views on whether an agent-based simulation offers capabilities that discrete-event cannot provide or whether all agent-based applications can, at least in theory, be undertaken using a discrete-event approach. Greasley and Owen (2015) compared a simple ABM using NetLogo with the corresponding DES versions implemented in ARENA software. The two versions of the discrete-time event model presented used a traditional process flow approach normally adopted in discrete-event simulation software and an agent-based approach to the model build. A real-time spatial visual display facility had to be developed to be embedded within the ARENA model. They found that DES can indeed be used to implement agent-based models but requires integration elements to provide the spatial displays associated with agent-based software.

Gonzales (2009) combined DES environment with an ABM to simulate a crisis response. The environment was modeled as a DES, and the crisis response agents were modeled as an ABM. It provided a high-level architecture suggesting the way in which DES and ABM could be combined into a single simulation in a simple way.

Ding et al. (2018) concluded that SD is a top–down modeling method that describes systems from a macro perspective, requiring knowledge of the system relations and causalities. ABM, on the other hand, is a bottom–up approach that models single acting entities of the system and the agents' interactions during simulation to determine the macro behavior of a system. They commented that a combination of both approaches might provide a powerful technique in the complexity simulation of construction waste management.

Zankoul et al. (2015) evaluated DES with ABM approaches to simulate construction earthmoving operations and concluded that a combination of both methods would provide a

better simulation of the system, using ABM to model road segments and trucks as agents, while modeling loaders, excavators, and their activities as regular DES processes.

A multi-paradigm simulation method was developed by Djanatliev and German (2013) to simulate healthcare decision-making by combining SD, DES, and ABM.

CHAPTER IV

MATERIALS AND METHODS

This work represents the development of a food-safety agent-based simulator (FS-ABS) to investigate cross-contamination of fresh-cut produce in a processing plant environment. The FS-ABS was developed using the simulation software NetLogo - 6.1.1, (Wilensky, 1999). The focus is set on green leafy processing plants and on the impact of cross-contamination, free-chlorine concentration on wash-water, and cold room temperature fluctuation strategies on pathogen growth. Microorganisms decay, growth, and transfer models in the wash water, equipment, and lettuces are embedded in a discrete event simulation model to deal with the growing complexity and uncertainties occurring in processing plants. Therefore, the FS-ABS of a processing facility for romaine lettuce based on cross-contamination, growth, and decontamination data for *E. coli* O157:H7 in lettuces was developed and evaluated using experimental data obtained from the literature (Buchholz et al., 2012a, b, 2014; Luo et al., 2011, 2012; Ding et al., 2009; Zeng et al., 2014).

4.1 Food Safety Agent-Based Simulation

Figure 4.1 shows the developed FS-ABS to investigate leafy green processing cross-contamination throughout daily operations. The simulator provides food safety insight to farms and processors because by integrating E. coli O157:H7 cross-contamination and growth data, it determines how many bags are contaminated, and how to reduce the number of contamination cases in the facility. The core of the system is a discrete event simulation, which models the processing facility with uncertainty present in both input (fresh harvested lettuce microorganism load) and in-the-facility cross-contamination including people, equipment, flume tank, and packaging area
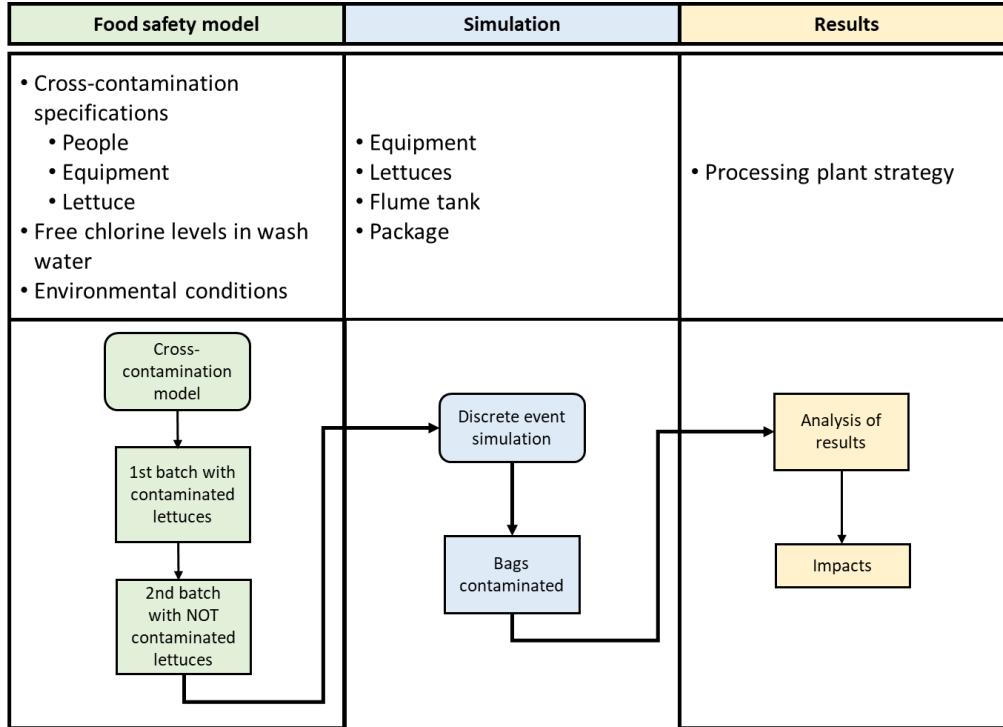
Figure 4.1: A schematic of the cross-contamination simulation of leafy greens processing facilities.

Data by Luo et al. (2011, 2012) were used to model the pathogen cross-contamination and inactivation by free chlorine in the wash water. Data from Buchholz (2012a,b, 2014) were used to develop statistical distribution models to describe cross-contamination at different pieces of equipment. The generic model presented by Baranyi (Baranyi and Roberts,1994) was used to predict microorganisms growth in lettuce based on the data by Ding et al. (2009). Those models are integrated to model cross-contamination and microorganism inactivation changes as well as microorganism's growth due to temperature abuse in the facility based on the different inputs. Figure 4.2 shows a schematic of lettuces arriving at the processing facility that was evaluated in this study.

Assumptions. The FS-ABS is initialized with a batch of lettuce heads arriving at the processing facility from the field. It is assumed that the lettuce heads are stored at 4oC before processing. The lettuce heads are then transported to the processing line, which consists of manual trimming by five workers, a shredder, a conveyor, a flume tank, a dewatering table, a centrifuge, and finally a packaging area where the shredded lettuce is randomly selected and packed in 500 g bags. It is also assumed that the lettuces and the equipment are independently transported and operated, respectively, during the process. Each piece of equipment as well as lettuces and workers have specified microorganism loads. Additionally, facility temperature and wash water free chlorine need to be defined to initialize the system.
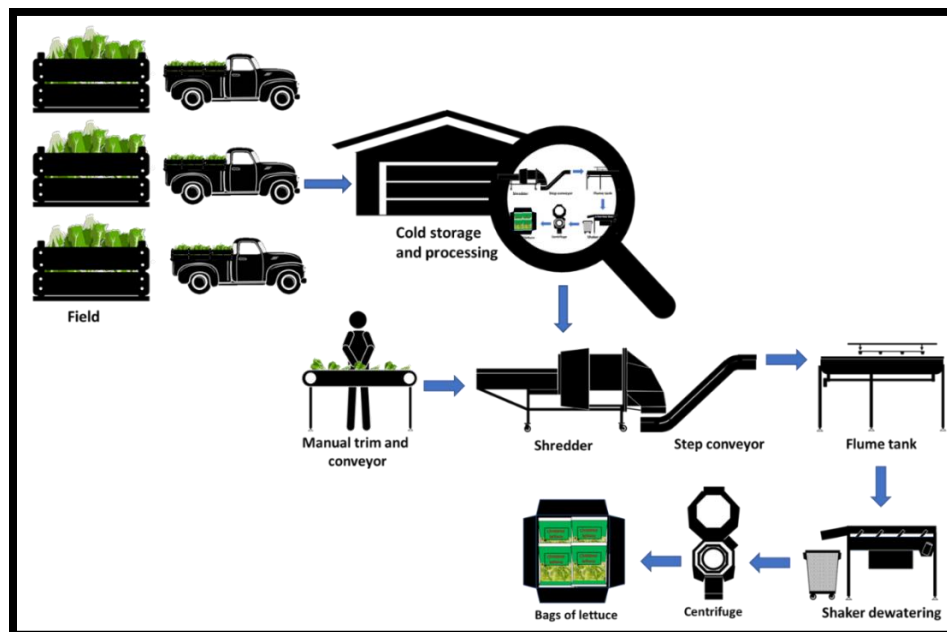
Figure 4.2: Modeled romaine lettuce processing facility structure.

An overview of the whole system in presented in Figure 4.3. The system models the flow of a product from trimming to packaging, i.e., the product is either cross-contaminated, non-contaminated, or cleaned completely in the flume tank.

Cold storage	Processing – Step 1	Processing – Step 2	Processing – Step 3	Processing – Step 4
Batches of whole lettuce heads are stored at 4°C	Lettuce are cut in half by 5 workers who can be contaminated or not with E.coli from contaminated lettuce or **table surface**	Half lettuce are cut into small pieces in the **shredder**, which can be contaminated or not with E. coli from lettuces	Lettuce pieces are washed in the **flume tank**, which can become contaminated or not with E. coli from lettuces	Lettuce pieces dewatered and centrifugated. These equipment can be contaminated or not with E. coli.
	Lettuce are inoculated with 0-10⁶ CFU/g of E. coli	Pieces are transported to the **step conveyor**, which can be contaminated or not with E. coli from lettuces	Water may contain or not chlorine, which is used to inactivate the pathogens	Lettuce pieces are randomly selected and packed in 500 g bags
				Count the number of bags contaminated

Figure 4.3: Process flow chart integrated within the FS-ABS.

Next is a description of the cross-contamination process modeling inputs and other parameters.

4.2. The Processing Line and Experimental Setup

The leafy greens process evaluated in this study consists of a small-scale commercial leafy green processing line capable of processing approximately 3,500 kg of shredded lettuce per hour as described in Buchholz et al. (2012a). The processing line is shown in Figure 4.2.

Non-inoculated romaine lettuce heads (22.7 kg) are fed into the trimming table for continuous processing followed by 22.7 kg of inoculated lettuce heads. The lettuces are then processed by shredding, conveying, fluming, shaker table dewatering, and centrifugal drying

before packaging in 500 g bags. Right after that, 90.8 kg of non-inoculated lettuce heads are similarly processed.

Whole heads of romaine lettuce (0.5 kg each) are processed at a rate of about 0.75 kg/s (45 kg per min, 0.14 m/s), with the entire 22.7 kg of product ready for centrifugal drying (60 s) after 126 sec (2.1 min) (Table 4.1). Figure 4.4 presents the number of bags (material balance) produced in the facility, which amounts to 90 bags of 500 g shredded lettuce per minute.

Table 4.1: Size (length) of each equipment piece and the time spent by the lettuce on each piece.

Equipment	Length [m]	Residence Time[s]
Trimming table	5.8	41
Shredder	3.8	27
Conveyor	2.9	21
Washing	3.6	26
Shaker	1.5	11
Centrifuge	--	60
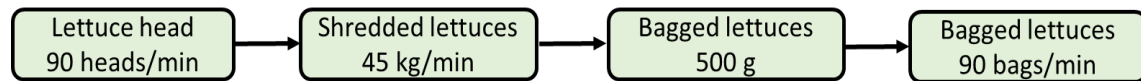
Figure 4.4: Material balance of romaine lettuce processed in the facility simulated in this study.

4.2.1 Trimming table, shredder, conveyor, shaker, and centrifuge

The processing of lettuce starts at the trimming table with five workers. The trimming process is limited to a user-defined time per batch and based on a specified arrival rate. The lettuce heads are cut in half and defined by an initial *Escherichia coli* O157: H7 load in Colony Forming Units per gram (CFU/g). According to a binomial distribution, this initial load varies between a predefined range to model biological variance present in the different processing line operations. Each worker is supposed to trim in average 1.5 head of lettuce per second. Thus, five workers can produce 7.5 heads of lettuce per second.

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In each step, the time the lettuce item goes through this process flow is measured. After the first batch with contaminated lettuce heads, cross-contamination transfer from inoculated lettuce to equipment (trimming table, shredder, conveyor) is updated following a triangular distribution as described in Mokhtari et al. (2018). For the second and third batches, cross-contamination transfer from equipment to lettuce is updated following a triangular distribution. After a change in the facility's ambien temperature, microbial growth is updated according to Baranyi's model (Baranyi and Roberts,1994).

4.2.2 Flume tank

At the flume tank, the level of cross-contamination from inoculated lettuce to the wash water and from there to the lettuce is updated based on free chlorine (FC) and chemical oxygen demand (COD) available as described by Luo et al. (2015). Depending on the level of contamination in the lettuce and water FC content, bacterial growth could be inactivated during this process.

4.2.3 Packaging

After the shredded lettuce is centrifugally dried, the produce is randomly selected and bagged into 500 g size bags. After a change in the facility's ambient temperature, microbial growth is updated according to Baranyi's model (Baranyi and Roberts, 1994).

4.3 Modeling of cross-contamination, pathogen growth, and free-chlorine in wash water

4.3.1 Cross-contamination transfer process

It is assumed that there is no net loss of microorganisms during cross-contamination. The total bacterial load is conserved during the transfer process between the two contacting surfaces. The population size is a discrete integer value and there are only i positive observations. The binomial probability distribution function is used to approximate the number of microbial loads to be transferred. The transfer coefficient ($0 < Tr < 1$) is the number of microbes that will be

moved from one contacting surface to the other, Surface A and Surface B (Tr_{AB}, Tr_{BA}). All the

parameters in Eqns. (4.1) to (4.4) are described in Table 4.2 (Mokhtari et al., 2018):

$$\chi \sim Binomial \ (N_{oA}, Tr_{AB}) \qquad\qquad [4.1]$$

$$\psi \sim Binomial \ (N_{oB}, Tr_{BA}) \qquad\qquad [4.2]$$

$$N_A = \ N_{oA} - \ \chi + \psi \qquad\qquad [4.3]$$

$$N_B = N_{oB} - \chi + \ \psi \qquad\qquad [4.4]$$

Table 4.2: Parameters for cross-contamination (Eqns. 4.1 to 4.4).

Parameter	Definition	Unit
N_{oA}	Initial *E. coli* population on surface A	CFU
N_{oB}	Initial *E. coli* population on surface B	CFU
N_A	*E. coli* population on surface A after cross-contamination	CFU
N_B	*E. coli* population level on surface B after cross-contamination	CFU
Tr_{AB}	Transfer coefficient from surface A to surface B	---
Tr_{BA}	Transfer coefficient from surface B to surface A	---
χ	*E. coli* population transferred from surface A to surface B	CFU
ψ	*E. coli* population transferred from surface B to surface A	CFU

Binomial distributions are difficult to calculate when the number of observations is large.

In NetLogo, a binomial distribution is generated from a Bernoulli process. The number (*i*) of

random binary values of size 1 and 0 are generated in series based on the probability/transfer

rate. This is an iterative process, a value of 1 or 0 is given *i* number of times in series, and a sum

of all the values size 1 is the new number. There is no easier way to execute this process if we

have 6 log CFU as the computer must process the data 10^6 times.

4.3.1.1 Cross-contamination transfer input probabilities

Table 4.3 shows the data used for the distribution for each equipment/hand/glove

situation (Buchholz et al., 2012a, b; Mokhtari et al., 2018).

Table 4.3: Cross-contamination rates, XC, between processing equipment and the produce (lettuce).

XC [%]	Direction	Location	ID	Distribution [%]
$XC_{H,Lh}$	Contaminated hands/gloves to lettuce head	Worker	L1	$T[3, 10, 30]$
$XC_{Lh,H}$	Contaminated lettuce head to hands/gloves	Worker	L1	$T[0, 1, 3]$
$XC_{K,Lh}$	Contaminated knife to lettuce head	Manual-trim	L2	$T[0, 29.6, 59.2]$
$XC_{Lh,K}$	Contaminated lettuce head to knife	Manual-trim	L2	$T[0, 2.5, 5.0]$
$XC_{S,Lh}$	Contaminated shredder to lettuce head	Shredder	L3	$T[16, 20, 28]$
$XC_{Lh,S}$	Contaminated lettuce head to shredder	Shredder	L3	$T[0, 0.25, 0.53]$
$XC_{Cb,Sl}$	Conveyor belt-shredded lettuce	Conveyor	L4	$T[15, 18, 22]$
$XC_{Sl,Cb}$	Contaminated shredded lettuce to conveyor belt	Conveyor	L4	$T[0, 0.62, 1.39]$
$XC_{St,Sl}$	Contaminated shaker table to shredded lettuce	Shaker	L6	$T[6, 28, 30]$
$XC_{Sl,St}$	Contaminated shredded lettuce to shaker table	Shaker	L6	$T[0, 0.06, 0.38]$
$XC_{C,Sl}$	Contaminated centrifuge to shredded lettuce	Dewatering	L7	$T[23, 27, 31]$
$XC_{Sl,Dc}$	Contaminated shredded lettuce to centrifuge	Centrifuge	L7	$T[0, 0.35, 1.59]$

T: Triangular distribution [min, most likely, max]

4.3.2 Microbial Growth Model

The Baranyi (Baranyi and Roberts, 1994) model (Eqns. 4.5 and 4.6) is a dynamic model

and in its differential form can be applied to estimate bacterial growth changes with temperature

(Velugoti et al., 2011).

4.3.2.1 Primary model

$$y(t) \ = \ y_0 \ + \mu_{max}F(t) - \ \ln\left(1 \ + \ \frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \qquad [4.5]$$

$$F(t) = t + \frac{1}{u_{max}}ln(e^{-u_{max}t} + e^{-h_0} - e^{u_{max}t - h_0}) \qquad [4.6]$$

Empirical data are used to determine the rate of change of these parameters at different

temperatures. Table 4.4 describes each model parameter and its units.

Table 4.4: Primary and secondary model parameters of the Baranyi model (Eqns. 3.5 and 3.6).

Growth Parameters	Name	Unit
$y(t)$	Microbial population	Log CFU/g
y_0	Initial microbial population	Log CFU/g
y_{max}	Maximum microbial population	Log CFU/g
μ_{max}	Maximum growth rate	Log CFU/g/h
λ	Lag time	h
h_0	$\mu_{max} \times \lambda$	Log CFU/g
T	Temperature	°C

4.3.2.2 Secondary model

Ding et al. (2009) data were used to describe the effect of temperature on the growth rate (Eqn. 4.7), lag time (Eqn. 4.8), and final microbial population (Eqn. 4.9) of *E. coli* O157:H7 growth on Ready-to-Eat (RTE) fresh cut lettuce stored at different temperatures (Table 4.5):

$$\sqrt{\mu_{max}} = 0.0169(T + 4.012) \qquad (R^2 = 0.96, RMSE = 0.0374) \qquad [4.7]$$

$$\lambda = 189.285 \times exp(-0.110 \times T) - 3.617 \quad (R^2 = 0.91, RMSE = 12.431) \qquad [4.8]$$

The changes in y_{max} are calculated using the sigmoidal function:

$$y_{max} = \frac{8.676}{1 + exp\left(-\dfrac{T - 3.52}{2.876}\right)} \qquad (R^2 = 0.92, RMSE = 0.537) \qquad [4.9]$$

Figure 4.5 shows that the Baranyi model (Eqns. 4.5 and 4.6) fit the experimental data from Ding et al. (2009) well for all temperatures tested. Plots of the secondary models are illustrated in Figure 4.6 indicating a good agreement between the parameters and the predicted models.
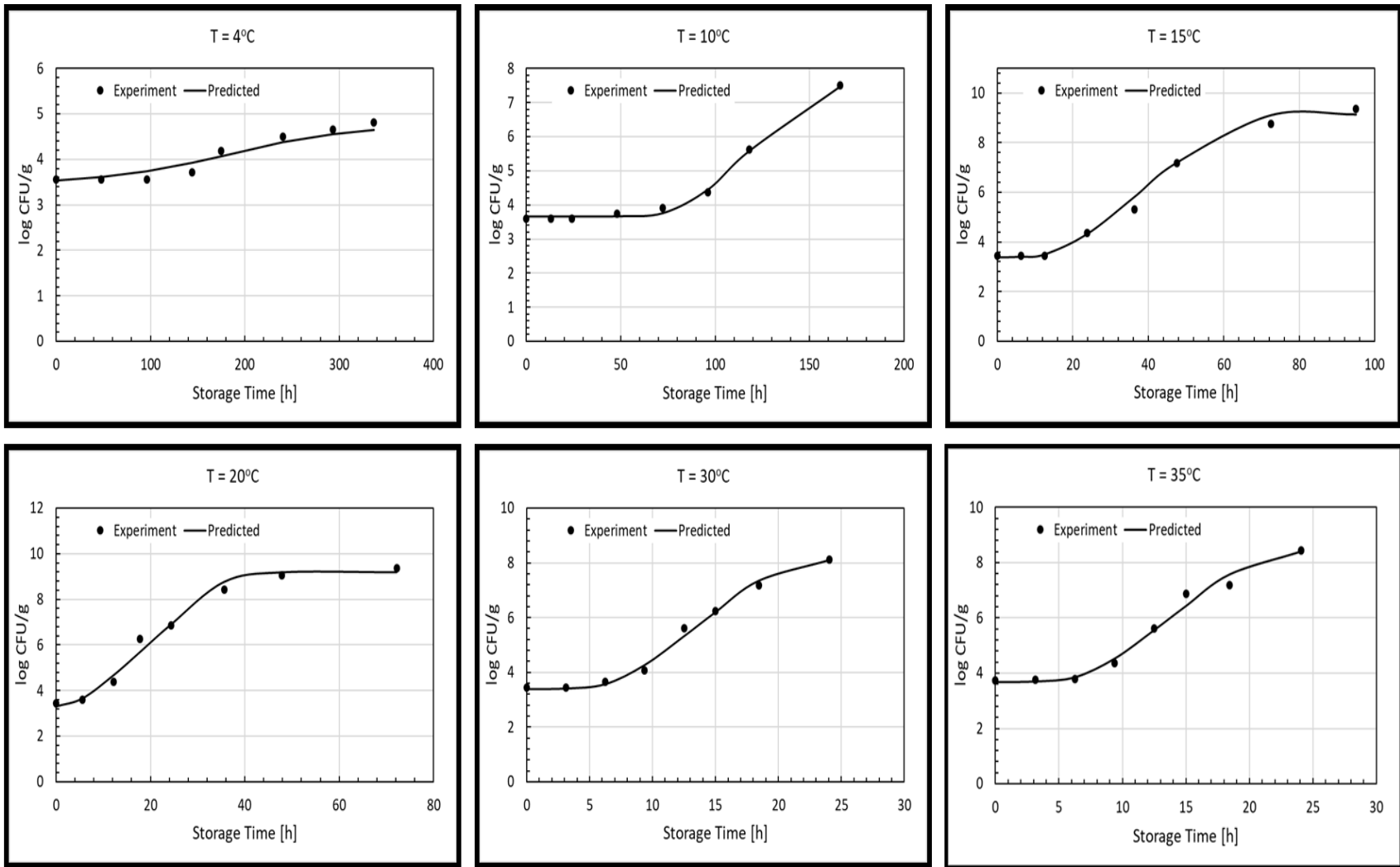
Figure 4.5: Curve fitting of experimental data using Baranyi model – Eqs. (3.5-3.6) for storage temperatures from 4°C to 35°C
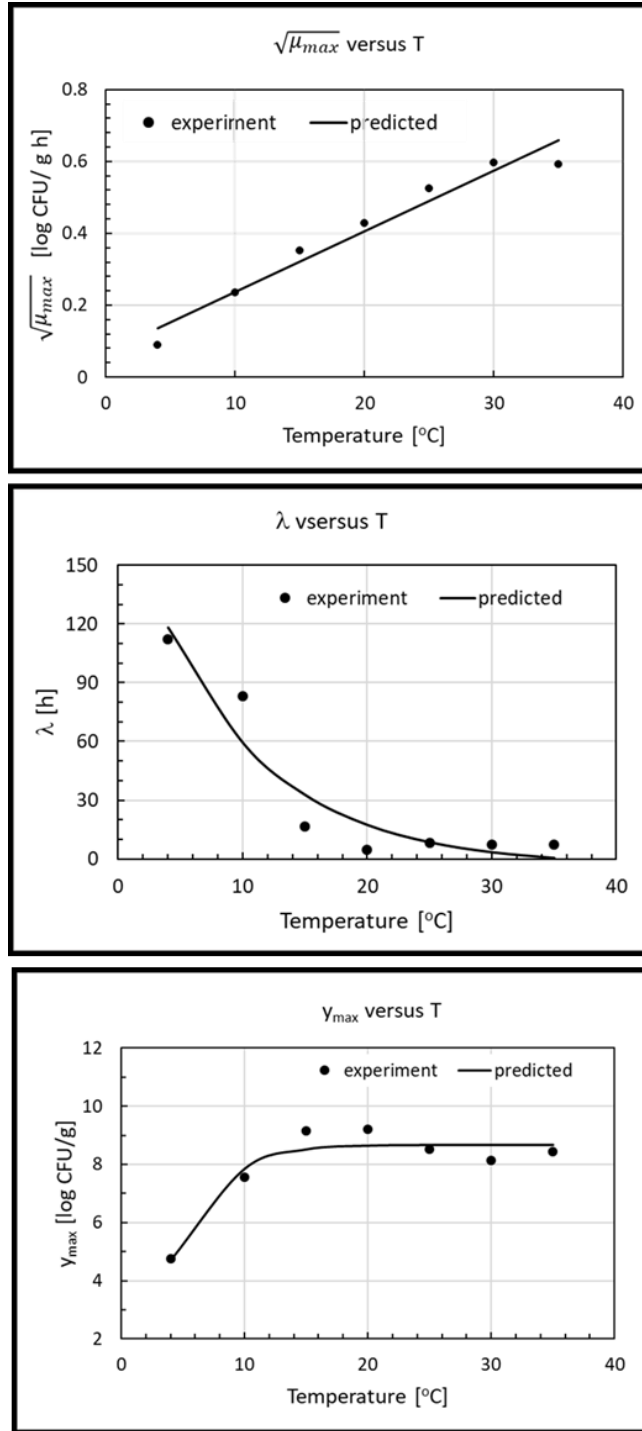
Figure 4.6: Secondary models (Eqns. 4.7 to 4.9) describing the effect of temperature on the growth rate, lag time, and maximum microbial population for E. coli *E. coli* O157: H7 growth on RTE fresh-cut lettuce.

Table 4.5: Growth parameters for *Salmonella* and *E. coli* O157: H7 inoculated in lettuce at different temperatures. Parameters were calculated using Eqns. (4.5) and (4.6).

Temperature [°C]	u_{max} [log CFU/g]	λ [h]	y_{max} [log CFU/g]	R^2	RMSE*
4	0.008±0.002	112.267±21.235	4.745±0.081	0.962	0.104
10	0.055±0.007	83.244±4.083	7.563±0.159	0.993	0.113
15	0.124±0.019	16.688±4.183	9.149±0.225	0.987	0.277
20	0.184±0.028	4.857±3.605	9.194±0.269	0.976	0.372
25	0.276±0.024	8.141±1.153	8.527±0.126	0.994	0.177
30	0.355±0.036	7.099±0.951	8.133±0.23	0.987	0.208
35	0.351±0.054	7.170±1.461	8.433±0.361	0.971	0.315

RMSE*: root means sum of squared error

4.3.2.3 Dynamic model

The differential growth model proposed by Baranyi and Roberts (1994) can be written as a set of two first-order differential equations:

$$\frac{dy}{dt} = \frac{\mu_{\max (T(t)}}{1 + \exp(-Q(t))} (1 - \exp(y(t) - y_{max}) \qquad [4.10]$$

$$\frac{dQ}{dt} = \mu_{max}(T(t)) \qquad [4.11]$$

with the following initial conditions: $y(0) = y_0$ $Q(0) = \ln(q_0)$, respectively. $Q(t)$ is the natural logarithm of $q(t)$, a variable related to the physiological state of the cells (Velugoti et al., 2011). Under isothermal conditions, the explicit solution for the above differential equations was given in Eqns. (4.5) and (4.6). The secondary model (Eqn. (4.7)) was substituted in Eqn. (4.11) assuming that the microorganisms respond is instantaneously to temperature changes if the cells are in exponential phase (Baranyi and Roberts, 1994). The above first order differential equations were then solved numerically using 4th-order RungeeKutta method in Excel (Microsoft 365, Microsoft Incorporation, WA). Figure 4.7 shows the comparison between the exact solution using the explicit and the Runge-Kutta solutions (MSE =0.0012, RMES = 0.036, and the exact and predicted values were not significantly different (p>0.05)).
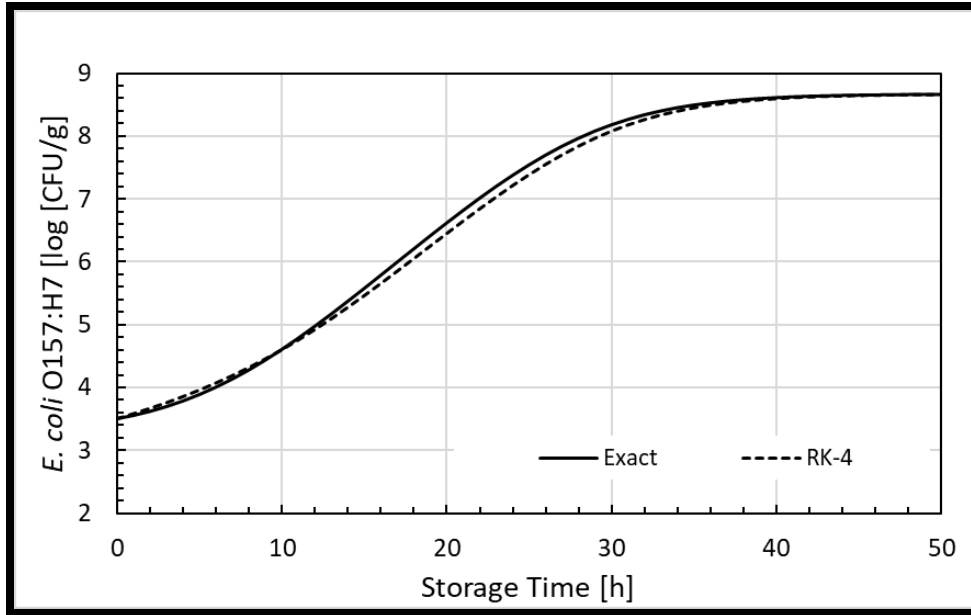
Figure 4.7: Exact solution and predicted solution using 4th-order Runge-Kutta for *E. coli* O157:H7 growth in fresh-cut romaine lettuce at 25°C storage temperature.

Figure 4.8 shows the simulation of *E. coli* O157:H7 growth estimated by the dynamic model for temperatures alternating between 2 and 22°C in a 15 h cycle for 60 h.
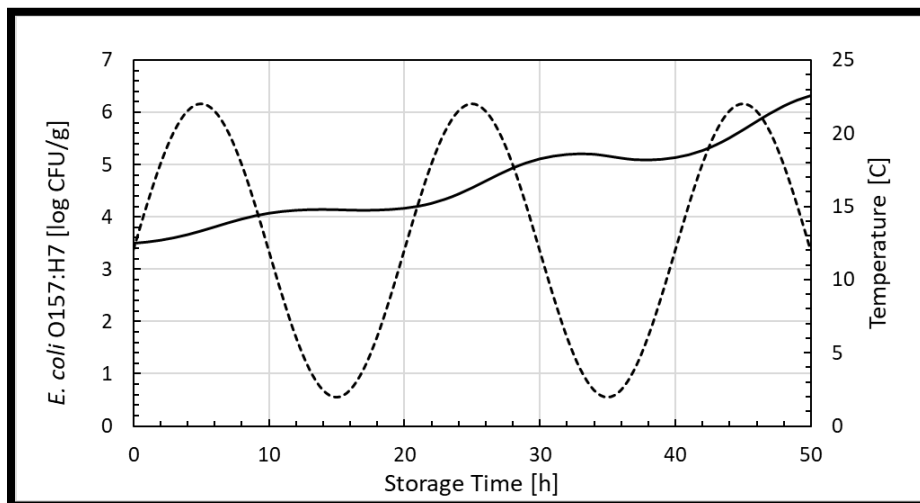
Figure 4.8: Simulation of the dynamic model for *E. coli* O157:H7 in fresh-cut romaine lettuce under short sinusoidal temperature profile (between 2°C and 22°C for 48 h with a period of 20 h/cycle).

4.3.3 Free chlorine (FC) and chemical oxygen demand (COD) in wash water models

The model developed in this study was based upon data from a washing facility that processed shredded lettuce (Luo et al., 2012). The model considers time as an independent variable while COD and FC increased and decreased, respectively, with time. The model can accurately predict free chlorine levels in a pilot plant scale washing process and considers chlorine dosages and the natural time decay of chlorine. The model was built fundamentally on two rate equations (Alradaan, 2018):

(1) The rate that organic exudates are deposited in water, a zero-order reaction:

$$\frac{dC_{O_2}}{dt} = k_o \qquad [4.12]$$

where ko = rate organic exudates are deposited in the water [mg/L-min] and CO2 the COD concentration [mg/L].

Integrating the chlorine decay rate differential Eqn. (4.12) yields:

$$C_{O_2} = k_o t + C_{O_{20}} \qquad [4.13]$$

The values of k_o were obtained by linear regression (R^2 =1.00):

$$C_{O_2} = 31.25t + 312.5 \qquad [4.14]$$

In the wash water, chlorine reacts with organic material to form chlorinated byproducts. Hypochlorous acid (HOCl), the free chlorine in the wash water, reacts with organic exudates to yield byproducts:

$$HOCl + Organic\ exudates \rightarrow byproducts$$
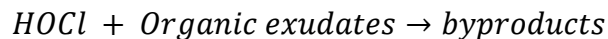
(2) The rate of depletion of free chlorine, which depends on the rate of organic load deposited, chlorine dosage, natural chlorine decay, a second order reaction:

$$\frac{dC_{FC}}{dt} = -k_c C_{FC} - \beta_c C_{FC} C_{O_2} \qquad [4.15]$$

where C_{FC} = FC concentration [mg/L]; k_c = natural decay rate of chlorine [L/min], which has a constant value of 0.0017 L/min (Munther et al., 2015); β_c = rate at which organic materials react with FC [L/min], a function of pH and temperature, though both the temperature and pH were constant during the experiment, so it is assumed that β_c is constant.

As there are different organic matter in the wash system, which react with chlorine such as lettuce extracts, bacteria, soil, etc., it should be considered that the reaction of chlorine with all those elements will have different rate constants; however, it is assumed that the constant β_c is the average of all those constants (Alradaan, 2018). Integrating the chlorine decay rate differential equation (Eqn. 4.15) yields (Alradaan, 2018):

$$C_{FC} = C_{FCo}exp\left[-k_ct - \beta_c\left(\frac{k_o}{2}t + C_{O_{2o}}\right)t\right] \qquad [4.16]$$

where the only variable is time. The value for β_c was obtained from curve-fitting of experimental data to the model Eqn. (4.16):

$$C_{FC} = C_{FCo}\exp\{[-0.0017t - 4.828 \times 10^{-4}(C_{O_2})t]\} \qquad [4.17]$$

The model fitted the experimental data well ($R2 = 0.98$) as shown Figure 4.9. Equation (4.17) works only if the pH, water temperature, and the total dissolved solids (TDS) are kept constant. Experimental data from Luo et al. (2007) show that measured levels of TDS and oBrix for 2, 10, and 18 kg washes remained constant for oBrix and TDS showed some increasing trend. Alradaan (2018) found that turbidity was not a good indicator of either COD or FC for romaine lettuce, and that the amount of organic load released by any produce depends on the produce type. The results showed that the higher the exposed surface area the higher the COD in the wash water, and thus more FC consumption. The βc parameter in Eq. (4.16) is independent of produce type or cut type, but may be dependent on temperature, or COD. This parameter will remain constant as long as pH, temperature, and TDS remain constant in the wash water.
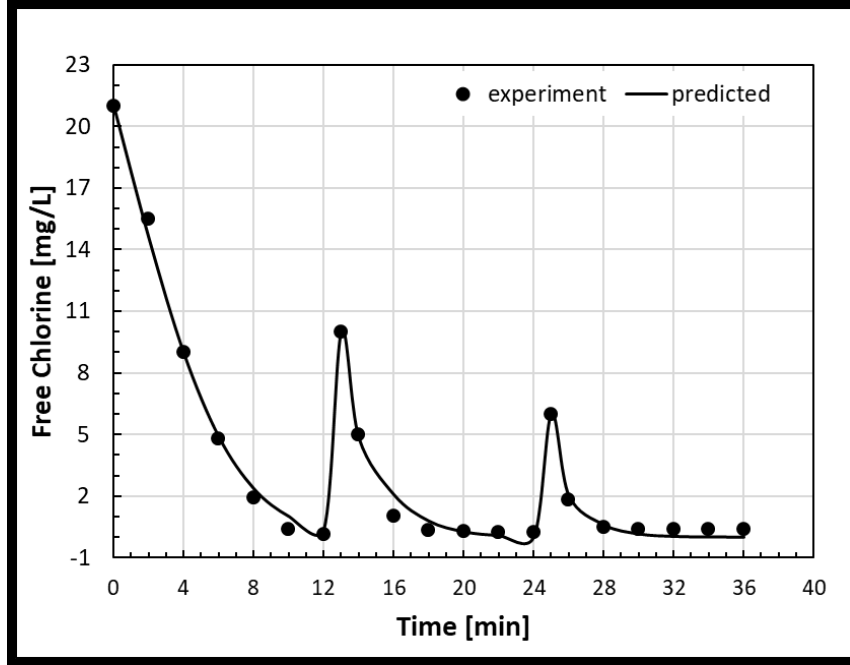
Figure 4.9: Curve fitting of experimental data using Eq. (4.17).

The experimental data indicated that lettuces remain in the flume tank for 26 seconds, and the FC will remain constant for that length of time. The samples were collected at every 2 minutes intervals and the lettuce flow rate was 45 kg/min. FC dose was applied at 12-minute intervals. The relationship between time and lettuce mass is linear with $R^2 = 1.00$:

$$m_p = 45 \times t \qquad [4.18]$$

where m_p is the mass of lettuce [kg] and t is the processing time in [min].

The rate of depletion of free chlorine as described by the differential Eqn. (4.15) was then solved numerically using 4th-order RungeeKutta method in Excel (Microsoft 365, Microsoft Incorporation, WA) with the initial condition $C_{FCo}(0) = 21 \; mg/L$ and C_{O2} as described by Eqn. (14). Figure 4.10 shows the comparison between the exact solution using the explicit and the Runge-Kutta solutions. The MSE = 0.87, RMSE =0.93 and t-test indicated that both values are not significantly different (p>0.05).
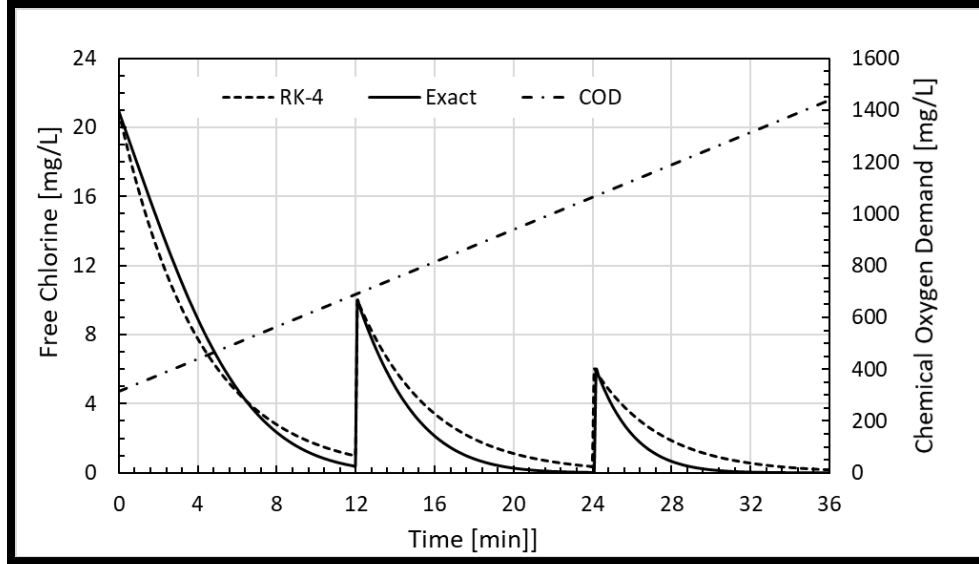
Figure 4.10: Simulation of the dynamic model for free chlorine kinetics in the flume tank under step free chlorine additional doses (21-10-6 mg/L) for 36 min with a period of 12 min/cycle.

4.3.4 Survival of microorganisms in fresh-cut lettuce washed in FC water solution

Data from Luo et al. (2011) were used to model the effect of free chlorine concentration on *E. coli* O157:H7 survival in shredded lettuces. The logarithmic equation was used to model the effect of FC in the wash water on the log reduction of microorganisms inoculated in shredded lettuce. The model fitted the experimental data well ($R^2 = 0.997$) for $0 \leq FC \leq 100$ mg/L:

$$\log R = [0.214 \times \ln(FC) + 0.220] \tag{4.19}$$

It was assumed that water alone would reduce approximately 1 log of the population (Luo et al., 2011), so the total log reduction was calculated as *TlogR = logR + 1*. The number of survival microorganisms was calculated using Eq. (4.20) below:

$$\log[N] = \log[N_o] - logR \tag{4.20}$$

where *N* is the survival population [CFU/g], N_o is the initial population [CFU/g], and CF is free chlorine content [mg/L]. It was assumed that washing only with water, without FC, reduces the microbial population by 84% (0.8 log reduction) (Luo et al., 2011).

Figure 4.11 shows the survival populations of *E. coli* O157:H7 in shredded lettuces indicating that washing in water alone reduced *E. coli O157:H7* populations by an average of 1.0 log CFU/g. Washing with solutions containing free chlorine at 1, 5, 25, and 100 mg/liter resulted in an additional 0.2, 0.6, 0.9, and 1.2 log CFU/g reduction in *E. coli O157:H7*, respectively, compared with water wash (0 mg/liter chlorine) (Luo et al., 2011).
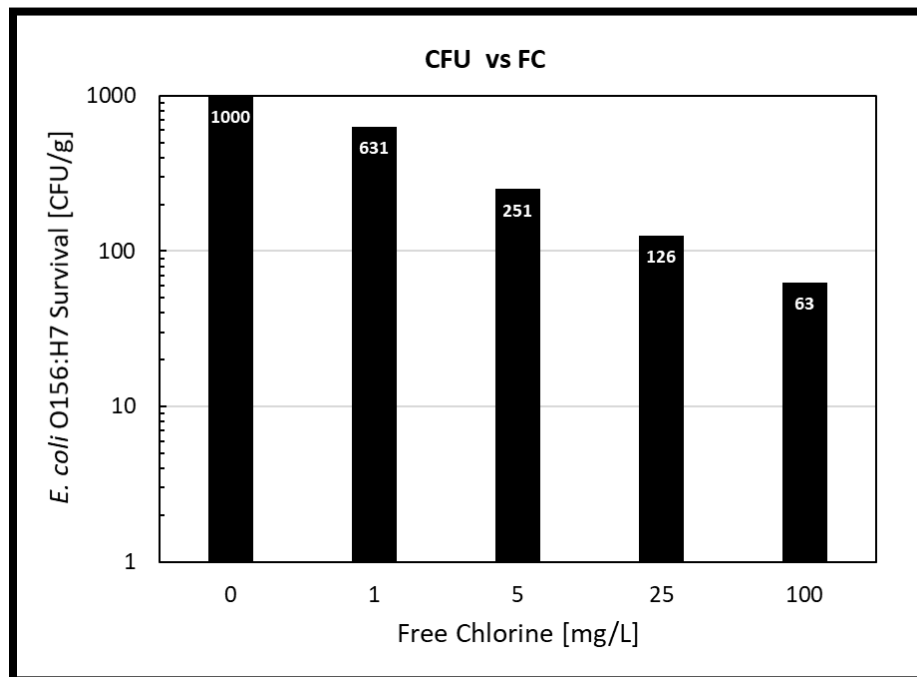
Figure 4.11: Survival E. coli O157:H7 populations from inoculated fresh-cut romaine lettuce after washing in solutions containing 0 to 100 mg/liter free chlorine (FC) (Luo et al., 2011).

4.4 NetLogo simulation and agent-based modeling

The traditional way to simulate manufacturing facilities is by using output analysis and treating the system as either terminating or non-terminating systems that are modeled using discrete event simulation (DES). That requires an input of objects into a system through some arrival rate and these objects are serviced by servers in the form of procedures. This type of model is a hybrid model that combines some elements of DES and a continuous system. Like

DES, lettuce heads will be coming in and exiting the system at a fixed rate of 45 kg per minute for an 8-hour shift.

4.4.1 Types of Agents

In NetLogo, there are four different types of agents: turtles, patches, links, and the observer. In this research, we only described the first three. Figure 4.12 illustrates examples of turtles, patches, and link agents. Turtles are dynamic agents that are free to move around the Netlogo environment (located in the interface tab) and come in multiple shapes and different sizes. In this work, lettuces are considered turtle agents that move in the equipment surface (patches). Patches are the two-dimensional grid, which is the surface the turtles move on; these patches only come in square form, and all the patches have the same size. In this work, equipment surfaces are patches agents. Links are agents that connect two turtles. In this study, water in the flume tank is considered a link agent.

4.4.2 Leafy greens processing facility design

4.4.2.1 Netlogo Facility Layout

A two-dimensional grid was used for fixed location agents (equipment and people). The equipment surfaces have reference points to represent processing locations. Figure 4.13 shows a 2-D grid of a fixed agent schematic. Table 4.6 describes each grid element of the facility and provides the location ID. The Netlogo layout differs from the experimental design described in session 4.2 by including 5 workers and a packaging area.
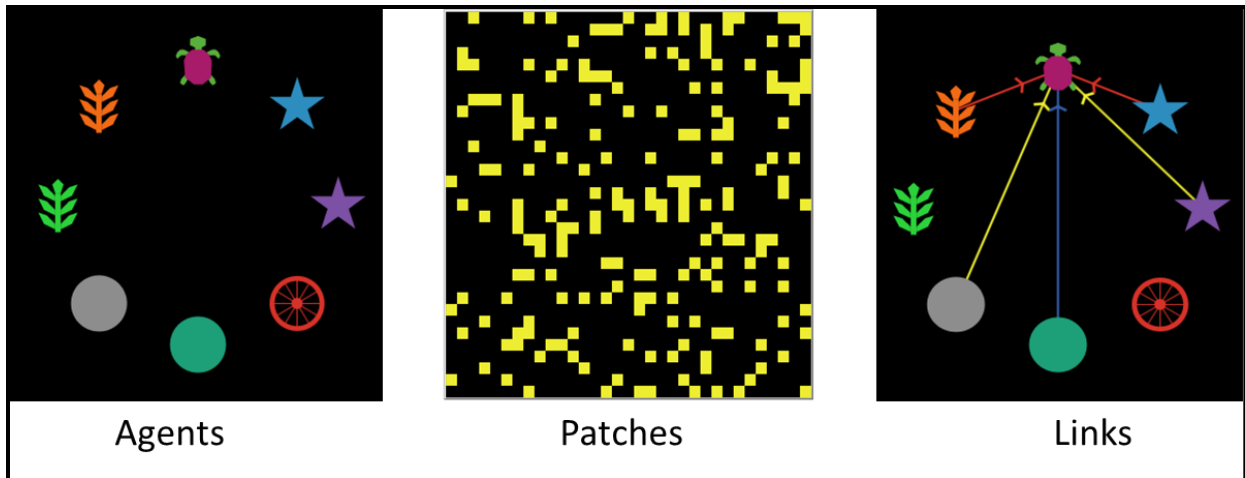
Figure 4.12: Illustration of agents, patches, and links.
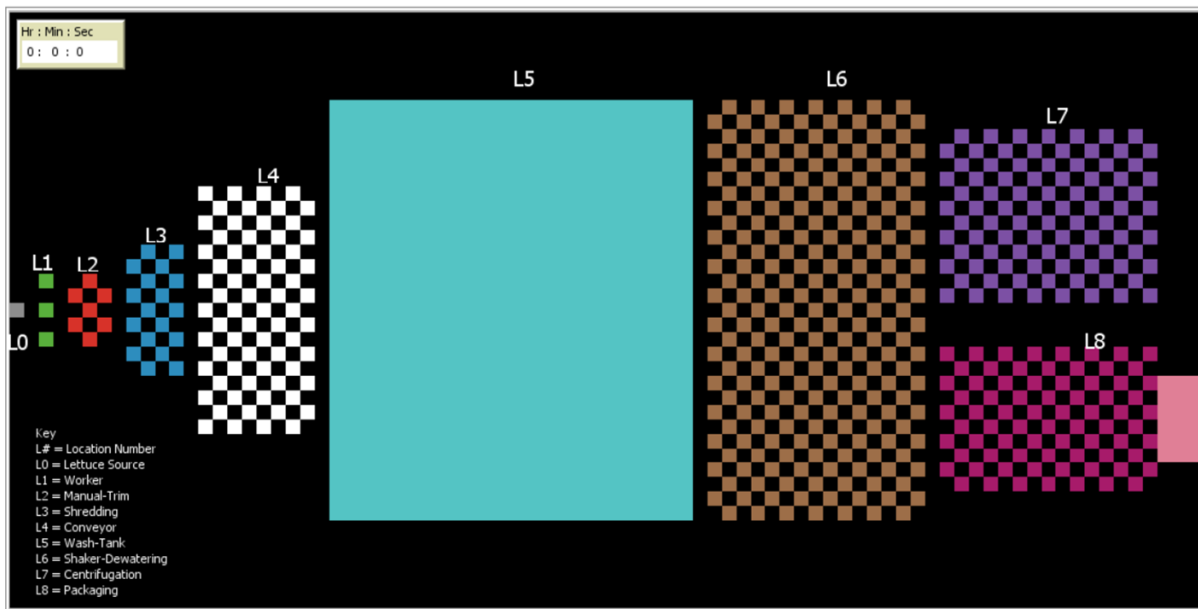
Figure 4.13: 2-D grid for the different fixed agent locations in the leafy-green processing facility simulated in this study.

Table 4.6: Description of each location ID in the processing facility

Location ID	Equipment	Number of patches
L0	Lettuce Source	1
L1	Worker	5
L2	Manual-Trim	15 (5x3)
L3	Shredding	36 (9x4)
L4	Conveyor	136 (17x8)
L5	Wash-Tank	-
L6	Shaker-Dewatering	360 (15x24)
L7	Centrifugation	180 (15x12)
L8	Packaging	150 (15x10)

Figure 4.14 illustrates a schematic of the lettuce processing facility and cross-contamination scenarios. The main agents include stationary agents (equipment and workers) and dynamic agents (lettuces). Non-contaminated lettuces can be contaminated by the pathogen via cross-contamination when it is transferred from contact with contaminated lettuces and/or equipment surfaces. The change in temperature in the facility affects the pathogenic population growth. In the flume tank, pieces of lettuces are washed, and the microbial population is reduced by 0.8-log at the most (Luo et al., 2012). Chlorine may be added to decontaminate the water as well as the lettuces.

The processing mass flow logic follows the same steps as described in section 4.2, up to the flume tank. Once the lettuce pieces leave the flume tank, they go to the dewatering shaker where they remain for 20 s until 18 kg of lettuce pieces are accumulated. Then, 15 kg of the samples is transferred to the centrifuge where they are dried for 60 s. Note that the mass flow rate is 45 kg/min, so in a minute ($20\ s\ \times \frac{1\ min}{60\ s} \times 3$) the centrifuge would dry 45 kg of lettuce pieces.

The next step is the packaging area, where 500 g of lettuce pieces are randomly selected, bagged, and the number of contaminated bags counted.
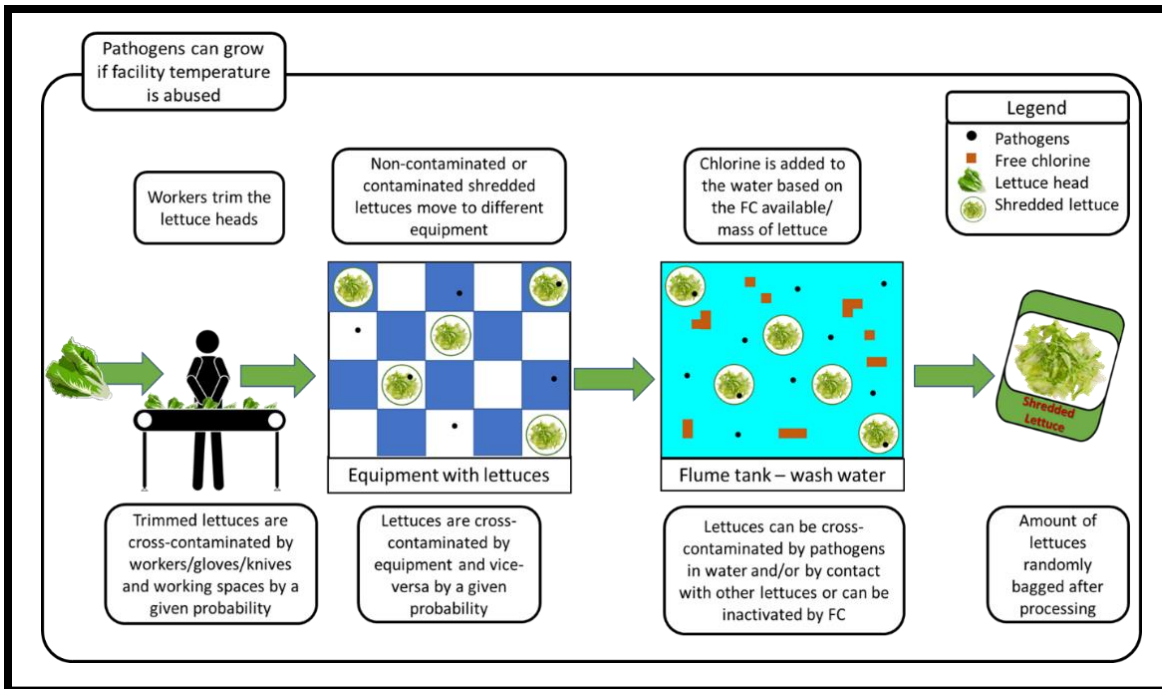
Figure 4.14: The leafy-green processing facility schematic.

The following is a description of each location depicted in Table 4.6. If there is bacteria load (CFU) in one of or both contacting agents, cross-contamination will take place between the two agents. Refer to Table 4.3 to determine the microbial transfer distributions between the contacting surfaces.

4.4.2.2 Lettuce Source [L0]

The single gray patch in Figure 4.12 is the only entry point of which lettuce agents, which are turtles and are circular, are introduced in the system from this location. Only a single lettuce head can occupy the L0 location.

4.4.2.3 Worker [L1]

Five workers are represented as patches: three in green color and two are located in-between the green patches. A single lettuce head moves from the L0 location to one of the five workers in L1.

4.4.2.4 Manual Trim [L2]

A single lettuce head is moved from the worker line to the manual-trim surface. During this moving process, the lettuce head is partitioned into 2 pieces. Each half is then portioned into 8 pieces with a total of 16 pieces at the most. These partitioned pieces are then each randomly placed on one of the fifteen patches that represent a manual-trim surface. Figure 4.15 illustrates lettuces (dynamic agents) sitting on different patches in a piece of equipment showing contamination levels (numbers) during cross-contamination.

4.4.2.5 Shredding [L3]

After the manual-trim process, the lettuce pieces that were located on each of the L2 surfaces are then moved to the shredding process at location L3. Like the manual-trim process, each lettuce piece is further partitioned into smaller pieces during the moving process. Each of these smaller lettuce pieces are randomly placed on one of the thirty-six patches that represent the shredding surface.
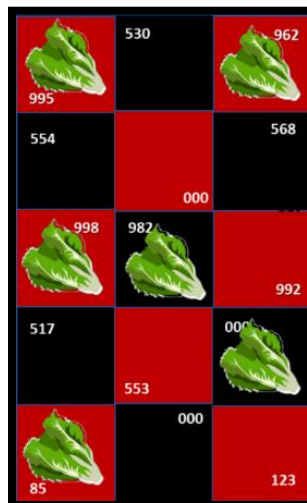
Figure 4.15: Turtle agents (lettuce) sitting on patches at a processing location, showing contamination loads on both agents (equipment and lettuce) during the process.

4.4.2.6 Conveyor [L4]

Each of the patches that make up the conveyor exists in two states, white or black. These patches are synchronized to alternate, and change color based on conveyor speed. After the shredding process, each of the small lettuce pieces is then moved to the conveyor with thirty-six white patches at that time interval.

4.4.2.7 Wash Tank [L5]

Small lettuce pieces are then moved from the conveyor to the wash tank. These small lettuce pieces are washed (bacterial removal) or contaminated, based on the presence of FC. Links are the transfer medium of bacteria population in CFU. The contaminated lettuce pieces will contaminate the links, and the links will contaminate the originally uncontaminated lettuce pieces. Figure 4.16-left shows the wash-tank with contaminated lettuces (red dots) when the FC concentration is high (> 0.5 mg/L). Figure 4.16-right shows the water tank when FC concentration is low and the links are active to transfer bacteria to the lettuces, and water (this example shows FC concentration below 0.5 mg/L).

4.4.2.8 Shaker Dewatering [L6]

After passing through the wash tank, lettuce pieces are then transferred to the shaker-dewatering equipment. Each lettuce piece is randomly placed on one of the 360 brown patches in the L6 location. Unlike the previous processes, here the lettuce pieces must wait in location to resemble dewatering.

4.4.2.9 Centrifugation [L7]

 Once 95 percent of the shaker-dewatering process is filled up with lettuce pieces, a limited number of the pieces is sent to the centrifugation process (180 patches, 15x12).
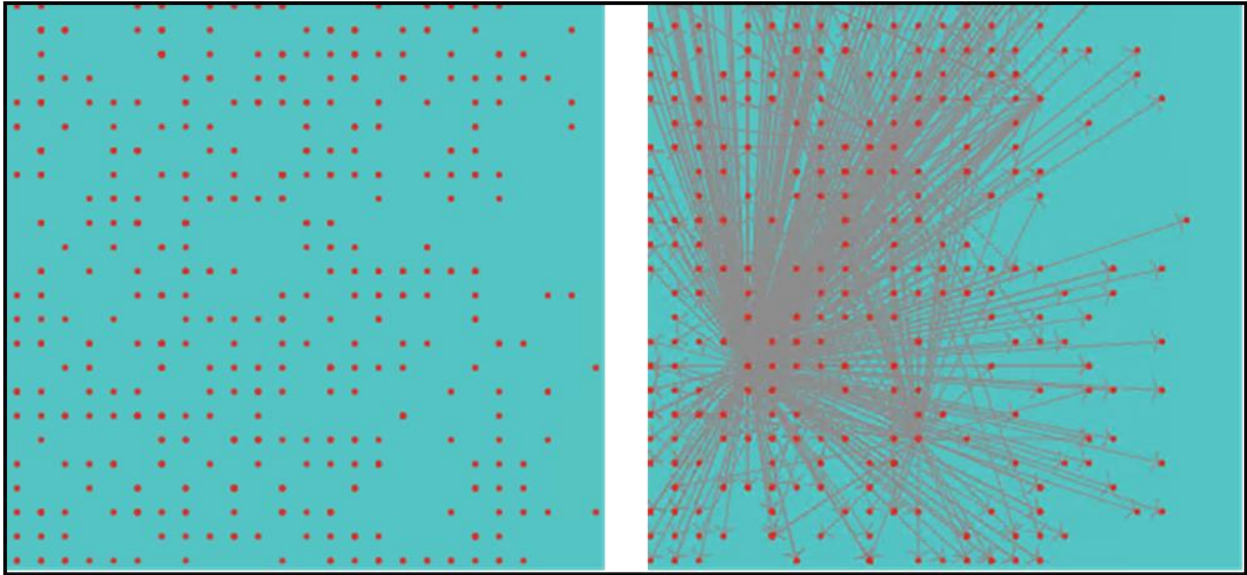
Figure 4.16: <u>Left</u> – Water tank patches showing red dots (contaminated lettuces) and cyan patches represent the water tank with full FC. <u>Right</u> – Water tank showing contaminated lettuces (red dots) and FC patches (cyan patches) linked during bacteria transfer.

4.4.2.10 Packaging [L8]

After the centrifugation step, the lettuce pieces are sent to the packaging area -- location L8 (150 patches, 15x10)-- where the lettuce pieces are bagged. During the packaging process, lettuce pieces are randomly selected (500 g/bag), the average bacteria CFU is measured and reported, and then the lettuce bags exit the system.

4.5 Simulation Logic

4.5.1 Bacteria Growth

The unit for bacteria population is CFU (colony forming units). Microbial population growth as affected by temperature and time is simulated using Eqns. 4.5-4.9.

4.5.2 Partitioning of Agents

The model contains processes that partition the produce into pieces, such as the manual-trim and the shredding process. The load of *E. coli* O157:H7 in each lettuce piece is defined in

terms of log CFU/lettuce which is randomly distributed in different pieces of lettuce. Partitioned lettuces are of the same size and weight. These are lettuce pieces with the same information as the original lettuce head, but smaller in size. During packaging, the lettuce pieces are clustered/packaged in group sizes that contain equal number of partitions. The average microbial load (CFU or log CFU) of a lettuce group represents the average contamination of the bagged shredded lettuce after processing. This assumption is made to normalize the bacteria population in a group.

4.5.3 Chlorination Process

The level of free chlorine (FC) in the wash water depletes based on how much lettuce has been loaded in the tank. FC depletion rate model was based on Luo et al. (2012) experimental data. Equations 4.14 and 4.17 are used to calculate the amount of COD and FC in the water based on the amount of lettuce in the flume tank during the washing process.

Chlorine depletion is a function of COD, which is directly proportional to the cumulative amount of lettuce mass (kg) that has been washed. To model this behavior, we have made FC depletion dependent on the cumulative mass that is processed (Eqn. 4.17). The FC dose is only applied once momentarily at 12-minute intervals. At every 12 minutes a new dose is added, and the FC value is calculated as above. The process is repeated three times until the final total lettuce load reaches 1620 kg.

4.5.4 Movement of Lettuces in the Flume Tank

A combination of random walk and Markov chain processes was used to simulate the movement of the lettuce pieces in the wash tank. The random walk process introduces the variance in time the lettuce pieces spend in the tank. Because the turbulence conditions in the tank (Luo et al., 2012), some pieces will randomly travel faster than others.

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4.5.4.1 Random walks

A random walk is a stochastic sequence $\{S_n\}$ defined by:

$$S_n = \sum_{\kappa=0}^{n} \Psi_\kappa \qquad\qquad [4.21]$$

where $S_0 = 0$ and $\{\Psi_\kappa\}$ are independent and identically distributed random variables.

A simple model (1-D) of movement using random walks assumes that the direction of movement is completely independent of the previous directions moved (i.e., uncorrelated) - the location after each step taken in the random walk is dependent only on the location in the previous step and the process is Markovian with regard to the location (Weiss 1994). Additionally, the direction moved at each step is completely random (i.e., unbiased). This process is essentially Brownian motion assuming that the movement to any direction is allowed.

Random walks can be assumed correlated if it involves a correlation between successive step orientations - 'persistence' (Patlak, 1953). This produces a local directional bias where each step tends to point in the same direction as the previous one, although the influence of the initial direction of motion progressively diminishes over time and step orientations are uniformly distributed in the long term (Benhamou, 2006).

An example of a probability distribution from a one-dimensional (1-D) random walk is illustrated below. Starting with few steps, each of unit length, we define the probability function $p_N(i)$ as the probability that in a walk of N steps of unit length, it randomly moves forward or backward along the line, beginning at 0 and end at point i. The sum of these probabilities over i must equal 1.

- For walk of no steps, $p_0(0) = 1$

- For walk of 1 step, $p_1(-1) = \frac{1}{2}$, $p_1(1) = \frac{1}{2}$

- For walk of 2 steps, $p_2(-2) = ¼$, $p_2(2) = ¼$

- For walk of 3 steps, $p_3(-3) = 1/8$, $p_3(3) = 1/8$

- For walk of 4 steps, $p_4(-4) = 1/16$, $p_4(4) = 1/16$

- For walk of 5 steps, $p_5(-5) = 1/32$, $p_5(5) = 1/32$

By factoring out the $(1/2^N)$, there is a pattern of all these probabilities as illustrated in Table 4.7, which is known as the Pascal's triangle. Every entry is the sum of the two diagonally above, and these numbers are in fact the coefficients of the binominal expansion of $(a + b)^N$. The row for $2^5 f_5(n)$, for example, mirrors the binominal coefficients. So, the total probability to reach -1 in five steps is equal to ½ $(6/2^4 + 4/2^4)$ or $3/16 + 2/16 = 5/16$. A modified Sterling's equation can be used to determine the random walks probabilities:

$$p(i) = \frac{2}{\sqrt{2\pi N}} e^{-i^2/2N}$$ [4.22]

Table 4.7: Probability distribution for each position in a 1-D random walk model with the initial position starting from 0, and with only the possibility of moving +1 or -1 based on even odds at each time step.

i	-5	-4	-3	-2	-1	0	1	2	3	4	5
$f_0(n)$						1					
$2f_1(n)$					1	0	1				
$2^2 f_2(n)$				1	0	2	0	1			
$2^3 f_3(n)$			1	0	3	0	3	0	1		
$2^4 f_4(n)$		1	0	4	0	6	0	4	0	1	
$2^5 f_5(n)$	1	0	5	0	10	0	10	0	5	0	1

Simulation logic:

The dimension of the wash-tank is shown in Figure 4.16. Each patch location is defined by its y and x coordinated point. The red-colored numbers are the y-coordinate that range from 14 to -14 and the white-colored ones are the x-coordinate.

As lettuce pieces enter the wash-tank, they are randomly placed at any point along the vertical line where $x = 21$ (Figure 4.17). Lettuce pieces travel horizontally until they leave the tank at $x = 45$. During the washing processes, air is pumped into the wash tank to generate turbulence to clean the produce more efficiently (Luo 2012). It takes, on average, 26 s for the lettuce pieces to travel through the washing process. The movement of lettuces is based on time and is assumed to be probabilistic to introduce randomness and mixing of agents in the tank.

To set the time in the simulation, it was assumed that it takes 2184 ticks/iterations (12 minutes) to process 540 kg of shredded lettuces in the flume tank (Luo 2012). All instances of time in the simulation were derived from this relation.
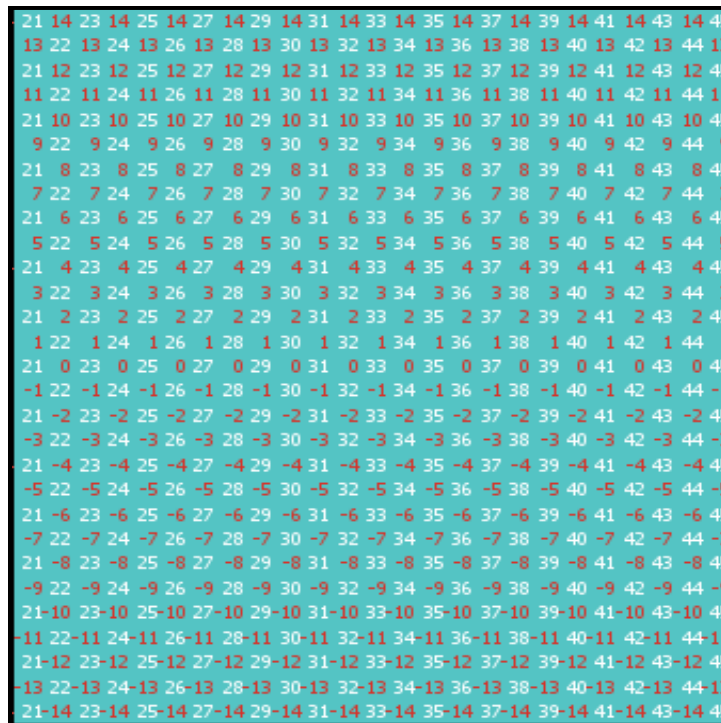
Figure 4.17: Flume tank patch locations. The red-colored numbers are the *y*-coordinate white-colored ones are the *x*-coordinate.

No matter the size of the lettuce piece agents, they can only occupy one patch in the tank. Their locations are fixed to one location all the time. The location coordinates are discrete, and the point of origin is the center of the square, which is rounded to the nearest integer.

The movement of lettuces in the tank is different from the movement in the other processes because in the tank, the lettuce piece moves on multiple patches and in the other processes, they only occupy one patch for the entire time they spend in the equipment. In the flume tank, a piece of lettuce is either moving (A) or stationary (B). There are 24 patches across the tank and the target residence time is 26 s. The model is updated at every iteration.

The lettuce pieces random walks simulation is described as follows. Consider the process described in Table 4.7 for 5 steps. Instead of having a probability of going backward (-1) distance, the lettuce pieces do not move, i.e., they stay in place. So, there are two possibilities, move (+1) or not move (0). The resultant probability distribution is shown in Table 4.8. Based on that probability distribution, the expected |E| average residence time (Table 4.9) for the lettuce pieces in the wash process for 25 steps is only 15.82 s, but the required value should be 26 s.

Table 4.8: Probability distribution for each position in a 1-D random walk model with the initial position starting from 0, and with only the possibility of moving forward (+1) or not moving based on even odds at each time step.

Steps [i]	0	1	2	3	4	5	6	7	8	9	10
$f_0(n)$	1										
$2f_1(n)$	1	1									
$2^2 f_2(n)$	1	2	1								
$2^3 f_3(n)$	1	3	3	1							
$2^4 f_4(n)$	1	4	6	4	1						
$2^5 f_5(n)$	1	5	10	10	5	1					

Table 4.9: Calculated parameters from random walk model

Parameter	Definition	Value	Unit
X	Length of the tank (horizontal distance Figure 4.14)	24	---
i_h	Number of iterations in an hour	10920	--
t_{step}	Length of time for each iteration = 0.33 sec	3600/10920	sec
p	Probability of taking a step	0.5	---
$|E|$	Expected time in tank during random walks	15.82	sec

$$|E| = t_{step} \times \left(\frac{X}{p}\right) \qquad [4.23]$$

4.5.4.2 Markov chains

Markov chains are a system of independent states that change in discrete time. The changes from one state to another are called transitions, and the probability of transitioning to the next state is independent and not related to the event before the change (Markov, 1971).

To add the delay to the random walk process that resulted in an average residence time expectation from 15.82 s to 26 s, the problem was turned into a Markov chain with two states, A and B. State A refers to 'not do' the random walks and state B to 'do' the random walks. The Markov diagram is shown in Figure 4.18 for the states A and B. Each number represents the probability of the Markov process changing from one state to another state, with the direction indicated by the arrow. For example, if the Markov process is in state A, then the probability it changes to state B is 0.6, while the probability it remains in state A is 0.4.
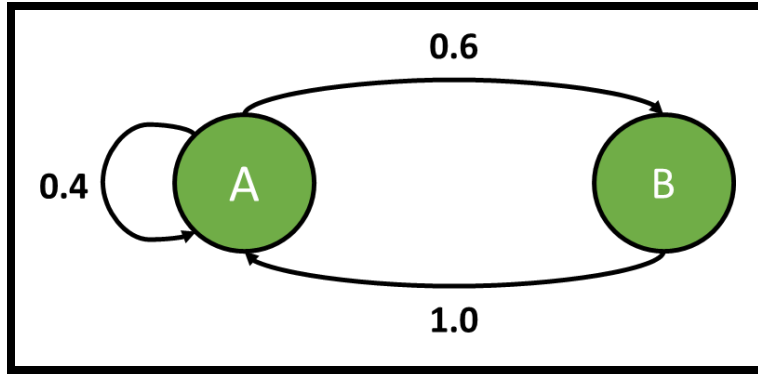
Figure 4.18: A diagram representing a 2-state Markov process, with the states labelled A and B.

The Markov process shown in Table 4.10 describes how to calculate the transitional probability so that the average residence time expectation is increased to 26 s. From Eqn. (4.23) it can be deduced that X/p is the expected number of iterations needed to travel the distance X, which is equal to 48. The Markov chain increases this number and as a result the expectation of 15.82 s

Table 4.10: Calculated parameters for transitional probability for 26 s.

Parameter	Definition	Value	Unit
N	Number of even iterations needed	80	---
t_{step}	Length of time for each iteration = 0.33 sec	3600/10920	sec
t	Desired time expectation	26	sec
$p(t)$	Transitional probability	0.6	---

$$N = \frac{t}{t_{step}} \qquad [4.24]$$

$$p(t) = \frac{48}{N} \qquad [4.25]$$

As an example, Figure 4.19 shows the calculation of the normal distribution experimental results of the time the lettuces were in the tank. There were 6 replications per experiments, which consisted of processing 500 kg of lettuces per experiment. The time the agents spent in the tank was recorded at the end of the experiment. The mass of a lettuce head was assumed to be 0.5 kg and the size of the lettuce pieces after manual trimming and shredding was 1/16 of the original mass. The total lettuce pieces processed were 184,000, calculated as:

$$184000 = 16 \left[\frac{pieces\ lettuces}{lettuce\ head}\right] \times 1 \left[\frac{lettuce\ head}{0.5\ kg}\right] \times 500 \left[\frac{kg}{experiment}\right]$$

$$\times\ 6\ [experiments]$$

The average time that the lettuce pieces stay in the tank was 26.02 s. Figure 4.19 gives the average time in the tank and its interval. In this case, 5% of the lettuce pieces spent 18.64 s in the tank, and 95% spent 33.40 s in the tank.
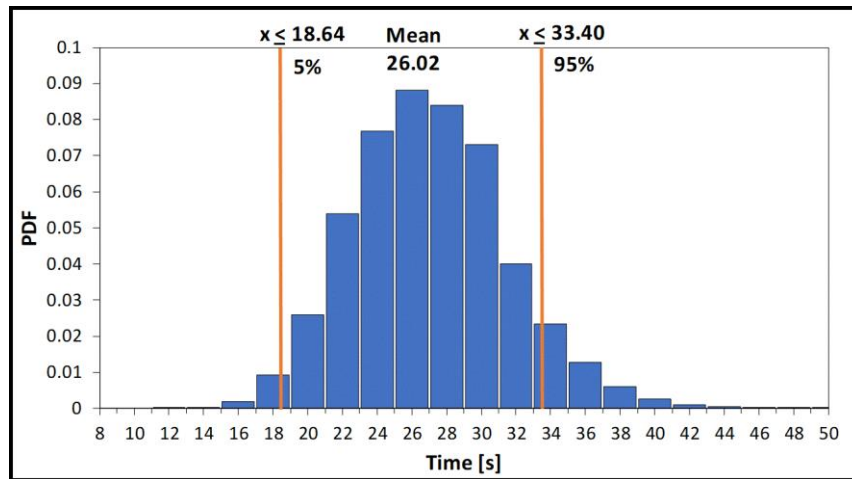
Figure 4.19: Probability density functions of the estimated lettuces time in the tank (s) including the 90% confidence interval.

The values defined in Tables 4.8 and 4.9 were used to simulate the process by more than 72,000 times. Figure 4.20 shows the good agreement between the NetLogo PDF distribution and the random walk distribution as described by Eqns. (4.20) to (4.22) above. The Chi-Square goodness of fit test resulted in $\chi^2 = 0.07$ for $p < 0.005$.
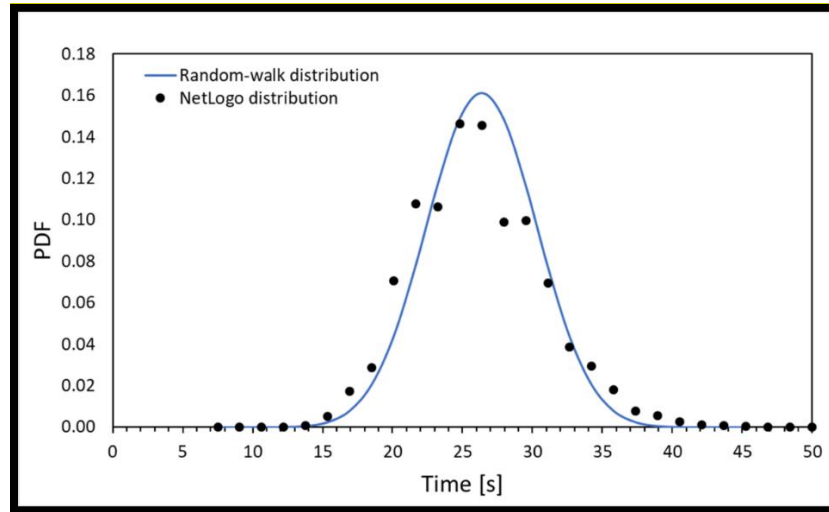
Figure 4.20: PDF curve for random walks Markov process calculated and predicted using NetLogo simulation.

4.5.5 Bacteria Transfer in the Wash Water

The phenomenon of bacteria transfer in water is not well understood. In this work, we simulated bacteria transfer as a diffusion process, where bacteria are transferred from high a concentration agent to a low concentration one. Following are the rules that govern bacteria transfer from lettuce to water when no FC is available in the water, i.e., transfer of bacteria in the water tank will only happen when there are bacteria survivors:

1. Once the lettuce pieces enter into the flume tank, it is assumed that 84% of bacteria population (CFU) from the lettuce pieces is transferred to the water (Luo et al., 2012) and then to the uncontaminated lettuce pieces.

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2. The average bacteria population (CFU) remaining in the lettuce pieces is then determined.

3. Links (water) are then created to target only the lettuce pieces that have bacteria populations lower than the average bacteria population in the lettuces as calculated in step 2.

   a. The number of bacteria in the water are then equally divided amongst the links.

   b. The links (water) then transfer a smaller percentage of their bacteria population to the lettuce pieces ~1% (Luo et al., 2012).

Those set of rules assume no total net gain or loss of bacteria population during the transfer process.

4.5.6 Inactivation of *E. coli O157:H7* in Shredded Lettuces in the Flume Tank

Bacteria decontamination in lettuces is simulated using Eqn. (4.16), which calculates the effect of FC concentration in the wash water on *E. coli* O157:H7 survival in shredded lettuces. The survival of bacteria depends on the concentration of FC and COD in the water. Based on the experimental data (Luo et al, 2012), *E. coli* O157:H7 did not survive in solutions with targeted free chlorine concentrations $\geq$ 0.5 mg/L with a 30 s to 1 min exposure.

There are two processes in the water, log-reduction, or transfer of bacteria. If FC $\geq$ 0.5 mg/L, log reduction takes place and if FC< 0.5, bacteria is transferred to the lettuce pieces. Links were used to simulate the transfer of bacteria from the water to the lettuce.

4.5.7 Setting the binomial and triangular distributions

NetLogo provides the following random distributions: uniform, exponential, gamma, normal, and Poisson. The cross-contamination process requires the following distributions: triangular and binomial. The binomial distribution was used to estimate the transfer amount

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between two agents given a probability. The given probability is calculated using a triangular distribution. The binomial distribution is explained in section 4.3.1.

The inverse-transform technique was applied to generate continuous random variates (pseudorandom number). The CDF or PMF is one of the two ingredients used for generating random variates. One primary requirement for any good random number generator is that the random variates being generated are IID (Independent and Identically Distributed). A second ingredient is a random number sampled from a uniform distribution.

The triangular distribution [min, most likely, max], represented as $T[a, c, b]$, has the following probability density function:

$$f(x) = \begin{cases} \dfrac{2(x-a)}{(b-a)(c-a)} & a < x < c \\ \dfrac{2(b-x)}{(b-a)(b-c)} & c \leq x < b \end{cases} \qquad [4.26]$$

The cumulative distribution function is as follows:

$$F(x) = \begin{cases} \dfrac{(x-a)^2}{(b-a)(c-a)} & a < x < c \\ 1 - \dfrac{(x-b)^2}{(b-a)(b-c)} & c \leq x < b \end{cases} \qquad [4.27]$$

The inverse transform technique consists in making $F(x) = u$, where $0 < u < 1$, and making x the subject of the formula:

$$F^{-1}(u) = \begin{cases} a + \sqrt{(b-a)(c-a)u} & 0 < u < \dfrac{c-a}{b-a} \\ b - \sqrt{(b-a)(b-c)(1-u)} & \dfrac{c-a}{b-a} \leq u < 1 \end{cases} \qquad [4.28]$$

The closed-form algorithm to generate random variables from a triangular distribution is the following:

1. Generate $U \sim U(0,1)$

2. If $U <$ than $(c - a) / (b - a)$ then apply:

$$X \leftarrow \quad a + \sqrt{(b-a)(c-a)u} \qquad\qquad [4.29]$$

1. If $U \geq$ than $(c-a) / (b-a)$ then apply:

$$X \leftarrow \quad b - \sqrt{(b-a)(b-c)(1-u)} \qquad\qquad [4.30]$$

where X is the random variable.

4.5.8 Cold Storage Room

The impact of temperature fluctuations during storage on the growth of microorganisms was also simulated. The assumptions made to simulate the lettuces bags in a cold storage room are listed below (Table 4.11):

- After processing, the lettuce bags are sent to the cold storage.

- The transfer of lettuce bags from the processing facility to the cold storage room is instantaneous.

- The storage sections can only house a maximum of 3240 bags.

- Each NetLogo patch houses a single lettuce bag (temperature is not uniform among stacked bags).

- The size of the storage room is 60 X 54 patches.

Table 4.11: Storage characteristics

Mass of lettuce at 45/min flow rate [rate]	1620
Total number of 500 g bags [ ]	3240
Room width x length [patches]	60 x 54
Processing time [min]	36
Storage time[days]	10*

*Zang et al. (2014), Gehringer et al. (2017)

To model the temperature fluctuations inside the cold storage room, the average temperature at a particular instant of time was calculated based on the temperature data given by Zeng et al. (2014). The temperature across the room was assumed to be non-uniform, ranging from 1°C and 18.2°C (Zeng et al., 2014). The mean temperature value was assumed to follow a triangular distribution with T [2, 8, 14]. Markov chain with a variable transition probability of changing the mean value was used to simulate how likely the mean temperature may change during storage.

The lettuce bags are transported from the processing facility packaging area to a current loading section in real time. The maximum storage time was assumed to be 10 days and the unit of time used in the simulation was minutes.

The storage facility was simulated as a continuous agent-based model, which runs parallel with the main program (processing facility). A different 'storage' area in the facility is created for each simulation run, for example, if they are 13 replications, 13 different storage places are created.

4.5.8.1 LevelSpace NetLogo Extension

To construct the storage facility, the NetLogo extension called *LevelSpace* (LS) was used (Hjorth et al., 2015). *LevelSpace* gives the user the ability to link multiple models that operate simultaneously. These models operate under a hierarchical structure. In this study, the *LevelSpace* has two hierarchies, the parent (the storage room) and the child (the processing facility) models. The parent model controls the child model.

From the cold storage model, the operation sequence consists of uploading the processing facility model and initiate the run command to start the processing simulation. Once the packaging step is complete, the shredded lettuce bags are then transported to the cold storage

room. After all the lettuces in the facility have been processed, the processing facility model is deactivated leaving only the cold storage model, which contains all the bags that were processed, running. This was done to save computational resources since the child model is no longer necessary to remain operating.

When the bags enter the cold storage room they are randomly placed to a location/patch inside the room. The goal is to simulate the temperature changes in every bag. Each patch has a different temperature value (mean and a range). *E. coli* O157:H7 growth in the surface of shredded lettuces was estimated by the dynamic model (Eqs. 4.10 and 4.11) for temperatures alternating between 5 and 25°C in a 20 h cycle.

Figure 4.21 shows the NetLogo model for the cold storage room showing the control buttons and the outputs.
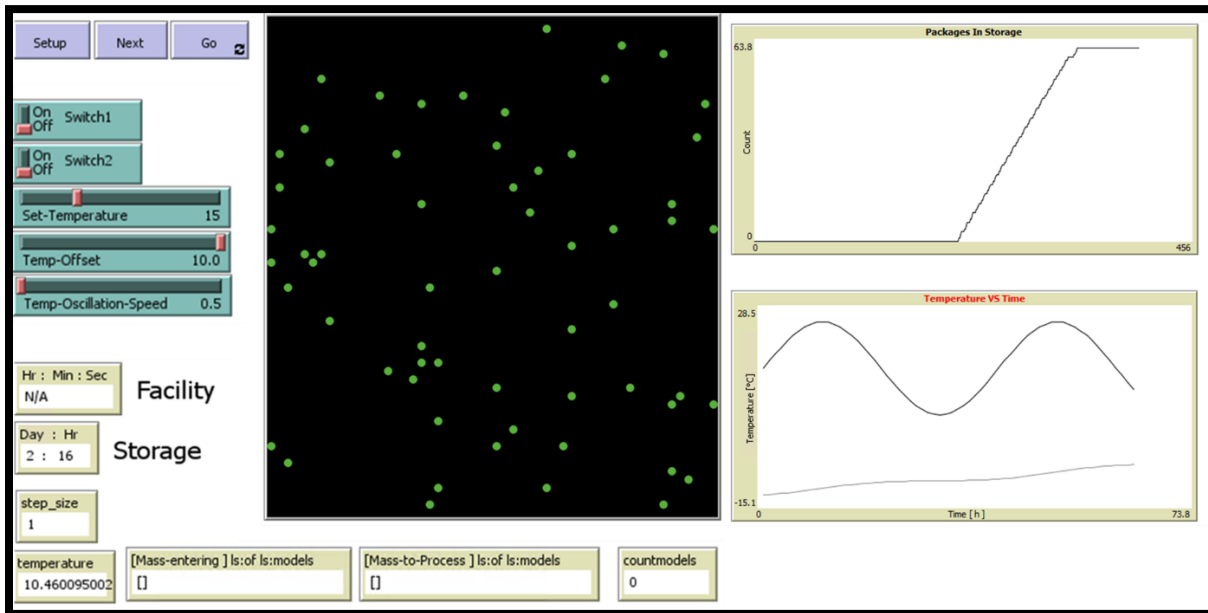
Figure 4.21: The NetLogo cold storage room model with temperature fluctuations

4.6 Verification, Calibration, and Validation

A simulation model is valid only if the model is an accurate representation of the actual system. In this process, we needed to compare the representation of a conceptual model to the real system.

The model was verified by comparing different results to ensure its accuracy. The model was tested to find and fix errors in the implementation of the model based on the model's specifications and assumptions. Furthermore, model output was examined carefully for reasonableness using various input combinations.

Calibration was performed to obtain robust solutions, so the calibrated model is not only able to fit the experimental data but is also able to predict reliable results with new input data (Liu et al., 2017). The experimental data of Buchholz et al. (2012a, b, 2014) was used to calibrate this model by adjusting parameters to fit the experimental data behavior.

The simulation results were compared to expected number of contaminated bags and average contamination level (log CFU/bag) using published literature data. Estimates were used from Buchholz et al. (2012a, b, 2014) and Luo et al. (2012, 2018) who report experimental data on cross-contamination and free chlorine decontamination; Mokhtari et al. (2018) and Rodríguez et al. (2011) who simulated cross-contamination in similar facilities using different approaches.

The program run in the TAMU Supercomputer. One 1000 replication was used per condition (3 total) to validate the model, which corresponded to 389 hours CPU time, taking about 20 hours to complete the job.

The number of agents created during the simulation is calculated as:

(1) **Mass of lettuces to be processed:**

60 kg during the warmup period + 22.5 kg for the 1st batch + 22.5 kg for

the second batch +90 kg for the 3rd batch = 195 kg

(2) **Number of agents created before shredding**

195 kg/(0.5 kg/agent) = 390 agents

(3) **Total number of agents created after shredding**

390*16 = 6,240 agents

(4) **Number of replications: 1000, number of factor: 3 (102, 104, 106 CFU/g)**

Number of experiments conducted = 1,000*3 = 3,000

4.7 Sensitivity Analysis

Sensitivity analysis was used to confirm that changes in the input parameters correspond with logical changes in the simulation results. We performed a deterministic 1-way sensitivity analysis where we examined the output and look for a significant change ($p < 0.05$) in the number of contaminated bags and contamination level (log CFU/bag). In this study, deciding the minimum level of contamination that would not lead to an infection is a complex process, requiring not just scientific judgment but also social values from consumers and manufactures (Puerta-Gomez et al., 2013). Therefore, two levels of tolerance were considered: (a) < 0.04 CFU/g (1 CFU/25 g) , which is based on the *no detectable level of viable organisms permitted* (Omac et all., 2017) and, (b) < 0.01CFU/g (1 CFU/100 g) based on the fact that more than 1% of probability infection is considered unsafe for food processors (Puerta-Gomez et al., 2013).

Therefore, bags of shredded lettuces bags were assumed safe if the average contamination levels where below those tolerance limits.

During a single simulation run, lettuce movements and various statistics results, e.g., current lettuce microbial load, are visualized. After each simulation run, the average cross-contamination at each equipment/lettuce, FC concentration in wash tank, number of lettuce bags decontaminated, and the average CFU/g in each bag are reported. Multiple runs with varying input parameters and stochastic factors were done. Subsequently, the aggregated results were analyzed to identify the impacts of those parameters on the processing performance. Two set of experiments were used in this study.

4.7.1 Experimental Design Case I

The implemented parameters for case I are listed in Table 4.12, representing the values of the base scenario in the computational experiments. The values in the variation column state the maximum and minimum values of the parameter as well as the steps the parameters are varied.

To facilitate a fair comparison of multiple varying simulation runs, a warmup, a processing and a cool down period were set. Based on extensive computational experiments, the first 60 kg of lettuces (1.28 min) the simulation act as a warm-up phase. During this period, the system is initialized to reach a steady-state and no statistics are collected to avoid distorted results. The subsequent 36 min is selected as the processing period in which all lettuce pieces are tracked, representing approximately 1620 kg of products per simulation run (3240 bags). This is repeated for 8-hour (21,600 kg = 43,200 bags). To simulate 8 hours, the program run for 13.33 times and the results averaged.

Once the processing period is over, a cool-down period (1.23 min) starts to enable all unprocessed lettuces to reach the packing area before the simulation ends. During this cool down period, no more lettuces can enter in the system. Once all lettuces enter within the processing

period leave the system, i.e., packaging area, the simulation stops, and results are reported to the user. To consider stochasticity, each experiment is replicated 13 times, i.e., reported results represent data from 8 hours of lettuce operations for each individual parameter setting. Table 4.13 shows the specific input parameters description and distribution and equations used to run the simulation.

To run those experiments, it took in average about 158 hours of CPU time, taking about 16 hours to complete the job. The number of agents created for Scenarion6, for example, during the simulation is calculated as:

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(1) **Mass of lettuces to be processed:**

  1,620 kg

(2) **Number of agents created before shredding**

  1620 kg/(0.5 kg/agent) = 3,240 agents

(3) **Total number of agents created after shredding**

  3,240*16 = 51,840 agents

(4) **Number of replications: 13, number of factors: 4 (0.3, 0.5, 0.7, 1)**

  Number of experiments conducted = 13*4 = 52

Table 4.12: Parameters of the simulation.

Group	Parameter	Base	Variation
Simulation	Replications	130	--
	Warmup time [min]	1.28	--
	Processing time [min]	2-3	--
	Waiting time in shaker [min]	0.33	
	Cool-down period [min]	1.23	
	Lettuce flow rate [kg/min]	45	--
	Lettuce processed [kg]	1620	--
	Run time [min]	36	--
	Facility operation time [h]	8	--
	Bag size [kg]	0.5	--
	Number of bag/day	42120	--
Probability on lettuce - L0	CP [%]	0.1	0.01, 0.1, 0.3, 0.5, 0.7, 1
Initial contamination level - L0	No [log CFU/g]	0.1	-2, -1, 0, 1, 2
Tr-worker to lettuce - L1	$XC_{H,Lh}$ [%]	T [3,10,30]	T [3,10,30]
Tr-worker to lettuce - L1	$XC_{Lh,H}$ [%]	T [0,1,3]	T [0,1,3]
Tr-knife to lettuce head - L2	$XC_{K,Lh}$ [%]	T [0,29.6,59.2]	T [0,29.6,59.2]
Tr-lettuce to knife - L2	$XC_{Lh,K}$ [%]	T [0,2.5,5]	T [0,2.5,5]
Tr-shredder to lettuce - L3	$XC_{S,Lh}$ [%]	T [16,20,28]	T [16,20,28]
Tr-lettuce to shredder - L3	$XC_{Lh,S}$ [%]	T [0,0.2,.53]	T [0,0.2,.53]
Tr-conveyor belt to lettuce - L4	$XC_{Cb,Sl}$ [%]	T [15,18,22]	T [15,18,22]
Tr-lettuce to conveyor belt - L4	$XC_{Sl,Cb}$ [%]	T [0,0.62,1.39]	T [0,0.62,1.39]
Tr-shaker table to lettuce - L6	$XC_{St,Sl}$ [%]	T [6,28,30]	T [6,28,30]
Tr-lettuce to shaker table - L6	$XC_{Sl,St}$ [%]	T [0,0.06,0.38]	T [0,0.06,0.38]
Tr-centrifuge to lettuce - L7	$XC_{C,Sl}$ [%]	T [23,27,31]	T [23,27,31]
Tr-lettuce to centrifuge - L7	$XC_{Sl,Dc}$ [%]	T [0,0.35,1.59]	T [0,0.35,1.59]
Free chlorine	FC [mg/L]	21-10-6	11-5-3 and 6-3-2
Temperature fluctuation	T [ºC]	7±3	7-35
Partition of lettuces – L1	M [g]	31.25	42, 50, 83, 125
Contamination level on equip.	$No\text{-}eq$ [log CFU/g]	0.1	2, 3, 4, 6
Probability in equipment L1-L7	$CP\text{-}eq$ [%]	0.01	0.1, 0.3 ,0.5 ,0.7 ,1
Type of lettuce	type	romaine	iceberg

Table 4.13: FS-ABS input parameters, distribution information, values, and sources of information for *E. coli* O157 cross-contamination, growth, reduction in wash water.

Description	Equation	Reference
Contamination probability	Uniform [0.05 0.15]	
Transfer – worker to lettuce - L1	T [3, 10, 30]	Mokhtari et al., 2018
Transfer – worker to lettuce - L1	T [0, 1, 3]	Mokhtari et al., 2018
Input - lettuce level of contamination [CFU/g]	Uniform [0.20 0.5]	
Temperature variation [°C]	7±3	
Transfer – knife to lettuce head - L2	T [0, 29, 0.6, 59.2]	Mokhtari et al., 2018
Transfer – lettuce to knife - L2	T [0. 2.5, 5.0]	Mokhtari et al., 2018
Transfer – shredder to lettuce -L3	T [16, 20, 28]	Buchholz et al., 2012a, b
Transfer – lettuce to shredder - L3	T [0, 0.25, 0.53]	Buchholz et al., 2012a, b
Transfer – conveyor belt to lettuce - L4	T [15, 18, 22]	Buchholz et al., 2012a, b
Transfer – lettuce to conveyor belt - L4	T [0, 0.62, 1.39]	Buchholz et al., 2012a, b
Transfer – shaker table to lettuce - L6	T [6,28,30]	Buchholz et al., 2012a, b
Transfer – lettuce to shaker table - L6	T [0,0.06,0.38]	Buchholz et al., 2012a, b
Transfer – centrifuge to lettuce - L7	T [23,27,31]	Buchholz et al., 2012a, b
Transfer – lettuce to centrifuge - L7	T [23,27,31	Buchholz et al., 2012a, b
Free chlorine [mg/L]	Eq. [4.15]	Data from Luo et al. (2012)
COD [mg/L]	Eq. [4.12]	Data from Luo et al. (2012)
Log reduction by FC [CFU/g]	Eq. [4.17]	Data from Luo et al. (2011)
Pathogens in the water [CFU]	Uniform [0.8 0.9]	Data from Luo et al. (2012)
Pathogen growth [CFU/g]	Eqs. 4.5 to 4.9	Data from Ding et al., (2009)

4.7.2 Scenarios

Tables 4.14 presents the different scenarios used to run the FS-ABS model. Note that for Scenario 7, the experiment assumed 18 batches of 90 kg each with the 1st batch only being contaminated from 0-6 log CFU/g in a normal distribution.

Table 4.14: Scenarios for Simulation of the Processing Facility

Parameter	Unit	Scenarios			
		Base	**1**	**2**	**3**
Contamination probability	[ ]	0.1	0.1	0.1	0.1
L0 – initial contamination level	log[CFU/g]	0.1	0.1	0.1	10^0 - 10^4
Tr-worker to lettuce at L1	[%]	T[3,10,30]	T[3,10,30]	T[3,10,30]	T[3,10,30]
Tr-worker to lettuce at L1	[%]	T[0,1,3]	T[0,1,3]	T[0,1,3]	T[0,1,3]
Tr-knife to lettuce head at L2	[%]	T[0,29.6,59.2]	T[0,29.6,59.2]	T[0,29.6,59.2]	T[0,29.6,59.2]
Tr-lettuce to knife at L2	[%]	T[0,2.5,5]	T[0,2.5,5]	T[0,2.5,5]	T[0,2.5,5]
Tr-shredder to lettuce at L3		T[16,20,28]	T[16,20,28]	T[16,20,28]	T[16,20,28]
Tr-lettuce to shredder at L3	[%]	T[0,0.2,.53]	T[0,0.2,.53]	T[0,0.25.0.53]	T[0,0.2,.53]
Tr-conveyor belt to lettuce at L4	[%]	T[15,18,22]	T[15,18,22]	T[15,18,22]	T[15,18,22]
Tr-lettuce to conveyor belt at L4	[%]	T[0,0.62,1.39]	T[0,0.62,1.39]	T[0,0.62,1.39]	T[0,0.62,1.39]
Tr-shaker table to lettuce at L6	[%]	T[6,28,30]	T[6,28,30]	T[6,28,30]	T[6,28,30]
Tr-lettuce to shaker table at L6	[%]	T[0,0.06,0.38]	T[0,0.06,0.38]	T[0,0.06,0.38]	T[0,0.06,0.38]
Tr-centrifuge to lettuce at L7	[%]	T[23,27,31]	T[23,27,31]	T[23,27,31]	T[23,27,31]
Tr-lettuce to centrifuge at L7	[%]	T[0,0.35,1.59]	T[0,0.35,1.59]	T[0,0.35,1.59]	T[0,0.35,1.59]
Free chlorine - L5	[mg/L]	21-10-6	21-10-6	0,11-5-3,6-3-2	21-10-6
T fluctuation	[ºC]	7±3	7±3	7±3	7±3
Partition of lettuces [2-16]	[g]	16	4, 6,10,12,16	16	16
No on equipment	log[CFU/g]	0.1	0.1	0.1	0.1
Probability in equipment	[%]	0.01	0.01	0.01	0.01
Parameter	Unit	**4**	**5**	**6**	**7**
Contamination probability	[ ]	0.3, 0.5,0.7,1	0.1	0	1
L0 – initial contamination level	log[CFU/g]	0.1	0.1	0	0, 1,2,3,4,5,6
Tr-worker to lettuce at	[%]	T[3,10,30]	T[3,10,30]	T[3,10,30]	T[3,10,30]
Tr-worker to lettuce at	[%]	T[0,1,3]	T[0,1,3]	T[0,1,3]	T[0,1,3]
Tr-knife to lettuce head at	[%]	T[0,29.6,59.2]	T[0,29.6,59.2]	T[0,29.6,59.2]	T[0,29.6,59.2]
Tr-lettuce to knife at	[%]	T[0,2.5,5]	T[0,2.5,5]	T[0,2.5,5]	T[0,2.5,5]
Tr-shredder to lettuce at	[%]	T[16,20,28]	T[16,20,28]	T[16,20,28]	T[16,20,28]
Tr-lettuce to shredder at	[%]	T[0,0.2,.53]	T[0,0.2,.53]	T[0,0.2,.53]	T[0,0.2,.53]
Tr-conveyor belt to lettuce at	[%]	T[15,18,22]	T[15,18,22]	T[15,18,22]	T[15,18,22]
Tr-lettuce to conveyor belt at	[%]	T[0,0.62,1.39]	T[0,0.62,1.39]	T[0,0.62,1.39]	T[0,0.62,1.39]
Tr-shaker table to lettuce at	[%]	T[6,28,30]	T[6,28,30]	T[6,28,30]	T[6,28,30]
Tr-lettuce to shaker table at	[%]	T[0,0.06,0.38]	T[0,0.06,0.38]	T[0,0.06,0.38]	T[0,0.06,0.38]
Tr-centrifuge to lettuce at	[%]	T[23,27,31]	T[23,27,31]	T[23,27,31]	T[23,27,31]
Tr-lettuce to centrifuge at	[%]	T[0,0.35,1.59]	T[0,0.35,1.59]	T[0,0.35,1.59]	T[0,0.35,1.59]
Free chlorine	[mg/L]	21-10-6	21-10-6, 0-0-0	21-10-6, 0-0-0	21-10-6, 0-0-0
T fluctuation	[ºC]	7±3	7±3	7±3	7±3
Partition of lettuces [2-16]	[g]	16	16	16	16
No on equipment	log[CFU/g]	0.1	1, 2, 3, 4	0, 3	0
Probability in equipment	[%]	0.01	0.1	0.3 ,0.5,0.7 ,1	0

Table 4.14: Scenarios for Simulation of the Processing Facility, cont..

Parameter	Unit	Scenarios
		8
Contamination probability	[ ]	0.05,0.25,0.5,0.75,1
L0 – initial contamination level	log[CFU/g]	1,2,3,4,5,6
Tr-worker to lettuce at L1	[%]	T[3,10,30]
Tr-worker to lettuce at L1	[%]	T[0,1,3]
Tr-knife to lettuce head at L2	[%]	T[0,29.6,59.2]
Tr-lettuce to knife at L2	[%]	T[0,2.5,5]
Tr-shredder to lettuce at L3		T[16,20,28]
Tr-lettuce to shredder at L3	[%]	T[0,0.2,.0.53]
Tr-conveyor belt to lettuce at L4	[%]	T[15,18,22]
Tr-lettuce to conveyor belt at L4	[%]	T[0,0.62,1.39]
Tr-shaker table to lettuce at L6	[%]	T[6,28,30]
Tr-lettuce to shaker table at L6	[%]	T[0,0.06,0.38]
Tr-centrifuge to lettuce at L7	[%]	T[23,27,31]
Tr-lettuce to centrifuge at L7	[%]	T[0,0.35,1.59]
Free chlorine - L5	[mg/L]	21-10-6, 0-0-0-0
T fluctuation	[ºC]	7 ± 3
Partition of lettuces [2-16]	[g]	16
No on equipment	log[CFU/g]	0
Probability in equipment	[%]	0
Batch size [number/1620 kg]	[ ]	60, 36
Number of batches	[ ]	27, 45

4.8 Statistical Analysis

The model was further validated by comparing the model output with the real system output described in Buchholz et al. (2012a, b, 2014) and Luo et al. (2011, 2012). The Mean Squared Error (MSE), Root Means Squared Error (RMSE), and t-test were used to compare the model to the experimental data. The two-sample t-test was used to evaluate the level of significance changes on the outputs from different experiments using R version 4.0.2 (2020-06-22).

NetLogo's BehaviorSpace was used to run simulations with different combinations of parameter values. Parameters were defined as global variables to identify key drivers of cross-contamination within the system. R version 4.0.2 (2020-06-22) was used to process all the data generate by the NetLogo model, i.e., all the experiments of all scenarios discussed in this study.

The two-sample *t*-test was used to evaluate the level of significance changes on the outputs from different experiments using R version 4.0.2 (2020-06-22).

Theil's inequality coefficient (TIC) was also used (Eqn. 4.31) to compare the experimental data and the simulated data for the validation studies (Van Haute et al, 2013). TIC values range from 0 to 1, and values below 0.3 indicate a decent agreement of the model with the experimental data (Audenaert et al., 2010).

$$TIC = \frac{\sqrt{\sum(Y_i - Y_{i,m})^2}}{\sqrt{(Y_i)^2} + \sqrt{(Y_{i,m})^2}} \qquad [4.31]$$

where *Y* is the predicted value, Y_m the measured value.

One-way analysis of variance (ANOVA) was used to determine the overall significant difference. The t-test ($\alpha = 0.05$) was used to compare all pairs of means and Dunnett's test was used for comparing all other means with a control group's means.

4.9 Summary of the Model Development

Cross-contamination of *E. coli* O157:H7 during post-harvest processing of fresh-cut leafy greens has become a major health concern worldwide. During commercial shredding, conveying, flume-washing, and drying leafy greens can become contaminated with *E. coli* and have been linked to many outbreaks in the US and Canada (CDC, 2020). To better understand the role of processing facility patterns on *E. coli* O157:H7 cross-contamination at romaine lettuce-processing equipment interface, we developed an agent based model for a pilot plant processing facility for fresh-cut romaine lettuce (Buchhlolz et al., 2012a) using the open-source NetLogo.

The model was designed to (1) track *E. coli* O157:H7 and lettuce movements in time, (2) evaluate microbial contamination in different piece of equipment (spatially explicit) and calculate the probability events of cross-contamination between agents (lettuces) and patches (equipment), and (3) determine the number of fresh-cut contaminate processed bags and their level of contamination at the end of the processing line. An extension was also added to the main model to determine *E. coli* O157:H7 growth due to temperature abuse in a cold storage facility.

4.9.1 Data

4.9.1.1 Experimental Data

The facility layout and cross-contamination data were based on information from (Buchholz et al., 2012a, b, 2014). Experimental data on the flume tank related to free chlorine (FC) and chemical oxygen on demand (COD) ware from Luo et al. (2011, 2012), modeling the flume tank FC consumption and COD generation was based om Munther et al., (2015). The growth of E.coli o157:H7 on fresh-cut lettuces at different storage temperature was modeled based on the experimental data of Ding et al. (2009). Temperature profile during storage was based on information from Zeng et al., 2014;). Cross-contamination transfer processes were based on the work of Mokhtari et al. (2018).

4.9.1.2 Probability Functions

The cross-contamination transfer process was based on the information described by Mokhtari et al., (2018) using a triangular distribution.

4.9.2 Model Description

4.9.2.1 State Variables and Scales

The components responsible for the dynamics of the cross-contamination of leafy greens are lettuces, space, and time. Agents are either mobile or patch agents. The lettuces are mobile agents that move from patch to patch. Patch agents are presented by a grid where each one

contains several values that are modified at each model run. Some of the attributes of the patch

agents change with time, for example, in the flume tank according to level of free chlorine and

chemical oxygen in demand.

In the flume tank, links are the transfer medium of bacteria population. The contaminated

lettuce pieces will contaminate the links, and the links will contaminate the originally

uncontaminated lettuce pieces.

Lettuces are transported through the processing line moving from patch to patch agents

that have their own attributes including levels of contamination and probability of microbial

transfers. In one of the processing line setups, about 1,620 kg lettuces are processed in 36

minutes (representing 3,240 bag of 0.5 kg each) for a total of 42,120 bags per day. Due to

computing constraints, one lettuce head is cut into 16 pieces of lettuces representing the shredded

produce. Each of the 16 lettuce pieces belongs to the same parent with the same attributes, but

they are each randomly placed on one of the total patches that represent an equipment.

Space is represented by a grid-based equipment with multiple attributes. Space provides

the environmental basis for movements and interactions of lettuces. Each equipment has a

different surface area. The number of patches per equipment are the following: trim-table has 15

patches (5x3), shredder 36 (9x6) patches, conveyor 36 patches (9x6), shaker 360 patches (15x24)

and it is where the lettuce pieces have to wait until the centrifuge is available, centrifuge 180

(15x12), and then the packing area that has 150 (15x120) patches all together.

The level of free chlorine (FC) in the wash water depletes based on how much lettuce has

been loaded in the tank. Chlorine depletion is a function of COD, which is directly proportional

to the cumulative amount of lettuce mass (kg) that has been washed. To model this behavior, we

have made FC depletion dependent on the cumulative mass that is processed. The FC dose is

only applied once momentarily at 12-minute intervals. At every 12 minutes a new dose is added, and the FC value is calculated as above. The process is repeated three times until the final total lettuce load reaches 1620 kg.

A combination of random walk and Markov chain processes was used to simulate the movement of the lettuce pieces in the wash tank. The random walk process introduces the variance in time the lettuce pieces spend in the tank. Because the turbulence conditions in the tank, some pieces will randomly travel faster than others.

This scale is a trade-off between a sufficient spatial resolution to represent the relevant agents involved in cross-contamination and computer processing time. Some spatial attributes vary through time like the flume tank chlorination concentration that changes according free chlorine and chemical oxygen demand levels. Other attributes of the patches are fixed parameters of the equipment, represented as raster data: contamination levels and transfer probabilities.

Time was represented by discrete time steps corresponding to one-third of a 2160 s (36 min) processing time (720-time steps).

4.9.2.2 Computer Simulation

At each time step, the time counter and/or free chlorine concentration is updated. The variables for the lettuces are updated as they move, get cross-contaminated, are decontaminated, and then packaged. The patch agent variables are also updated accordingly as they become cross-contaminated. The variables are updated immediately during model runs.

4.9.3 Designing Concepts

4.9.3.1 Observations

A user-friendly interface was created to follow spatial and temporal variations in model outputs. Global level outputs, such as the number of contaminated bags, the lettuce

contamination levels, were computed and visualized on plots and diagrams. Diagrams

representing equipment variables were also produced to track changes in these variables.

4.9.3.2 Agent Interactions

A main feature of the modeled processing facility is the cross-contamination between

different agents. Spatial-temporal dynamics of contacts are influenced by changes in the global

state of the system, which affect interactions between lettuces and the equipment.

Random events were introduced in the model to represent the equipment heterogeneity. A

realistic pattern of variability was introduced into deterministic rules by defining probability

distribution functions. For example, the stochasticity of mobile agents was represented by

varying their locations in the equipment during initialization of each model run by introducing a

random element in agent movements. The lettuce dynamics include a random selection of

lettuces that are cross-contaminated at each time step.

The storage facility was simulated as a continuous agent-based model, which runs

parallel with the main program (processing facility). A different 'storage' area in the facility is

created for each simulation run, corresponding to a batch of lettuce processed.

CHAPTER V

RESULTS AND DISCUSSION

5.1 Model Validation, Calibration, and Verification

Figures 5.1 and 5.2 compare the experimental contamination levels of *E. coli* O157:H7 in a pilot plant facility (Buchholz et al., 2012b) with the simulated contamination levels generated by the FS-ABS model for initial contamination loads of 10^6 CFU/g and 10^2 CFU/g for incoming romaine or iceberg lettuces heads (prior to shredding), respectively. The model fitted the experimental data well. A *t*-test revealed that the experimental and the simulated data were not significantly (p>0.05) different. Table 5.1 shows the statistical analysis for all the experimental data presented. All TIC (Theil's inequality coefficient) values indicate a good agreement of the model with the experimental data (Audenaert et al., 2010).

The difference between simulated and experimental data was high for the initial microbial load of 2 logCFU/g since the transfer-rate data were more variable than for the 4 logCFU/g and 6 log CFU/g loads (Figures 5.1 and 5.2). In these experiments, all the data points that were near the non-detectable level of < -1.4 log CFU/g) were set at log -1.4 CFU/g, thus reducing the model's accuracy in quantifying the transfer of viable cells to previously uncontaminated products (Buchholz et al., 2012b). This issue is observed for the 2 log CFU/g results where for most of the uninoculated samples, the data were recorded as log -1.4 CFU/g. In the present study, we did not use -1.4 CFU/g as a point of no detection, and the model predicted the experimental data well when the experimental results were above the detection limit. Therefore, for the sake of consistency and the lack of more accurate experimental data, the developed model is assumed to behave as expected, even when the level of contamination is low (2 CFU/g).

79
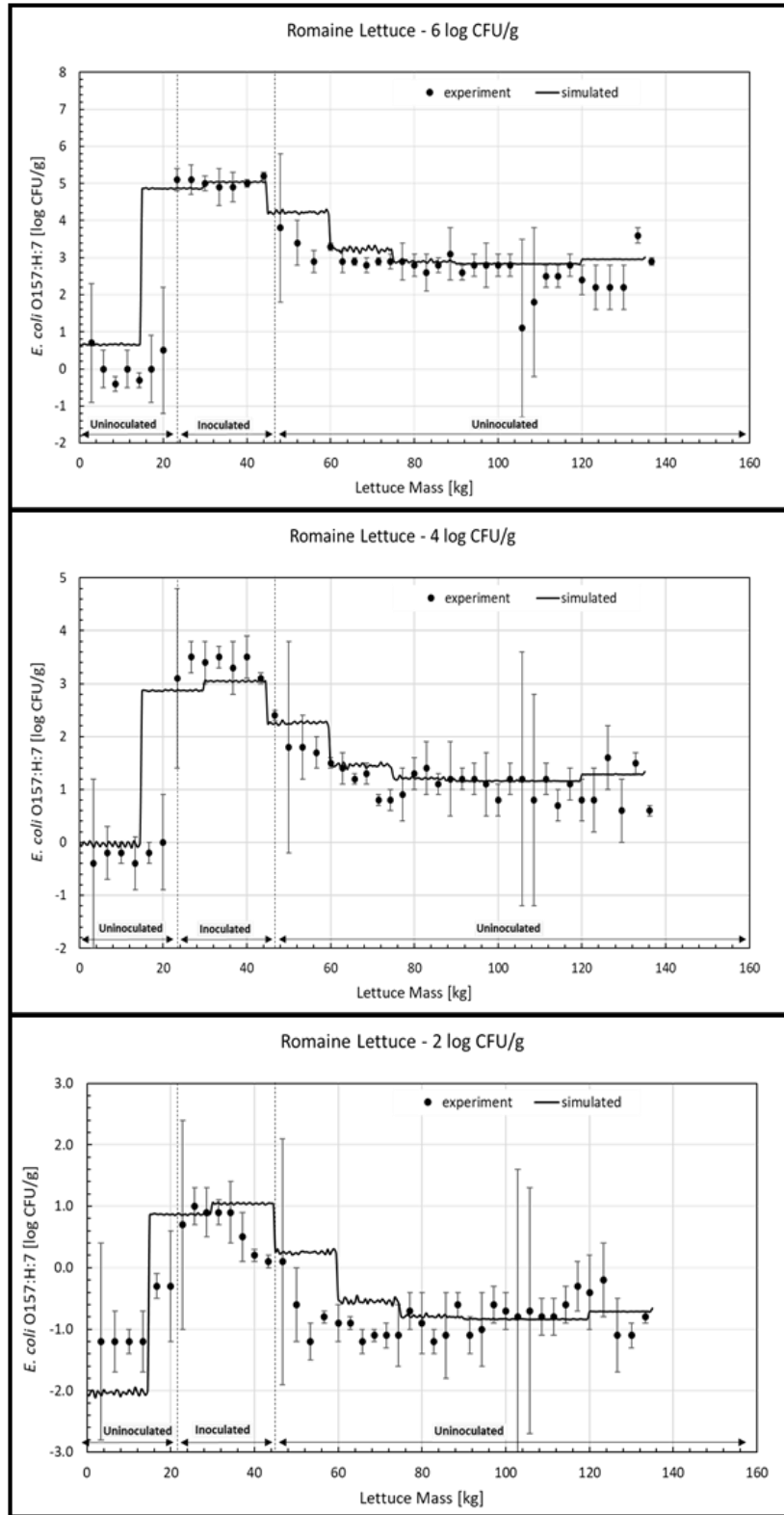
Figure 5.1: Predicted (solid line) and experimental data (dots) of *E.coli* O-157:H7 contamination level (log CFU/g) for different initial microbial load on shredded romaine lettuce.
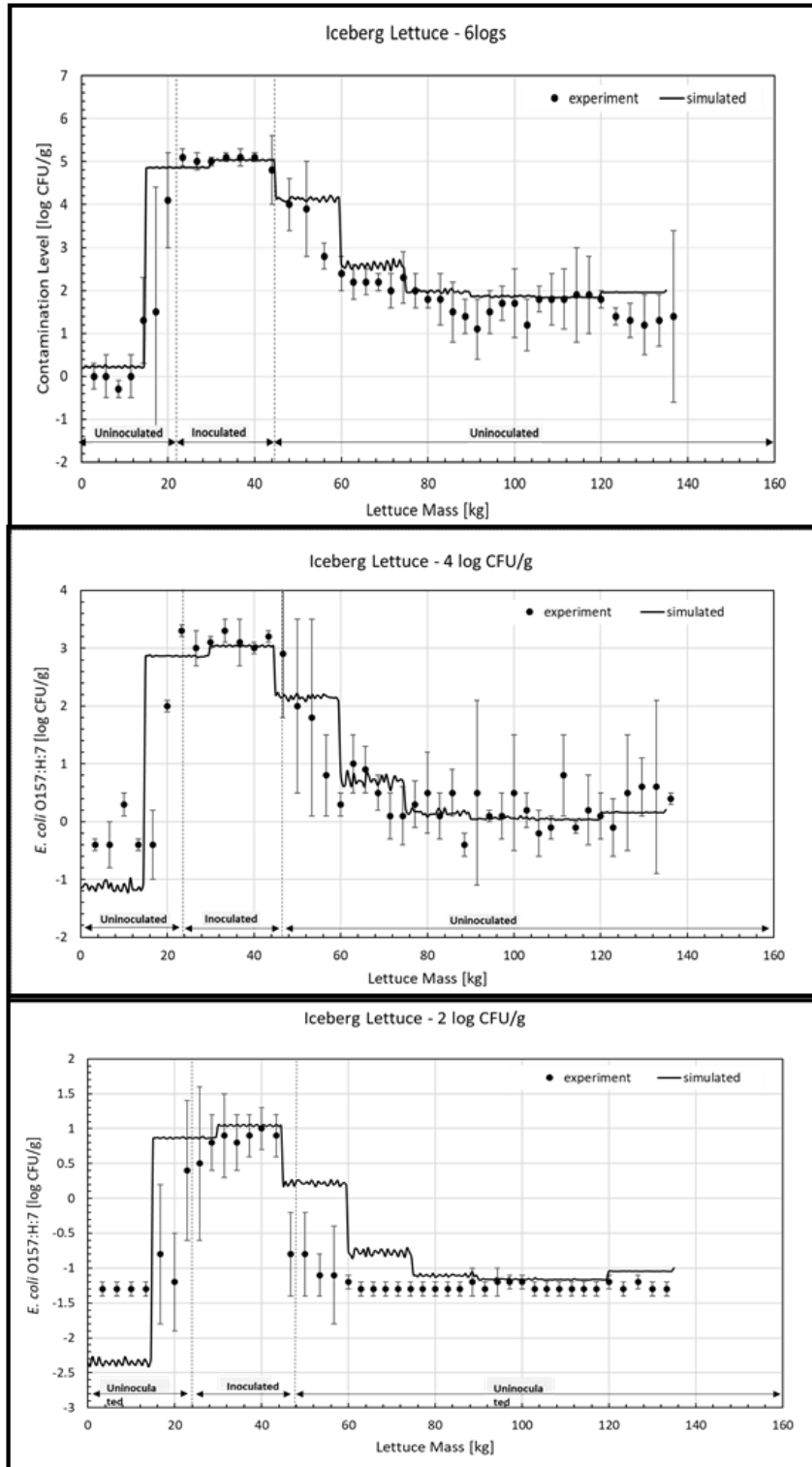
Figure 5.2: Predicted (solid line) and experimental data (dots) of *E.coli* O-157:H7 contamination level (log CFU/g) for different initial microbial load on shredded iceberg lettuce.

Buchholz et al. (2012 a, b) discussed this problem with the experimental data, emphasizing the fact that if sanitizers had been used in the pilot plant, *E. coli* O157:H7 populations in the flume water would have been reduced to non-detectable levels, thus decreasing the ability to quantify the transfer of viable cells to the uncontaminated product. Even at low contamination levels, the authors consistently detected *E. coli* O157:H7 in the flume water at levels between -1.5 and -2.0 log CFU/g. Nevertheless, based on an estimated oral infectious concentartion of less than 100 cells for *E. coli* O157:H7 (Puerta-Gomez et al., 2013), the presence of any viable pathogenic cells in the flume water, as seen in their study, is reason for concern if appropriate intervention strategies are not applied to prevent product cross-contamination.

Figure 5.3 shows the FS-ABS model validation results based on the experimental data by Buchholz et al. (2012a) for *E. coli* O157:H7 populations on the surface of lettuces inoculated at different loads. Based on the *t*-test, the experimental and predicted data were not significantly different ($p > 0.05$). Therefore, the FS-ABS model predicts the experimental data well.

Table 5.1: Theil's inequality coefficient (TIC) values for validation of the experimental data for E. coli O157:H7 inoculated on romaine and iceberg lettuces for different inoculum loads.

Initial Load [log CFU/g]	Romaine Lettuce	Iceberg Lettuce
6	0.182	0.126
4	0.213	0.248
2	0.310	0.248
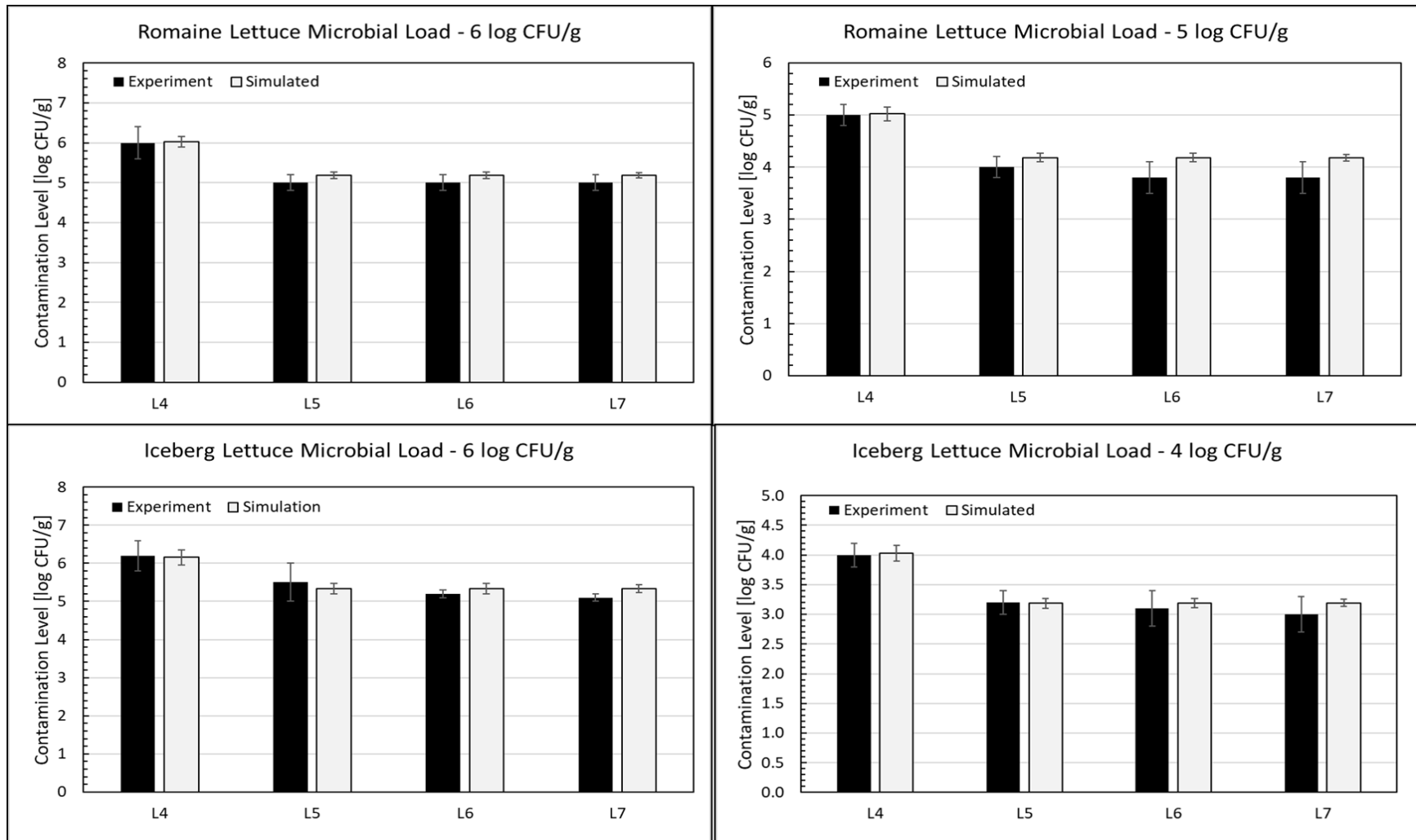
Figure 5.3: Predicted (solid line) and experimental data (dots) of contamination level (log CFU/g) for different initial microbial load and lettuce types. Bars represents standard deviations

5.2 Sensitivity Analysis for Case I

5.2.1 Base Scenario

Table 4.14 describes the different scenarios evaluated in this study. Figure 5.4 shows the results for simulation using the input data for the base scenario. The effect of cross-contamination level in every step of the processing line is not significantly different ($p>0.05$). However, differences ($p<0.05$) were found in the levels of contamination of lettuce pieces inside the bags between steps L4 (step conveyor) and L5 (flume tank), mainly due to the decontamination step in the flume tank. These results show the cross-contamination level in each step when the initial contamination load is 0.1 log CFU/g or 1.26 CFU/g and the probability of contamination in each batch is 10%. The log reduction of *Escherichia coli* O156: H7 in the flume tank was 1.26 ± 0.00 CFU/g for free chlorine (FC) concentration levels of 21-106 mg/L.
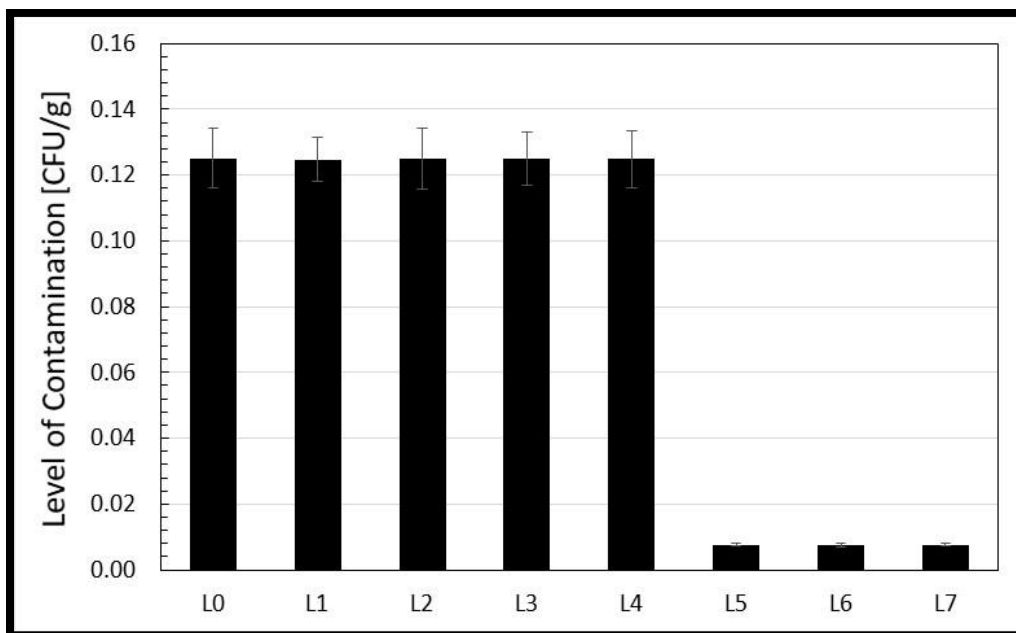
Figure 5.4: Shredded romaine lettuces level of contamination in each step in the processing line during 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 =

shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Error bars are standard deviation values.

Figure 5.5 illustrates the level of cross-contamination in the flume tank (L5), where larger contamination variation is observed compared to the shaker (L6) and the centrifuge (L7), mainly because of the chlorination process. In the step conveyor (L4), lettuce spend a long time crossing the equipment, which results in accumulation of lettuces and therefore larger variation in the cross-contamination mean compared to those at L1 - L3 that only have a few lettuce pieces.
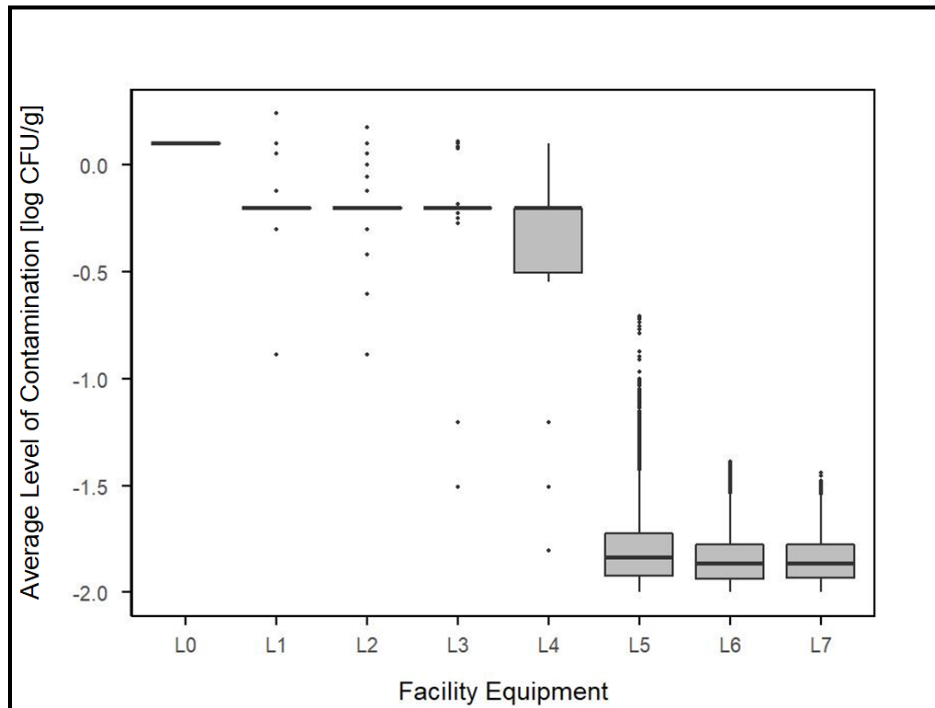
Figure 5.5: level of contamination in Romaine lettuces at each step in the processing line with FC concentrations of 21-10-6 mg/L, during an 8-hour processing shift, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge.

Without chlorination (Figure 5.6), the contamination level shows higher mean values at the flume tank (L5), shaker (L6), and centrifuge (L7) steps compared to the values shown in

Figure 5.5. However, the distribution is narrower resulting in only 0.8-1 log reduction range of

pathogens from the produce surfaces.

The average number of contaminated bags processed depends on the minimum

acceptable contamination level. Two minimum tolerance levels were considered, low acceptable

limit as < 0.01 CFU/g (1 CFU/100 g) and high acceptable limit as < 0.04 CFU/g (1 CFU/25 g).

Therefore, any bag with an average contamination level higher than those two limits is

considered to be contaminated with the pathogen.
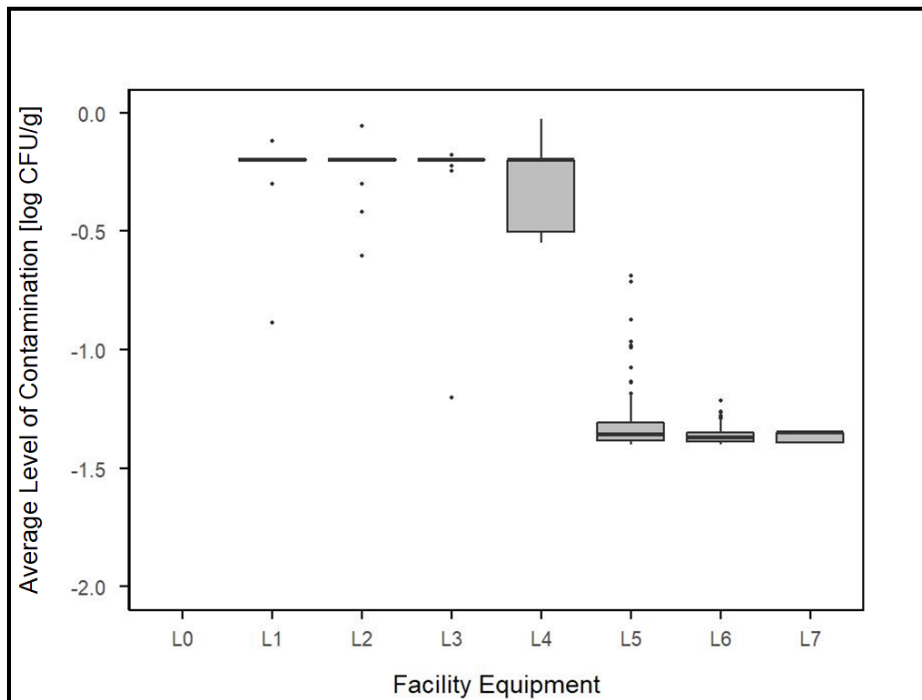
Figure 5.6: Average level of contamination in Romaine lettuces at each step in the processing line
without chlorination (FC = 0), where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 =
shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge.

Table 5.2 shows that in 8 hours, only 1% of the total bags of romaine lettuces processed

were above the safety limit of 1 CFU/ 25 g (0.04 CFU/g) compared to 73% of bags above the

safety limit of 1 CFU/100 g (0.01 CFU/g). Recall that the tolerance limit of 1 CFU/25 g is based on the *no detectable level of viable organisms permitted* (Omac et al., 2017) and 1 CFU/100 g is based on the fact that more than 1% of probability infection is considered unsafe by food processors (Puerta-Gomez et al., 2013). Although the results show significant differences ($p<0.05$) between the two limits, using the more rigorous tolerance limit would be safer for the consumer.

It is also important to notice that, depending on the acceptable level of contamination, around 30% of the bags could have been sold "as safe" when they would actually be unsafe for consumption. These results illustrate the importance of having reliable methods to detect low levels of contamination in fresh produce. Until then, prevention of microbial contamination at all steps from production to distribution is important when following the current Good Manufacturing Practice (GMP), Hazard Analysis (HACCP), and Risk Based Preventive Controls for Human Food (RBPC) (FDA, 2018).

Table 5.2: Average number of contaminated and uncontaminated shredded romaine lettuce bags after 8 hours processing.

	< 0.01 CFU/g	< 0.04 CFU/g
Contaminated bags (0.5 kg)	$_x11,431 \pm 943$	$_y358 \pm 171$
Safe bags (0.5 kg)	$_x30,689 \pm 943$	$_y41,762 \pm 171$
Total number bags in 8 hours period	42,120	42,120
Percentage of contaminated bags [%]	27	1
Percentage of safe bags [%]	73	99

Different letters within the same $_{xy}$row for each component indicate significant difference ($p < 0.05$) according to one-way ANOVA and t-test test ($\alpha = 0.05$).

The whisker plot in Figure 5.7 shows that the median number of contaminated bags differs significantly ($p<0.05$) based on the minimum contamination limit used to calculate the number of processed bags with an average *E. coli* O157:H7 contamination level above the tolerance limit. Less contaminated bags are produced when the tolerance limit is assumed to be 1 CFU/25 g of product.

Figure 5.7: Number of contaminated shredded lettuce bags per batch for 8-hour processing time as a function of minimum acceptable contamination limit.

Based on the above results, the FS-ABS was run to determine the impact of using different minimum values of contamination to calculate the transfer of viable cells to the uncontaminated product or equipment surfaces during lettuce processing. Figures 5.8-A and 5.8-B indicate that, using a minimum value of -3 log CFU/g, the calculated number of bags remains constant around 1100 and 1300, for the 1 CFU/100 g limit, and around 40 and 80 for the 1 CFU/25g minimum contamination level. These findings demonstrate that selecting the -1 log CFU/g limit would decrease the accuracy of the model to quantify the transfer of *E. coli* O157:H7 to products and equipment surfaces during the simulated process. In the present study, the minimum level of -10 log CFU/g was used to simulate the process of cross-contamination of *E. coli* O157:H7 on romaine or iceberg lettuces and equipment surfaces in the processing line described in this study.

Figure 5.8: The effect of different minimum contamination limits on the NetLogo model FS-ABS model to calculate the number of contaminated bags based on (A) -2.0 log CFU/g and (B) -1.4 CFU/g contamination limits.

5.2.2 Effect of Sample Sizes (Scenario 1)

Figure 5.9 shows the average contamination (> 1 CFU/100 g) levels of *E. coli* O157:H7 on shredded romaine lettuces after 8-hour processing time. The size of the lettuce pieces did not affect ($p < 0.05$) the level of contamination of the shredded lettuces in the processing line (Buchholz et al., 2012a).

Figure 5.9: Average level of contamination in Romaine lettuces at each step in the processing line for different sample sizes after 8 hours processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

Figure 5.10-A shows that the mean number of contaminated bags per batch is around 900 for the 1 CFU/100 g safety limit. For the 1 CFU/25 g safety limit (Figure 5-10-B), the mean value varied between 20 and 40 for sizes 10 to 16, but it was higher for the lower sizes.
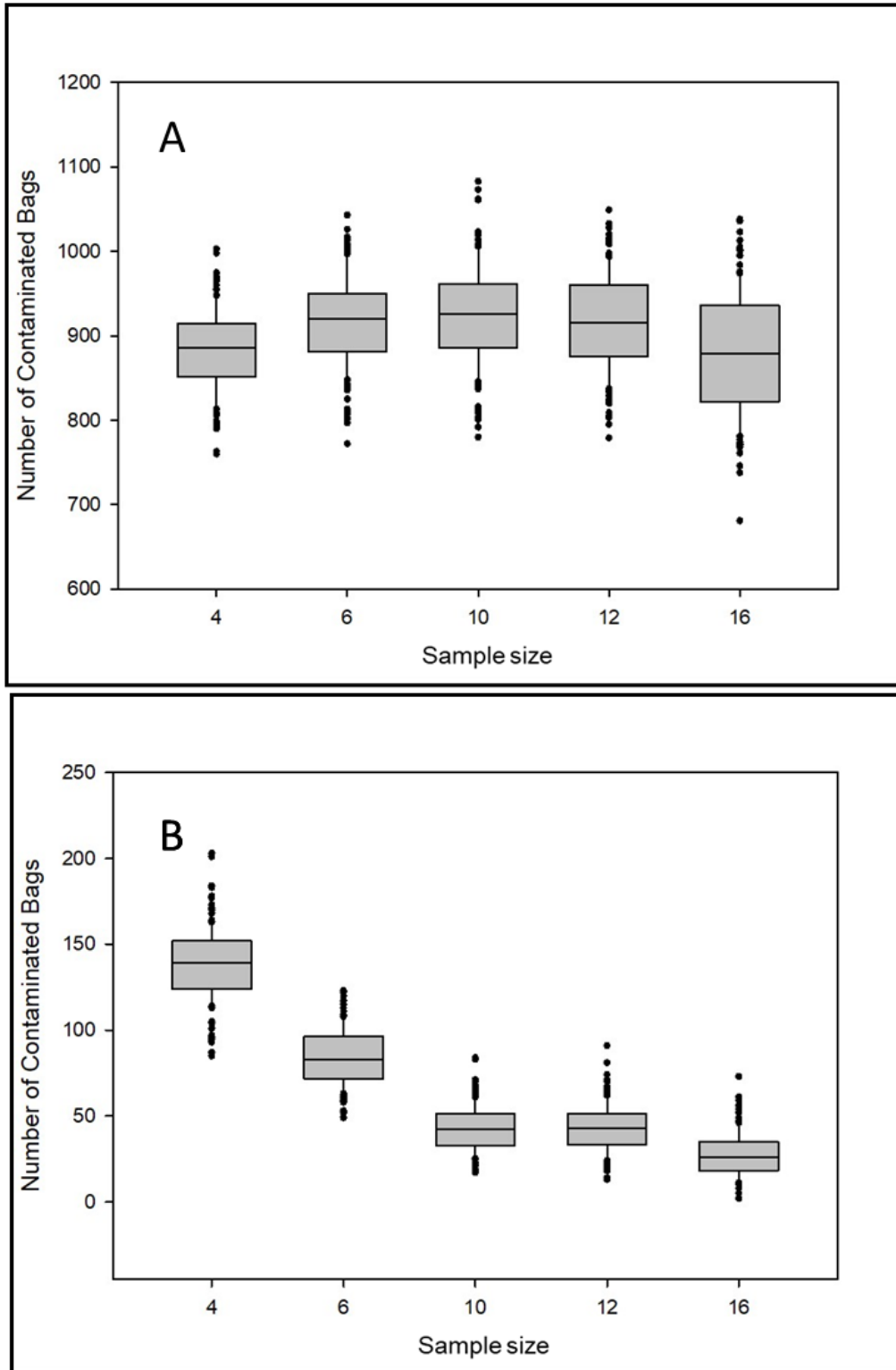
Figure 5.10: Whisker plot highlighting the relationship between sample size and the number of contaminated shredded lettuce bags per batch for 8-hour processing time (A) 1 CFU/100 g and (B) 1 CFU/25 g contamination limits.

5.2.3 Effect of Free Chlorine (FC) Concentrations in the Flume Tank (Scenario 2)

Figure 5.11 shows the effect of FC concentration in the wash water on the level of contamination in the flume tank (L5), shaker (L6), and centrifuge (L7). The FC concentrations affect ($p<0.05$) the level of contamination of shredded romaine lettuces at L5, L6, and L7. As expected, the level of contamination increases with decreased FC. The level of lettuce contamination increases by three times when no FC is used in the flume tank compared with the higher FC concentrations (21-10-6 mg/mL).
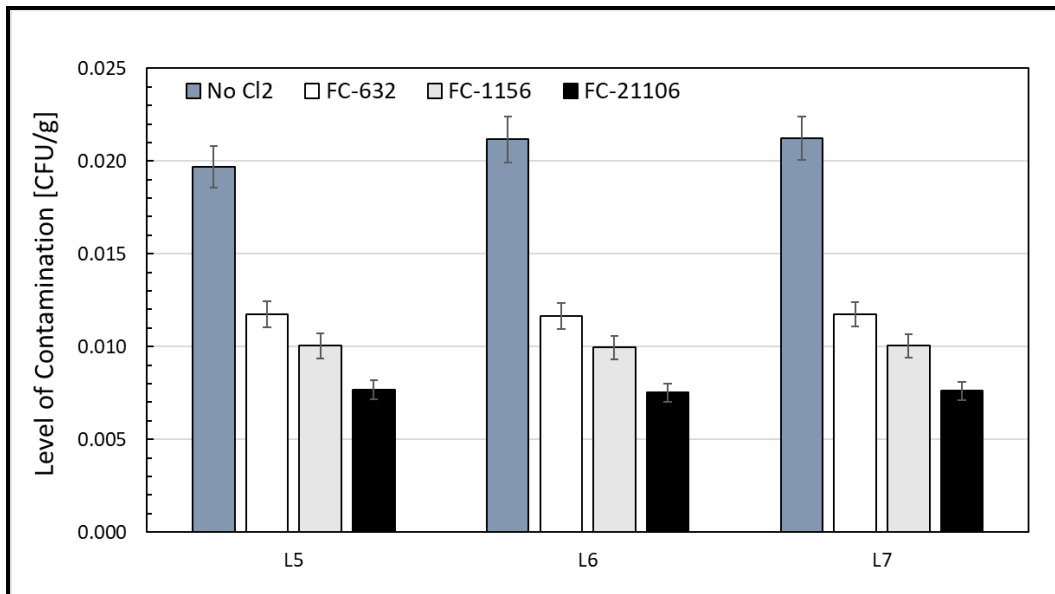
Figure 5.11: Shredded romaine lettuces level of contamination after being washed in the flume tank after 8-hour processing, where L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Error bars are standard deviation values.

Figure 5.12 shows the average contamination level for different FC concentrations on the average levels of *E. coli* O157:H7 on shredded romaine lettuces after 8-hour processing time (safety limit < 1 CFU/100 g). Adding FC to the wash water helps to reduce the number of pathogens in the produce surface after the chlorination process. The median level of

contamination was about -1.6 log CFU/g when no chlorine was used compared to -1.8 log

CFU/g for higher concetration treatment (21-10-6 mg/L). See the Appendix for the results

obtained for a safety limit < 1 CFU/25 g.

Figure 5.13 shows the whisker plot highlighting the relationship between the average

level of contamination at each step in the processing line for different FC concentrations after 8-

hours. The average level of contamination decreases with increased FC concentrations in L6 and

L7 after the flume tank step, but not significantly ($p>0.05$). However, as discussed before, the

mean level of contamination reduction is not sufficient to make the product safer for

consumption. Puerta-Gomez et al. (2013) discussed that other alternatives, like exposure to 1.0

kGy irradiation concentration after packaging could reduce the number of tainted leafy-greens

samples from 84% to 0.1%, for highly cross-contaminated lots (3 log CFU/g).
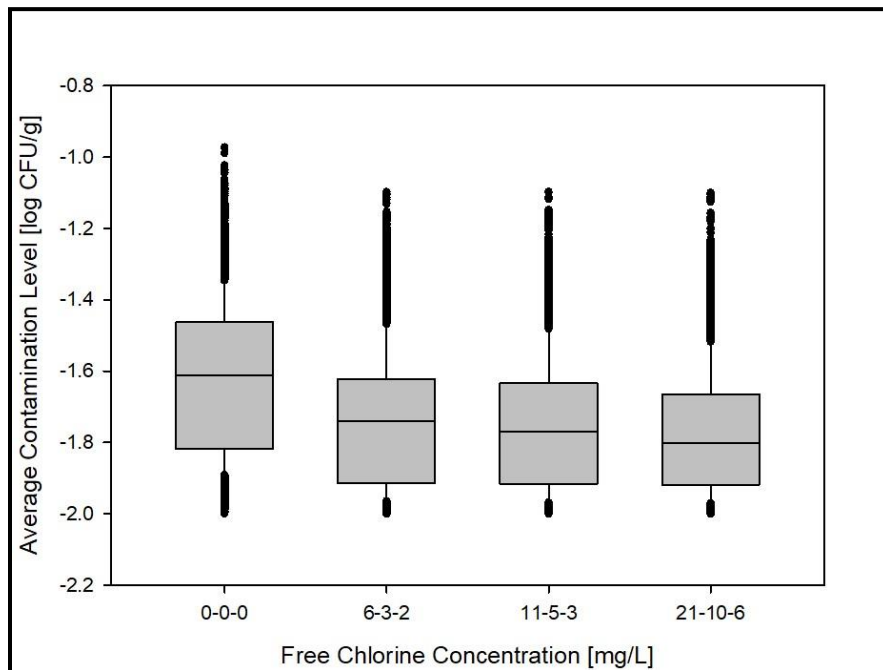
Figure 5.12: The effect of free chlorine concentration concentrations on the average levels of *E. coli* O157:H7 on shredded romaine lettuces for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

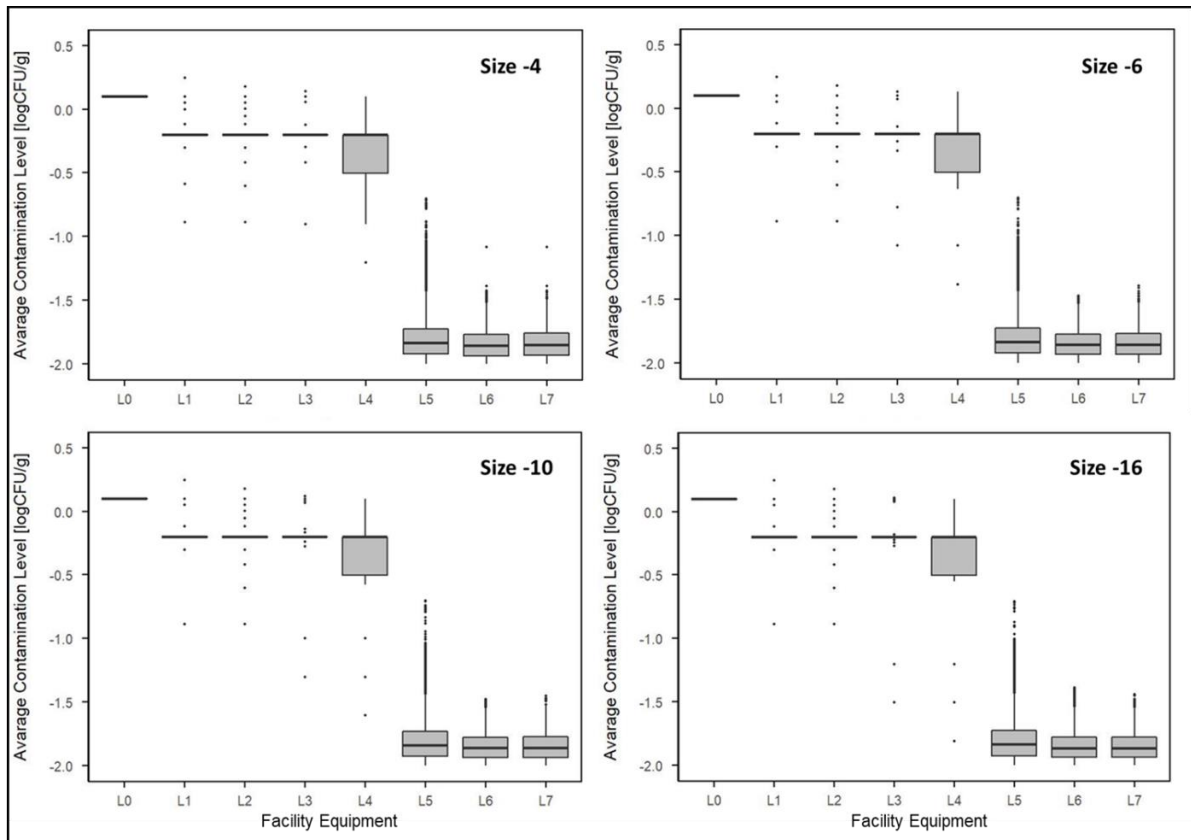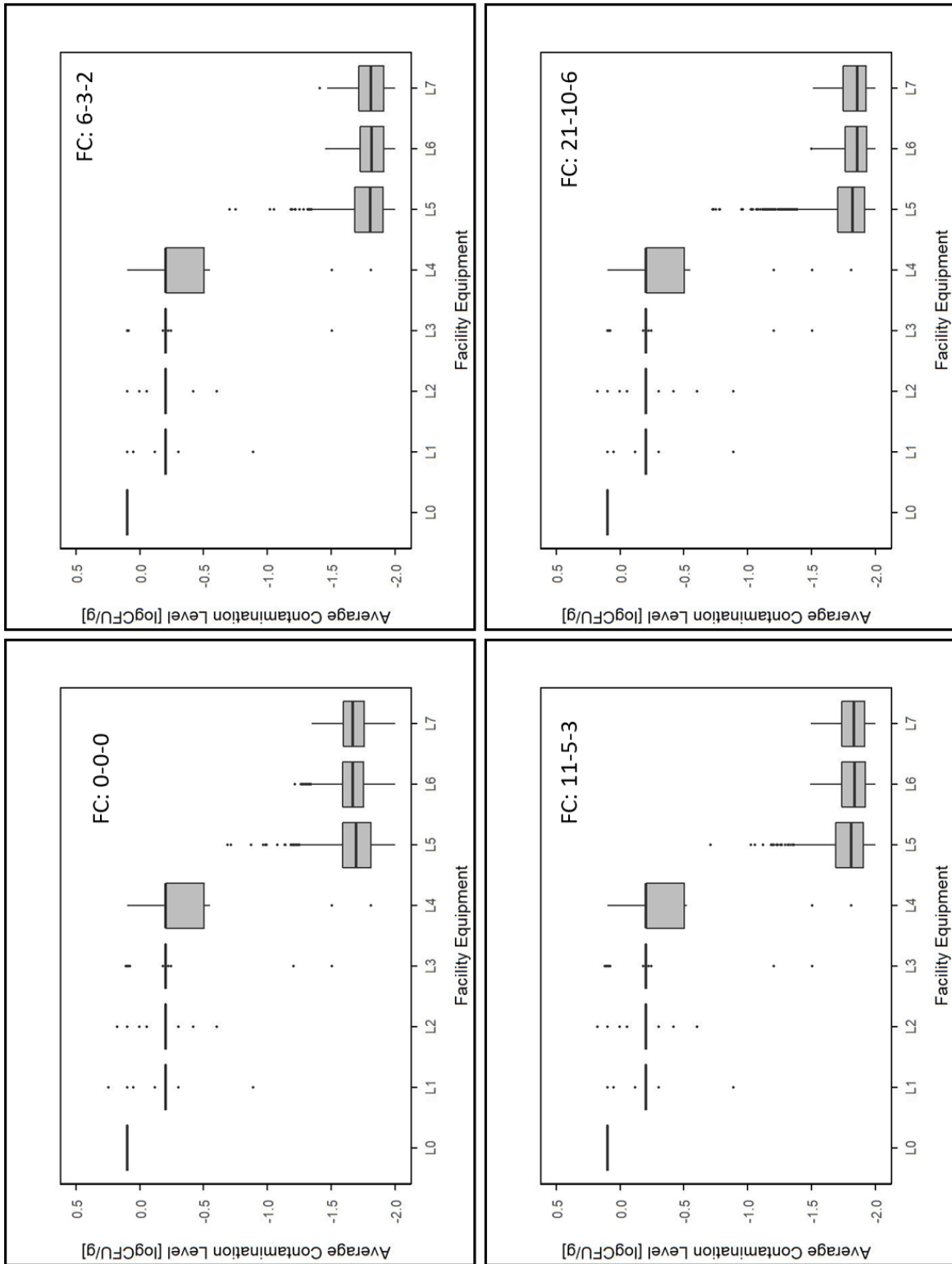**Figure 5.13:** Average level of contamination in Romaine lettuces at each step in the processing line for different FC concentration concentrations for 8-hour processing, where L0 =lettuce

source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

The level of FC significantly ($p<0.05$) affected the average number of contaminated bags. (Table 5.3). The percentage of contaminated bags above 1 CFU/100 g safety limit (-2 log CFU/g) were 79%, 47%, 39%, and 27% for lettuces treated with no FC, lower, medium, and higher levels of FC, respectively; and 10%, 3%, 2%, and 1%, respectively, for contaminated bags above 1 CFU/25 g safety limit (- 1.4 log CFU/g).

Table 5.3: Number of contaminated shredded romaine lettuces bags after 8-houra processing as a function of chlorination concentration concentrations.

FC concentration levels [mg/L]	< 0.01 CFU/g	< 0.04 CFU/g
	Average Number of Contaminated Bags $\pm$ std	
21-10-6	$_x11{,}431 \pm 943^a$	$_y364 \pm 169^a$
11-5-3	$_x16{,}536 \pm 1027^b$	$_y793 \pm 247^b$
6-3-2	$_x20{,}332 \pm 1066^c$	$_y1{,}131 \pm 286^c$
No FC	$_x33{,}215 \pm 962^d$	$_y4{,}147 \pm 702^d$

Different letters within the same $_{xy}$row and the same column[a,b,c,d] for each component indicate significant difference ($p < 0.05$) according to one-way ANOVA and t-test test ($\alpha = 0.05$).

The whisker plot in Figure 5.14 shows that the median number of contaminated bags (> 1 CFU/100g) per batch was approximately 2,500 when using fresh water with no FC added. The values decrease as FC was added to the flume tank, resulting in a 64% reduction (to approximately 900 bags) in contaminated bags/batch when higher FC concentrations (21-10-6 mg/L) are used during the processing. Even if the incoming lettuces were contaminated with 0.01 log of CFU/g, the probability of having contaminated bags can be high if low chlorination concentration concentrations are used.

When considering a higher minimum contamination level (1 CFU/25 g), the whisker plot (Figure 5.15) shows significant spread of E. coli O157:H7 into lettuce bags for the lettuces treated only with water. A larger reduction – between 74 and 90% -- in the number of bags per

batch occurred when higher addition concentrations of FC were used during the processing, yielding approximately 32 contaminated bags per batch.
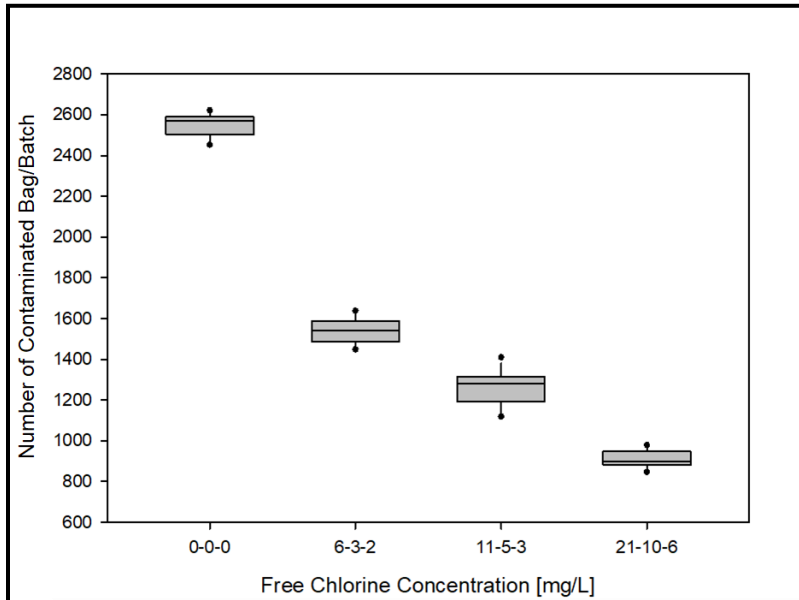
Figure 5.14: Whisker plot highlighting the relationship between the FC addition concentration rate and the number of contaminated shredded lettuce bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

The average log-reductions for the different levels of FC concentrations in the flume tank is presented in Figure 5.16. Increasing FC concentrations in the wash water increases significantly ($p < 0.05$) the log reduction of *Escherichia coli* O156: H7 in the lettuce pieces. Now, increasing the FC concentration by 63%t resulted in only 11% in log reduction. By doubling the amount of FC, the log reduction of the pathogen counts was still around 13%. These results clearly demonstrate that chlorine alone will not reduce the total microbial load in leafy greens, as observed by Puerta-Gomez et al. (2013). Lettuce has a large surface area that facilitates growth of microorganisms and increased potential for pathogen internalization in structures such as the

stomata. Once internalized, pathogens are difficult to remove using surface treatments such as

washing (Buchanan, 2006).
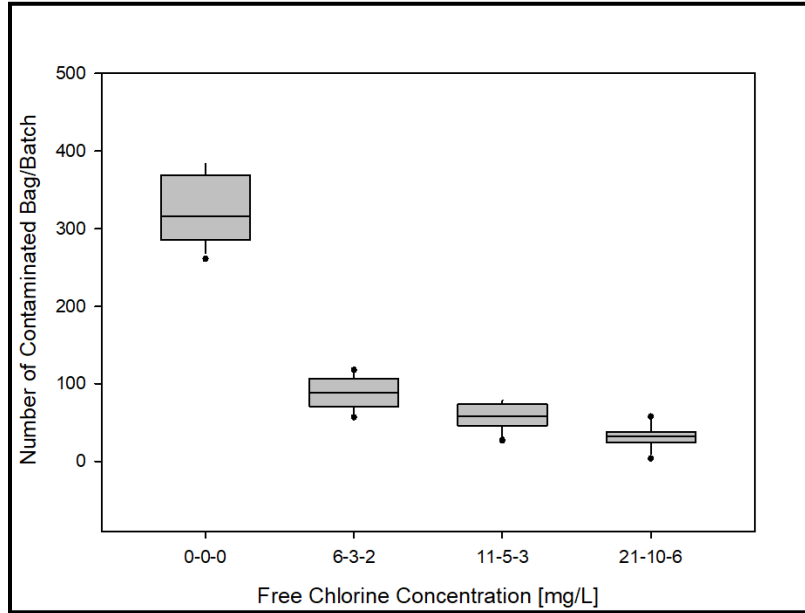
Figure 5.15: Whisker plot highlighting the relationship between the FC addition rate concentration and the number of contaminated shredded lettuce bags per batch for 8-hour processing time (contaminated bags above 1 CFU/25 g safety limit).
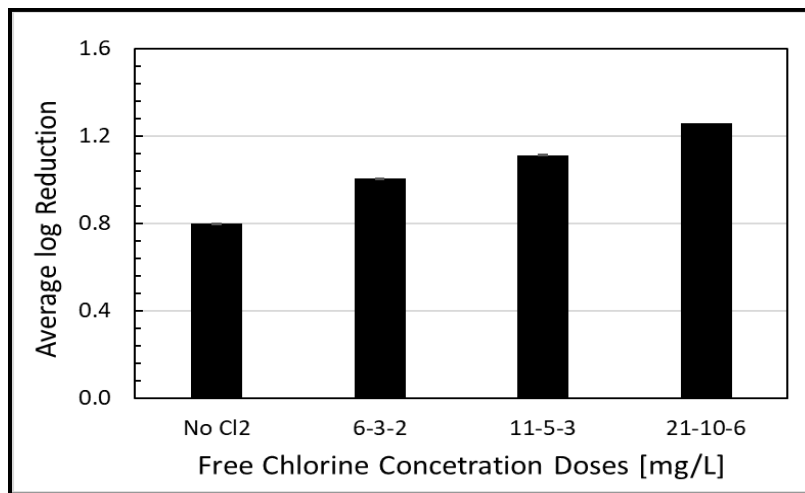
Figure 5.16: Average log reduction of E. coli O156 on Romaine lettuce pieces after washing in the flume tank. Error bars are standard deviation values.

5.2.4 Effect of Lettuce Head Initial Contamination Load (Scenario 3)

Figure 5.17 presents the effect of initial microbial load on Romaine lettuce heads on the average contamination level after 8-hour processing for a safety limit of 1 CFU/100 g. There is a significant effect difference ($p<0.05$) among treatments. The level of contamination in the shredded lettuces increase linearly with the initial microbial load on lettuce heads. Similar results were obtained with the 1 CFU/25 g limit (see Appendix).

Figure 5.17: Effect of initial E. coli O157:H7 contamination load on the heads of Romaine lettuce heads on the average level of contamination on shredded lettuces after 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

Figure 5.18 shows the effect of initial lettuce contamination load on the mean level of contamination (-2 to 4 log CFU/g) at each step in the processing line. The average level of contamination decreases significantly ($p<0.05$) after the chlorination process (L5) but increases

significantly ($p<0.05$) from L1 to L4 with increased initial contamination level on lettuce heads. Probability of cross-contamination increases in L1-L4 for initial contaminations above or equal to 2 log CFU/g (Figure 5.19).

Figure 5.18: Average level of contamination in Romaine lettuces at each step in the processing line for different initial level of contamination for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

Figure 5.19: Average level of contamination in Romaine lettuces at each step in the processing line for different level of initial contamination for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g

The average number of contaminated bags is not significantly (p>0.05) affected by initial

contamination loads on the lettuce heads (Table 5.4) in the range of 10^2 to 10^4 CFU/g. The

percentage of contaminated bags above 1 CFU/100 g is only 19% of the total processed for an

initial microbial population of 1 CFU/g compared to 78% and $82\pm1\%$ for an initial load of 10

and 10^2 to 10^4 CFU/g, respectively. For the higher tolerance level of contamination (1 CFU/25 g

safety limit), these percentages are 2%, 52%, and $81\pm1\%$, respectively.

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Table 5.4: Average number of contaminated shredded romaine lettuce bags after 8 hours processing
as function of initial microbial load on the lettuce heads.

Initial Population [CFU/g]	< 0.01 CFU/g	< 0.04 CFU/g
	Average Number of Contaminated Bags $\pm$ std	
1	$8,126 \pm 871^a$	$1,001 \pm 532^a$
10	$32,952 \pm 562^b$	$21,837 \pm 794^b$
10^2	$34,090 \pm 603^c$	$33,600 \pm 534^c$
10^3	$34,357 \pm 872^c$	$33,951 \pm 921^c$
10^4	$34,754 \pm 902^c$	$34,801 \pm 797^c$

Different letters within the same column[a,b,c,d] for each component indicate significant difference (p < 0.05) according
to one-way ANOVA and t-test test (α = 0.05).

The whisker plot in Figure 5.20 shows the number of contaminated bags (above 1

CFU/100g) per batch. The number of contaminated bags increases with the initial contamination

level reaching a median value of ~ 2,600 bags/batch for initial contamination load higher than 1

log CFU/g. See the Appendix for the 1 CFU/25 g safety limit.

The average log-reduction was 1.26 ± 0.00 and it was not affected (p>0.05) by the initial

contamination load. These results confirm that FC alone is ineffective in reducing the microbial

load in leafy greens, being only capable of 1 to 2 log CFU/g reductions at the most (Barrera et

al., 2012).
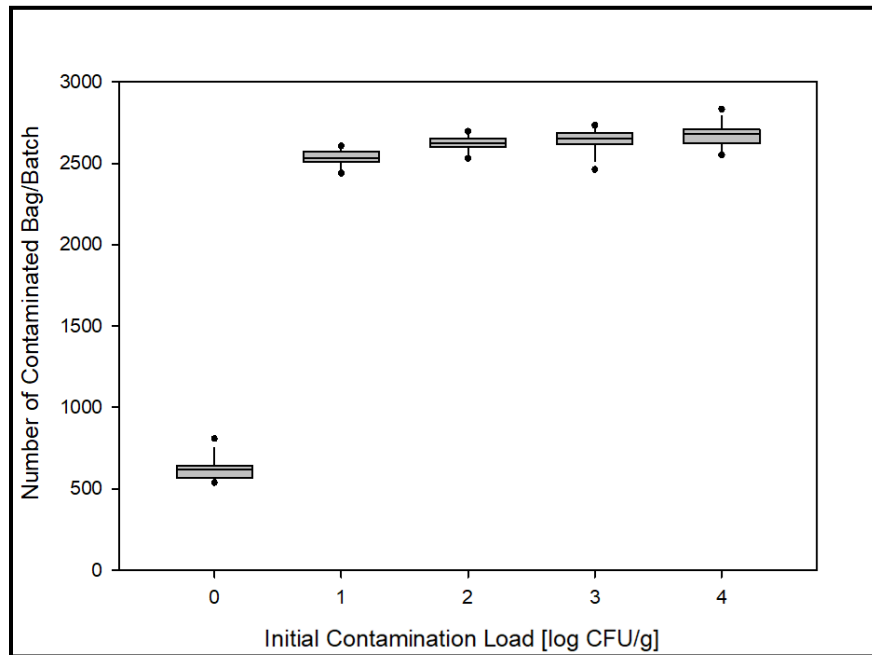
Figure 5.20: Effect of initial E. coli O157:H7 contamination load on the heads of Romaine lettuce heads on the number of contaminated shredded lettuce bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

These results show that the effect of initial level of contamination on incoming lettuce heads affect the number of contaminated bags, however the level of FC (Table 5.3) has the most significant effect ($p < 0.05$) on the number of contaminated bags.

5.2.5 Effect of Initial Contamination Probability (Scenario 4)

Figure 5.21 presents the effect of initial contamination probability on Romaine lettuce heads on the average contamination level after 8-hour processing for a safety limit of 1 CFU/100 g. There is a significant effect ($p < 0.05$) among treatments. The level of contamination in the shredded lettuces increases with increased contamination probability.
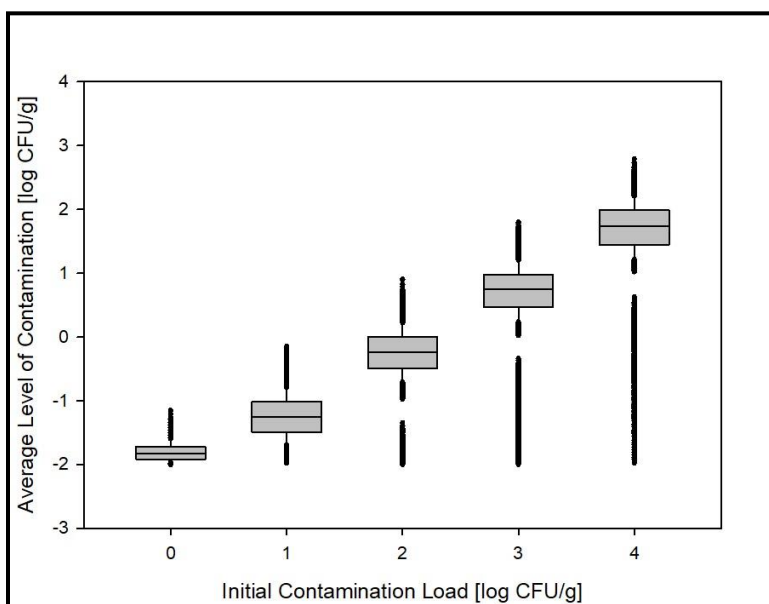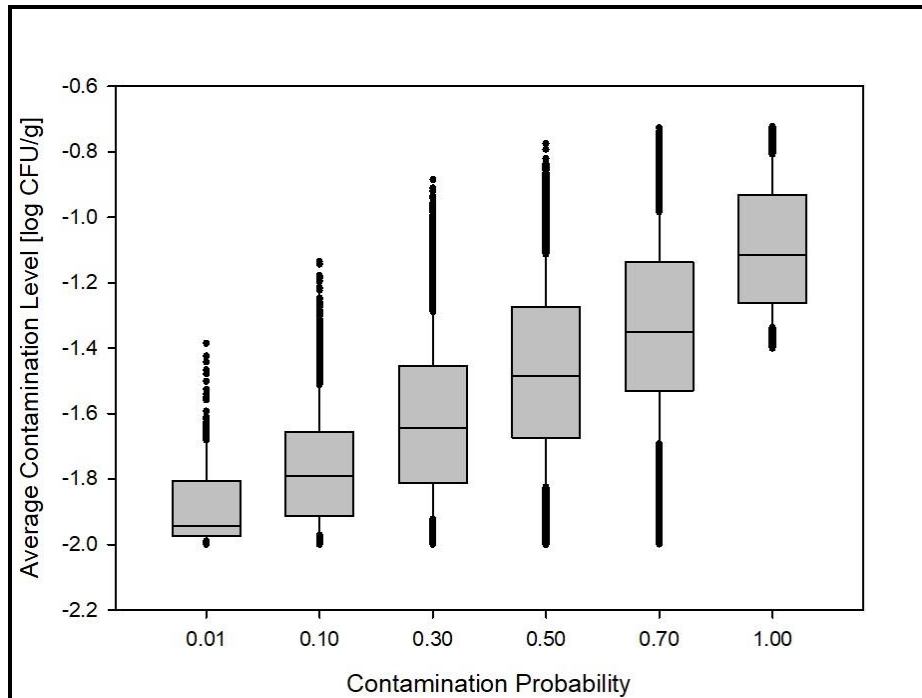
Figure 5.21: Effect of initial E. coli O157:H7 contamination probability on the heads of Romaine lettuce heads on the average level of contamination on shredded lettuces after 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

Figures 5.22 and 5.23 show the relationship between initial contamination probability on lettuce heads (from 0.01 to 0.3 and from 0.5 to 1.0, respectively) on the average level of contamination on shredded lettuces after 8-hour processing time. As the probability increases the mean level of contamination also increases and affects the contamination distribution on the different equipments in the processing line. The sanitizer helps reduce the level of contamination to a certain point (L6-L7) as discussed before and cross-contamination on L6 and L7 have similar distributions. The higher the probability of lettuce heads being contaminated the higher the level of cross-contamination in L1-L4 thus increasing the number of contaminated lettuces by the end of the process. Therefore, these pieces of equipment should be sanitized frequently to eliminate the probability of cross-contamination of uncontaminated lettuces.
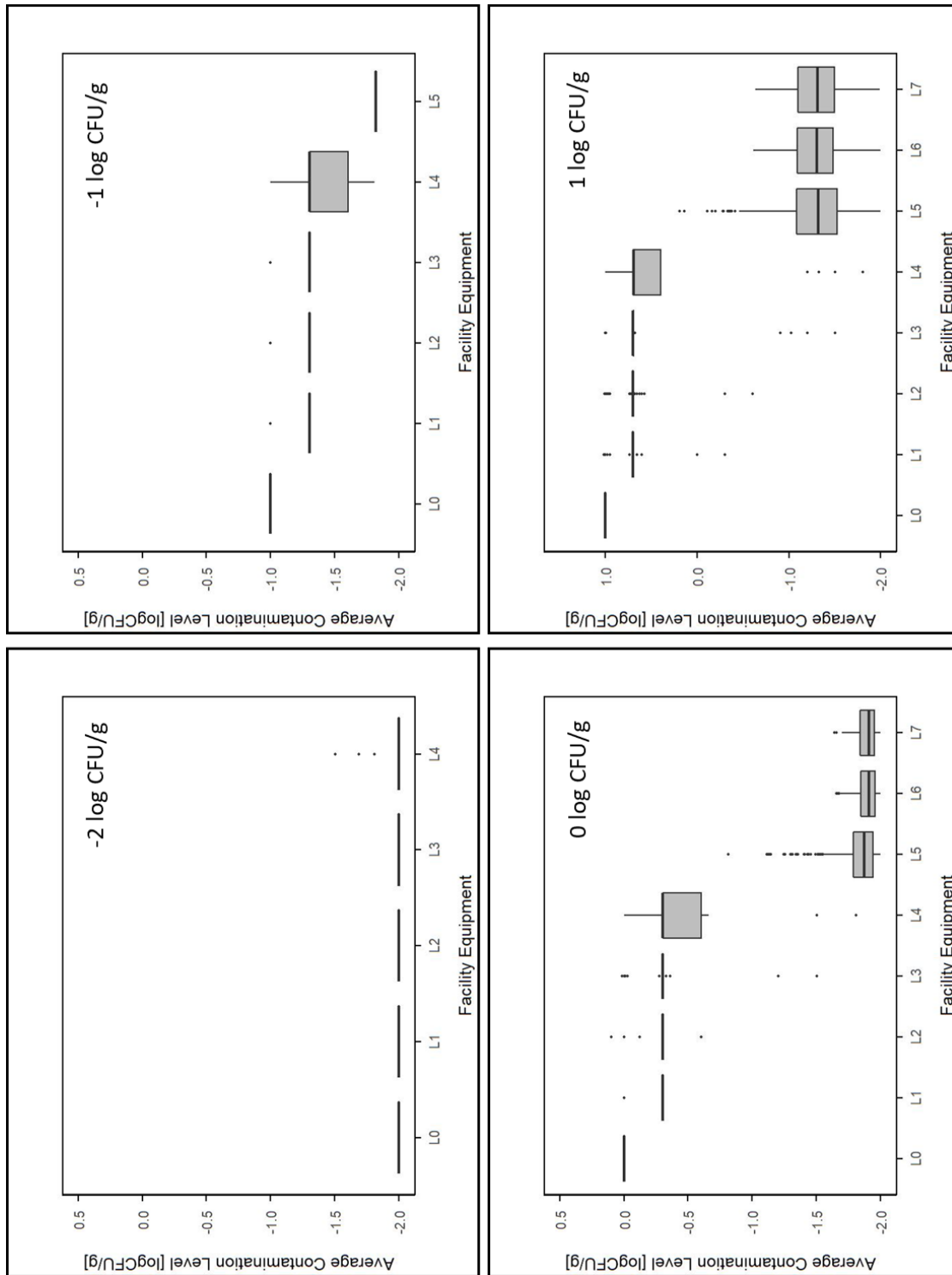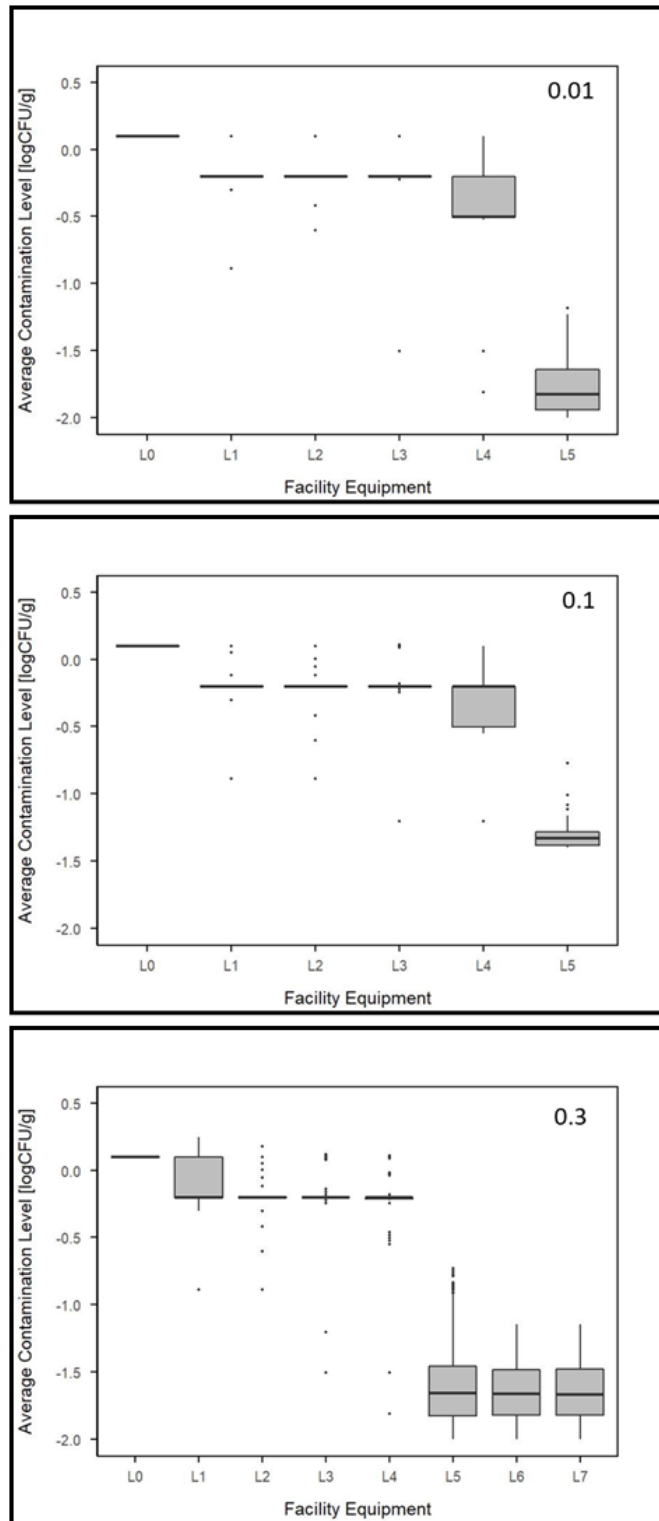
Figure 5.22: Average level of contamination in Romaine lettuces at each step in the processing line for different level of probability of contamination for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.
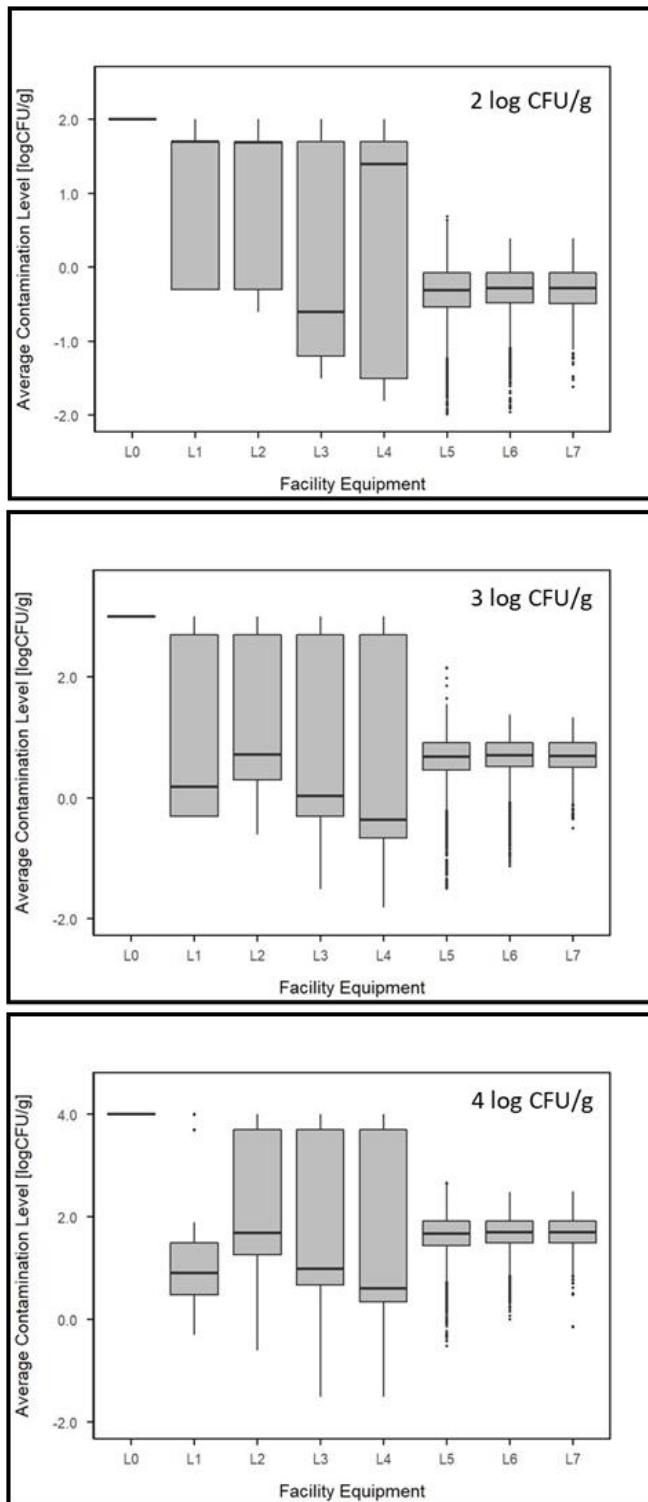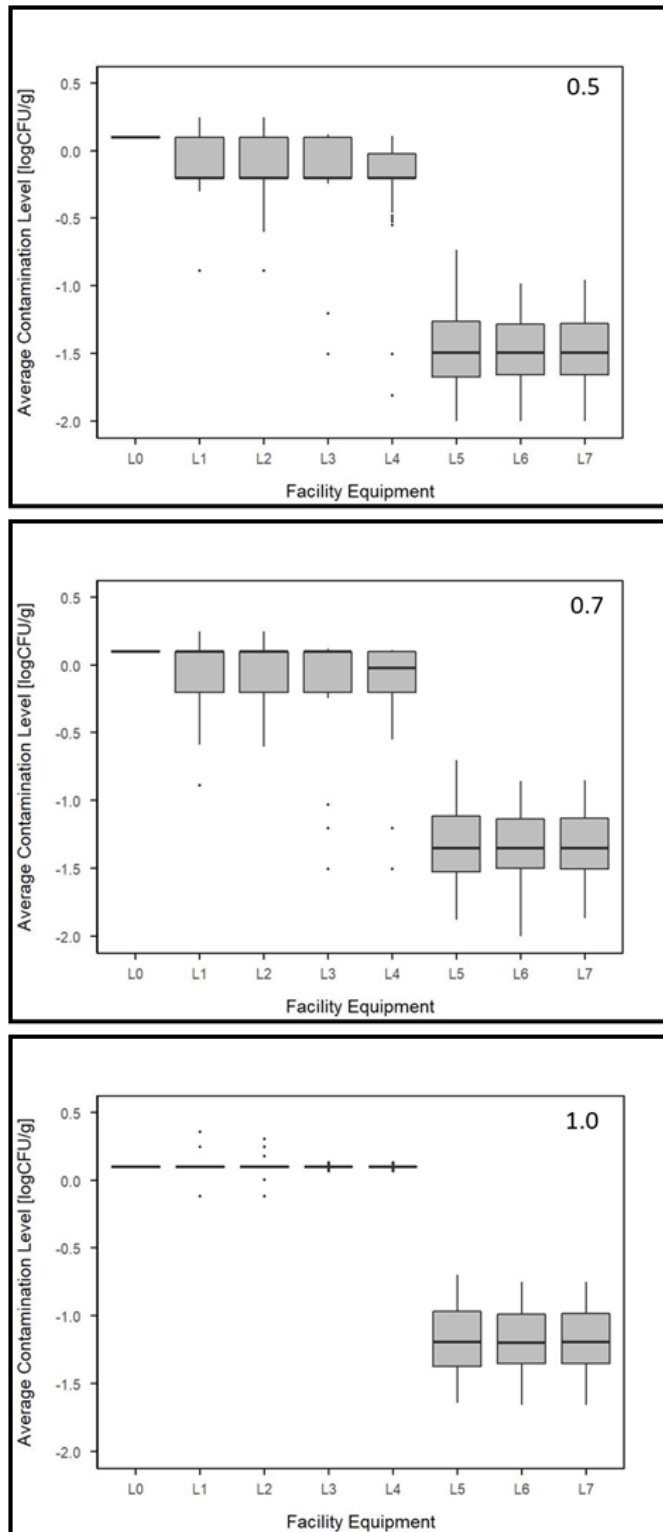
Figure 5.23: Average level of contamination in Romaine lettuces at each step in the processing line for different probability of contamination for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

Table 5.5 indicates that the average number of contaminated bags is affected (p<0.05) by the initial probability of contamination of lettuce heads. The percentage of contaminated bags above 1 CFU/100 g is only around 2% of the total processed bags for a probability that only 1% of the total lettuce heads are contaminated compared to 29% and 77% when the probability increases to 10% and 30%, respectively. At 50% probability and above, most of the bags are contaminated. For the higher tolerance level of contamination (1 CFU/25 g safety limit), more bags would be safe and therefore the risk of infections would increase.

Table 5.5: Average number of contaminated shredded Romaine lettuce bags after 8 hours processing as function of probability of contamination of lettuce heads.

	< 0.01 CFU/g	< 0.04 CFU/g
Probability of contamination	**Average Number of Contaminated Bags $\pm$ std**	
0.01	$_x710 \pm 324^a$	$_y126 \pm 0^a$
0.1	$_x12,052 \pm 1,014^b$	$_y407 \pm 145^b$
0.3	$_x32,500 \pm 637^c$	$_y6,437 \pm 631^c$
0.5	$_x40,456 \pm 299^d$	$_y15,893 \pm 578^d$
0.7	$_x42,029 \pm 52^e$	$_y23,852 \pm 534^e$
1.0	$_x42,120 \pm 0^f$	$_y33,416 \pm 110^f$

Different letters within the same column[a,b,c,d] for each component indicate significant difference (p < 0.05) according to one-way ANOVA and t-test test (α = 0.05).

Figure 5.24 shows that the number of contaminated bags per batch increases with increased probability of contamination in lettuce heads before processing (1 CFU/100 g limit). Only 2% or 29% of the bags were contaminated when the probability of contamination is 1% or 10 %, respectively. When 50% of the total lettuce heads was contaminated, the number of contaminated bags increased (p<0.05) to 96%. For the 1 CFU/25 g limit, the relationship between the number of contaminated bags per batch and the probability of contamination was more linear (see Appendix).

As expected, the average log-reduction was only 1.26±0.00 and it was not affected (p>0.05) by the probability of contamination. Even for a small initial *E. coli* O157:H7 contamination level of 1.3 CFU/g in the incoming lettuce heads, the probability of contamination impacted (p<0.05) the number of contaminated bags. Cross-contamination in the system and the limitations of the chlorination process all contributed to these results. Thus, it is not recommendable to have more than 30%contamination of the incoming lettuces as it would result in 50% of all processed bags being contaminated with the pathogen.
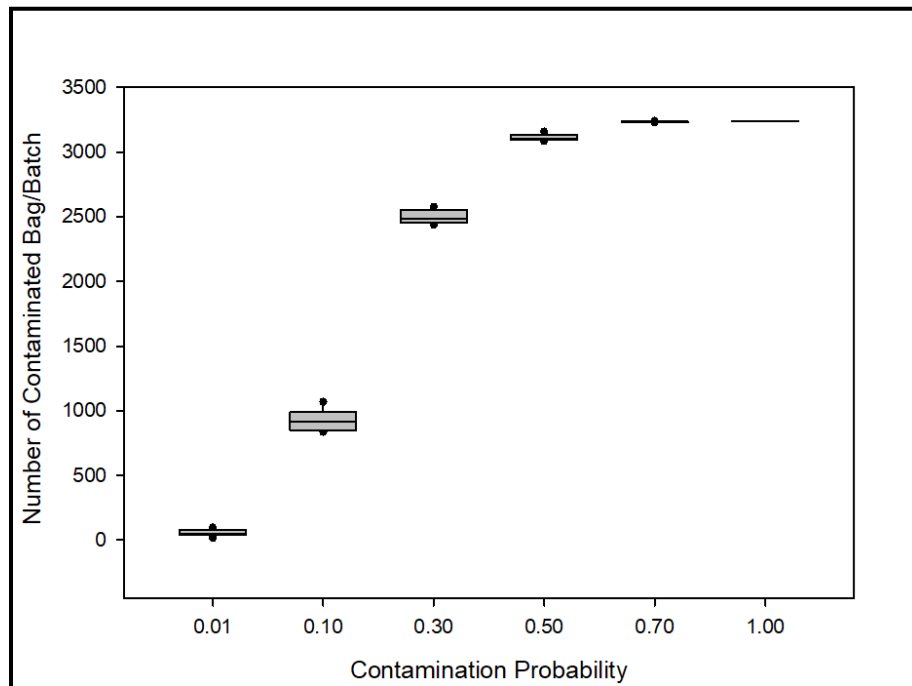
Figure 5.24: Effect of probability of E. coli O157:H7 contamination on the heads of Romaine lettuce heads on the number of contaminated shredded lettuces bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit).

These results show that the number of contaminated bags is affected by the probability of contamination, however the level of chlorination (Table 5.3) affects significantly (p<0.05) more the number of contaminated bags process.

5.2.6 Effect of Contamination Level on the Equipment L1-L4 (Scenario 5)

Figure 5.25 presents the effect of contamination level on the equipment (worker, trim table, shredder, conveyor) on lettuce average contamination level after 8-hour processing for a safety limit of 1 CFU/100 g. The level of *E. coli* O157:H7 contamination in the shredded lettuces increases with increased contamination level on L1 to L4. These results were higher ($p<0.05$) for the contamination levels above or equal to 3 log CFU/g. Adding FC in the flume tank (21-10-6 mg/L) helps to reduce the level of contamination (Figure 5.25-B) compared to the shredded lettuces that are only washed in sanitizer-free water (Figure 5.25-A). Figure 5.26 shows that the mean level of contamination on the lettuces increases with increased level of contamination in equipment L6 and L7, regardless of FC being used or not in the flume tank (L5).

Figures 5.27 and 5.28 show that for washing without FC (non-chlorination case), the level of contamination on the lettuces increases more ($p<0.05$) for contamination levels of 3-4 log CFU/g at L1 to L4. Chlorination helps reduce the level of contamination in the lettuces at L6 and L7 for the range of input used in this study.

Figures 5.29 and 5.30 illustrate that the level of contamination on lettuces increases with increased level of contamination at L6 to L7 for the non-chlorination cases. The overall range of contamination level at L6 and L7 gets more dispersed when the contamination level of 4 log CFU/g increases at the L6 to L7 equipment. Chlorination helps reduce the level of contamination in the lettuces at L6 and L7, but the overall range of contaminated level data increases at L6 and L7 as the contamination level in L6 to L7 increases from 2 to 4 log CFU/g. These results imply that a high contamination level in the shaker or centrifuge is problematic because it can cause large variation in the contamination level at that equipment and consequently increase the number of contaminated bags.
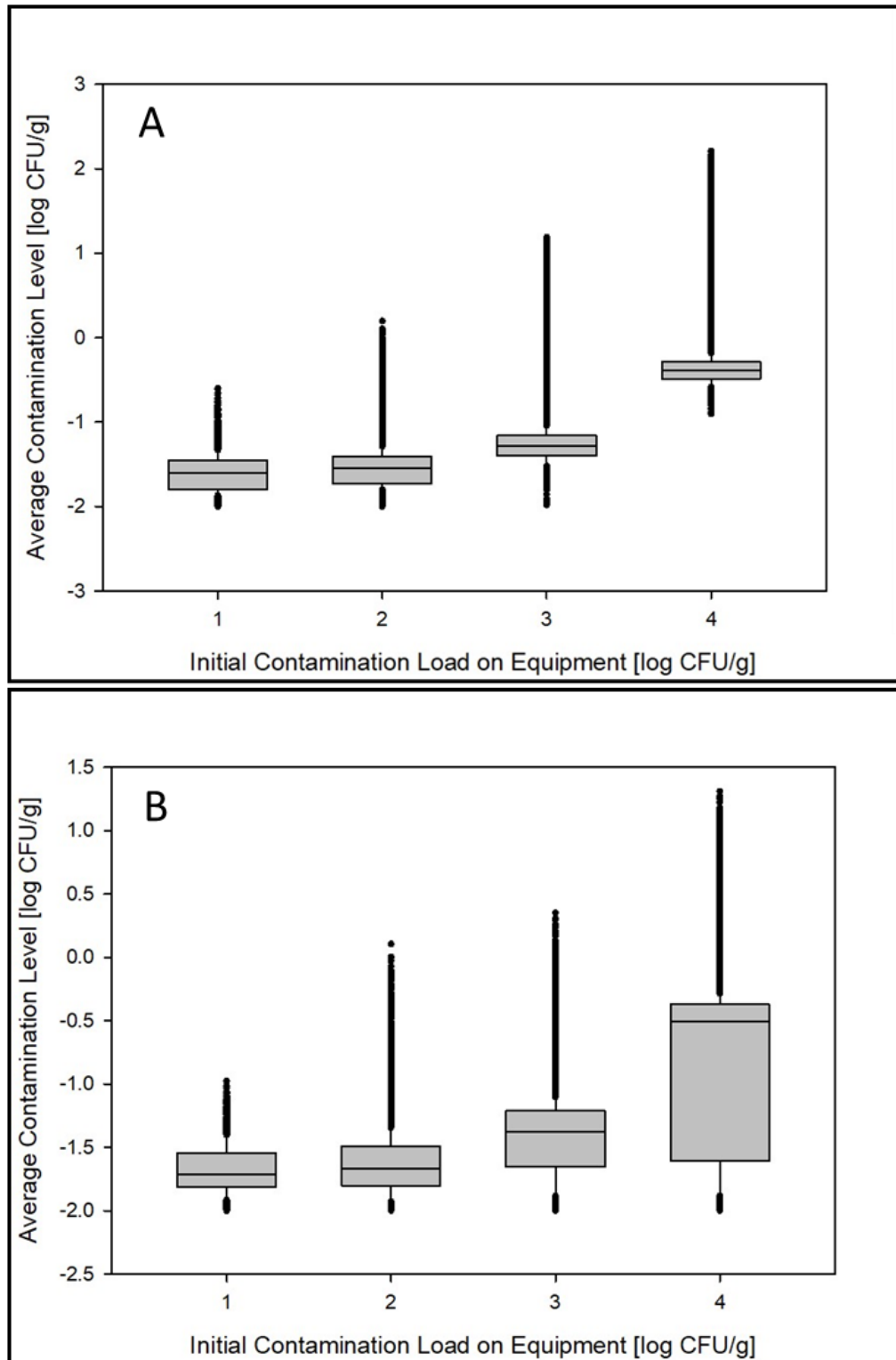
Figure 5.25: Effect of *E. coli* O157:H7 contamination level on equipment L1-L4 on the average level of contamination on shredded lettuces after 8-hour processing time. (A) no chlorination and (B) with chlorination treatment. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor.
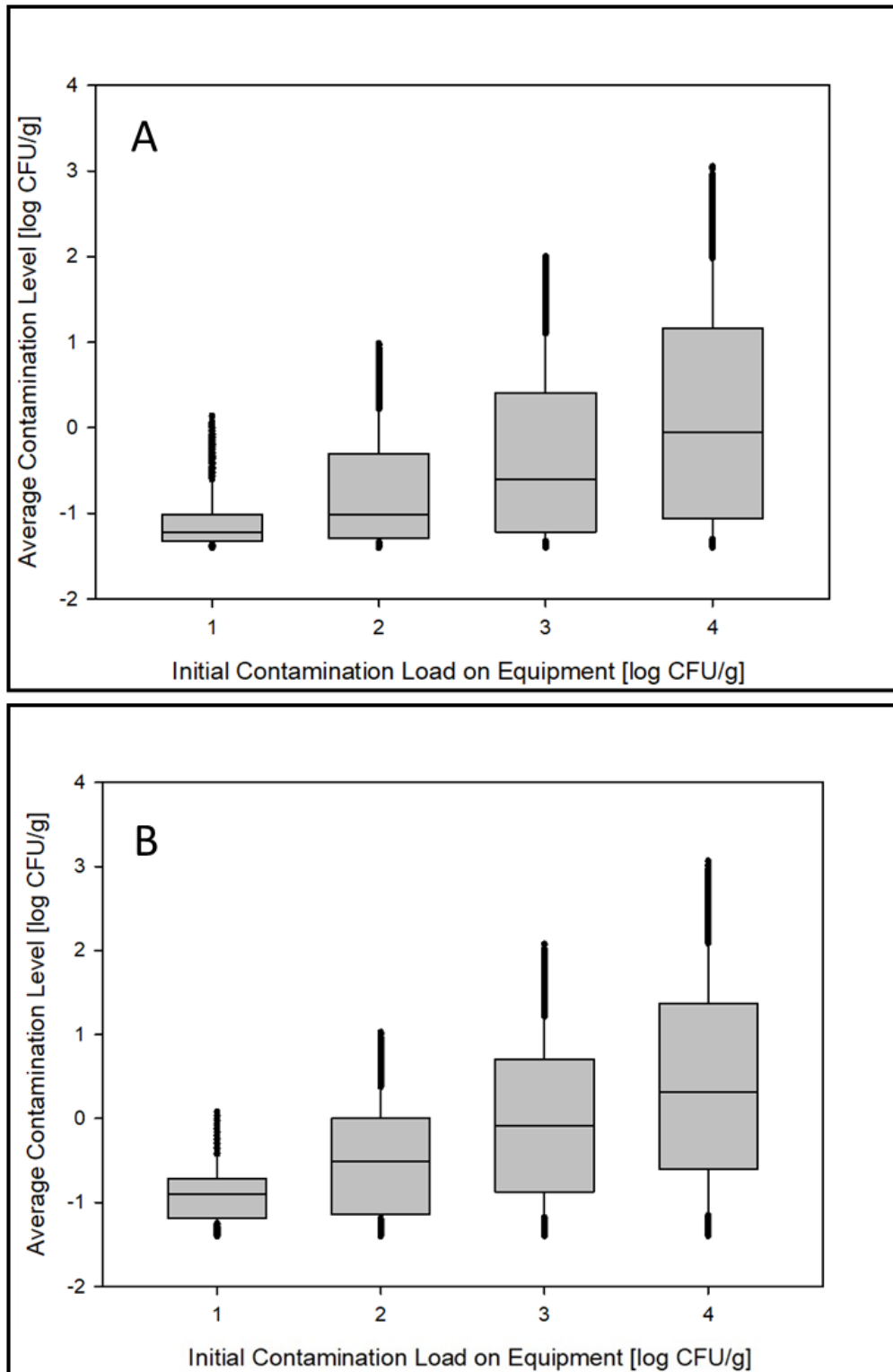
Figure 5.26: Effect of *E. coli* O157:H7 contamination level on equipment L6-L7 on the average level of contamination on shredded lettuces after 8-hour processing time. (A) no chlorination and (B) with chlorination treatment. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor.
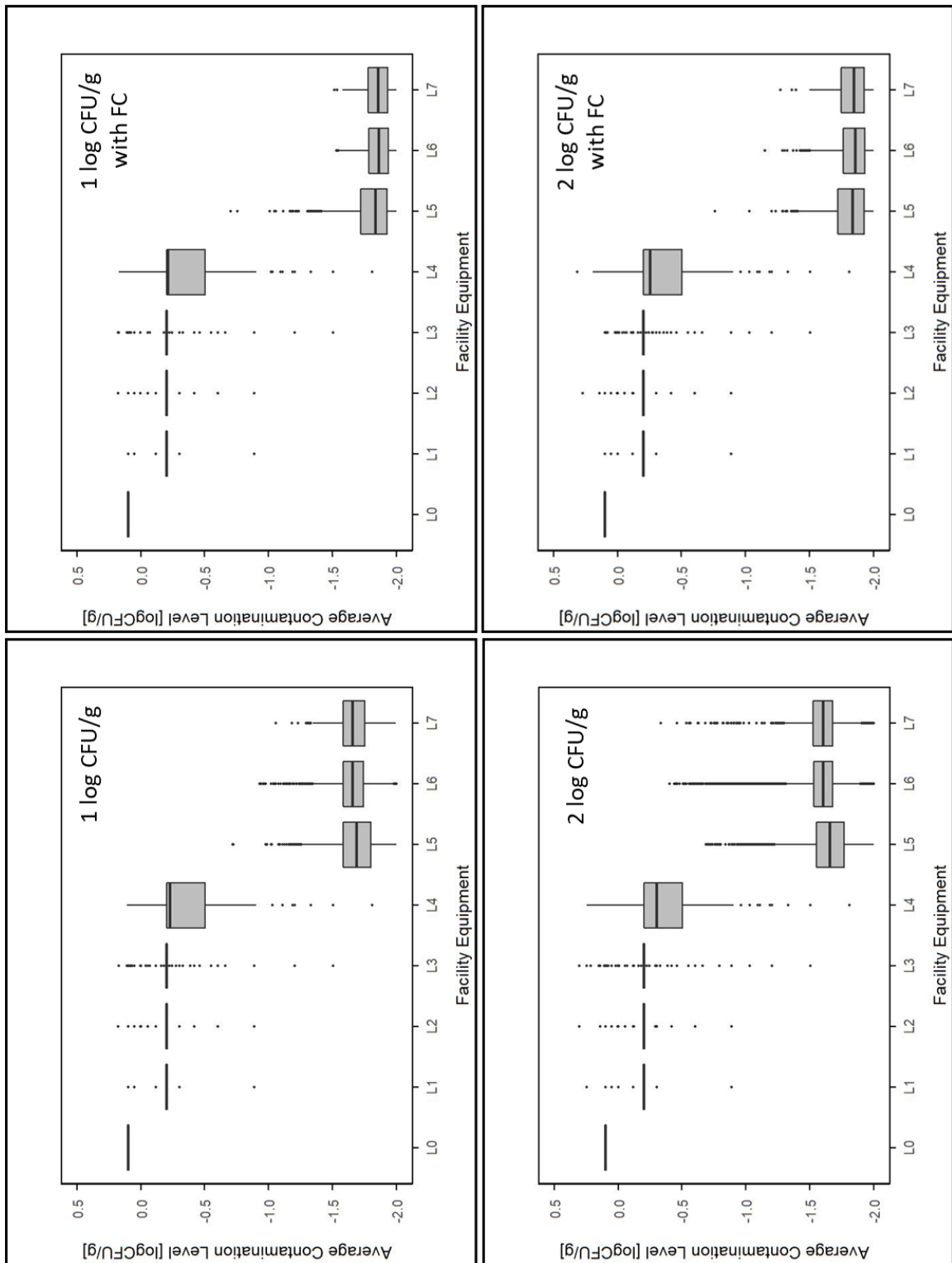
Figure 5.27: Effect of E. coli O157:H7 contamination level (1-2 log CFU/g) on equipment L1-L4 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.
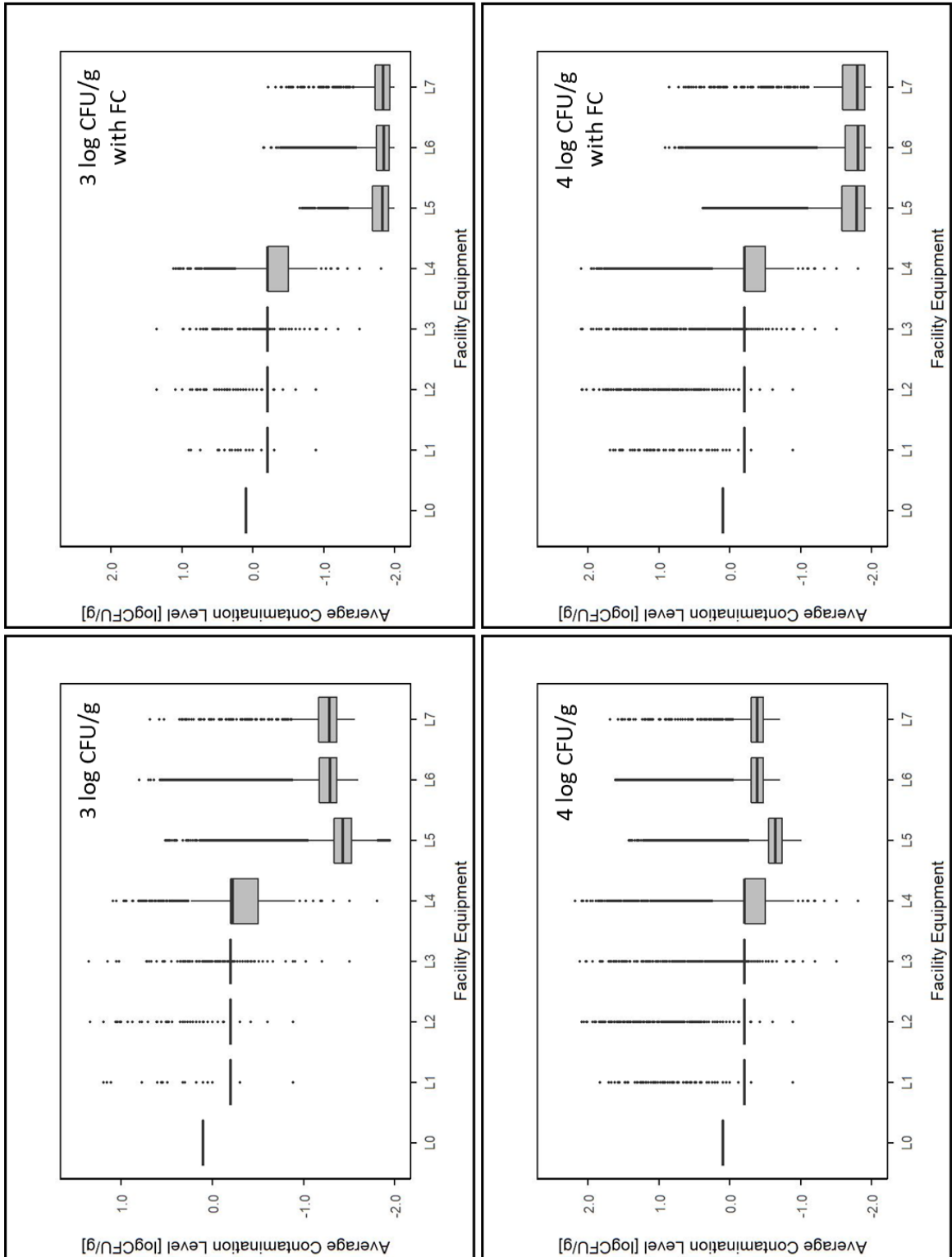
Figure 5.28: Effect of *E. coli* O157:H7 contamination level (2-3 log CFU/g) on equipment L1-L4 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.
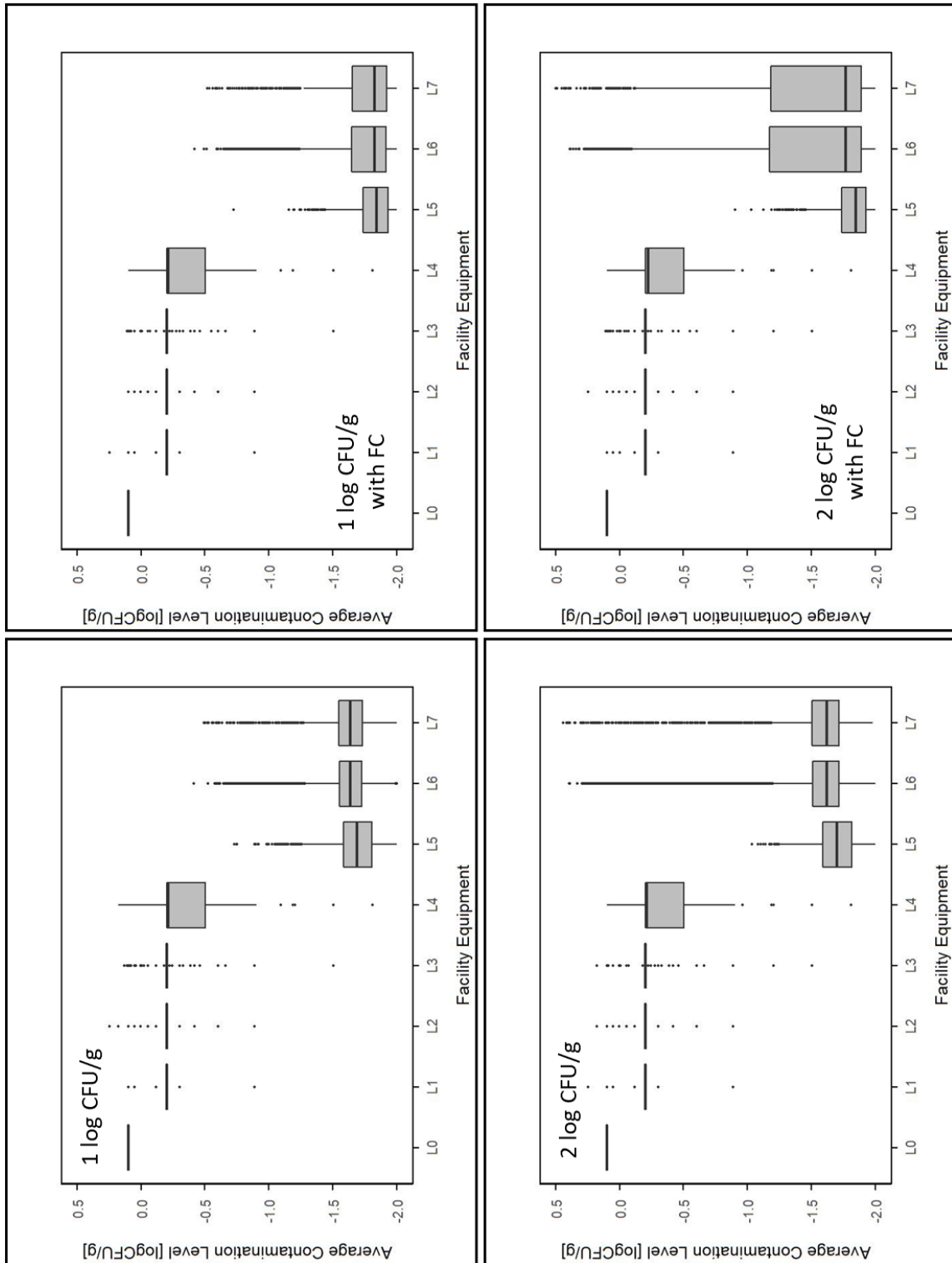
Figure 5.29: Effect of *E. coli* O157:H7 contamination level (1-2 log CFU/g) on equipment L6-L7 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.
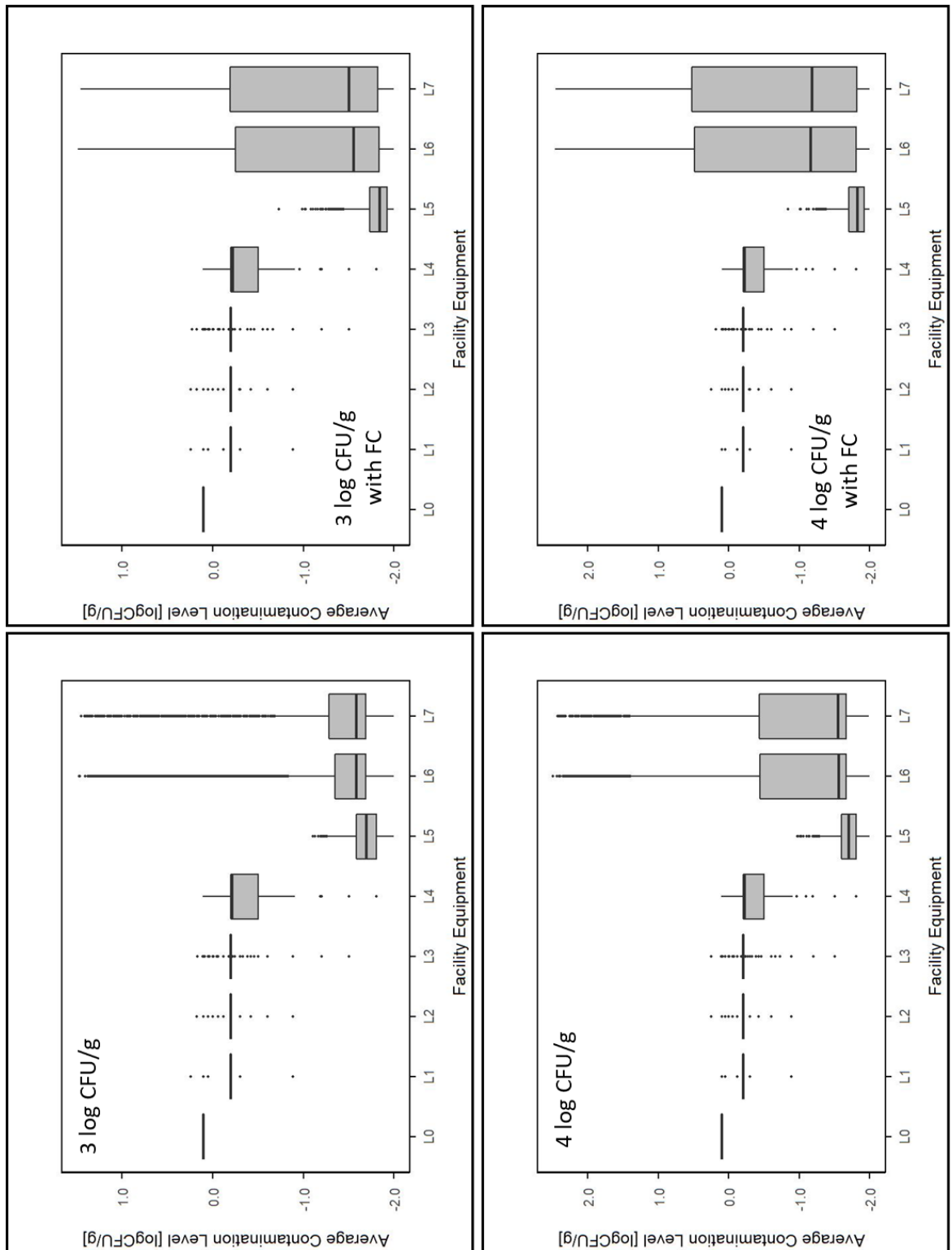
Figure 5.30: Effect of *E. coli* O157:H7 contamination level (3-4 log CFU/g) on equipment L6-L7 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

Table 5.6 shows that the average number of contaminated bags is affected (p<0.05) by the *E. coli* level of contamination on the equipment at steps L1 to L4. When the lettuces are washed with no FC, the number of contaminated bags can range from 80 to 100% (1 CFU/100 g safety limit) or from 12 to 100% (1 CFU/25 g safety limit) depending on the level of contamination. By adding FC to the wash water, the number of contaminated bags can be reduced by 65% (1 CFU/100 g safety limit) or by 92% (1 CFU/25 g safety limit).

Table 5.6: Average number of contaminated shredded Romaine lettuces bags after 8 hours processing as a function of contamination levels in equipment L1-L4.

Contamination level [log CFU/g]	< 0.01 CFU/g	< 0.04 CFU/g
	Average Number of Contaminated Bags $\pm$ std	
No Free Chlorine		
1	$_x33,436 \pm 676^a$	$_y4,826 \pm 676^a$
2	$_x34,359 \pm 1,313^b$	$_y8,021 \pm 1,339^b$
3	$_x42,120 \pm 0^c$	$_y31,499 \pm 8,333^c$
4	$_x42,120 \pm 0^c$	$_x42,120 \pm 0^d$
With Free Chlorine		
1	$_x11,648 \pm 1118^a$	$_y896 \pm 86^a$
2	$_x13,104 \pm 793^b$	$_y1,008 \pm 61^b$
3	$_x14,339 \pm 1,131^c$	$_y1,103 \pm 87^c$
4	$_x15,964 \pm 767^d$	$_y1,228 \pm 59^d$

Different letters within the same $_{xy}$row and same column[a,b,c,d] for each component indicate significant difference (p < 0.05) according to one-way ANOVA and t-test test ($\alpha = 0.05$). L1 = worker, L2 = trim table, L3 = shredder, L4 = step conveyor.

The number of contaminated bags per batch increases (p<0.05) with increased contamination level in L1-L4 when no FC is used (Figure 5.31-A). One hundred percent of the bags are above 1 CFU/100 g safety limit when the contamination levels are between 3 and 4 log CFU/g. The mean number of contaminated bags per batch increases linearly with the level of contamination in L1-L4 when FC is used (Figure 5.31-B). The number of contaminated bags can be reduced if more efficient intervention treatments are introduced after the flume tank, before the product is stored, to inactivate the remaining pathogens.

Figure 5.31: Effect of *E. coli* O157:H7 contamination level in equipment L1-L4 on the number of contaminated bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit). (A) no chlorination and (B) with chlorination treatments. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor.

Table 5.7 shows that without FC added about $82\pm2\%$ of the bags are contaminated
(above 1 CFU/100g) when L6 and L7 are contaminated with 1 to 4 log CFU/g. When FC was
used, the number of contaminated bags was affected ($p<0.05$) by the level of contamination in
L6 and L7. The number of contaminated bags (above 1 CFU/25 g) increased ($p<0.05$) from 16%
to 36% with increasing contamination levels when no FC was used. Processing with free
chlorine reduces the number of contaminated bags by a 7% to 30% range.

Table 5.7: Average number of contaminated shredded Romaine lettuce bags after 8 hours processing
as function of contamination levels in equipment L6-L7.

	< 0.01 CFU/g	< 0.04 CFU/g
Contamination level [log CFU/g]	**Average Number of Contaminated Bags $\pm$ std**	
No Free Chlorine		
1	$_x33,833 \pm 850^a$	$_y6,795 \pm 735^a$
2	$_x34,396 \pm 818^a$	$_y9,232 \pm 769^b$
3	$_x35,214\pm 798^a$	$_y12,200 \pm 768^c$
4	$_x36,062 \pm 715^b$	$_x15,245 \pm 0^d$
With Free Chlorine		
1	$_x14,128 \pm 618^a$	$_y3,052 \pm 196^a$
2	$_x16,873\pm 873^b$	$_y5,910 \pm 198^b$
3	$_x19,384 \pm 1,470^c$	$_y9,080 \pm 442^c$
4	$_x22,663 \pm 981^d$	$_y12,691 \pm 458^d$

Different letters within the same $_{xy}$row and same column[a,b,c,d] for each component indicate significant difference ($p < 0.05$) according to one-way ANOVA and t-test test ($\alpha = 0.05$). L1 = worker, L2 = trim table, L3 = shredder, L4 = step conveyor.

The number of bags per batch contaminated increases linearly with increased
contamination level in equipment L6-L7 with or without chlorination treatment (Figure 5.32-A
or 5.32-B). Free chlorine treatment reduces ($p<0.05$) the number of contaminated bags (above 1
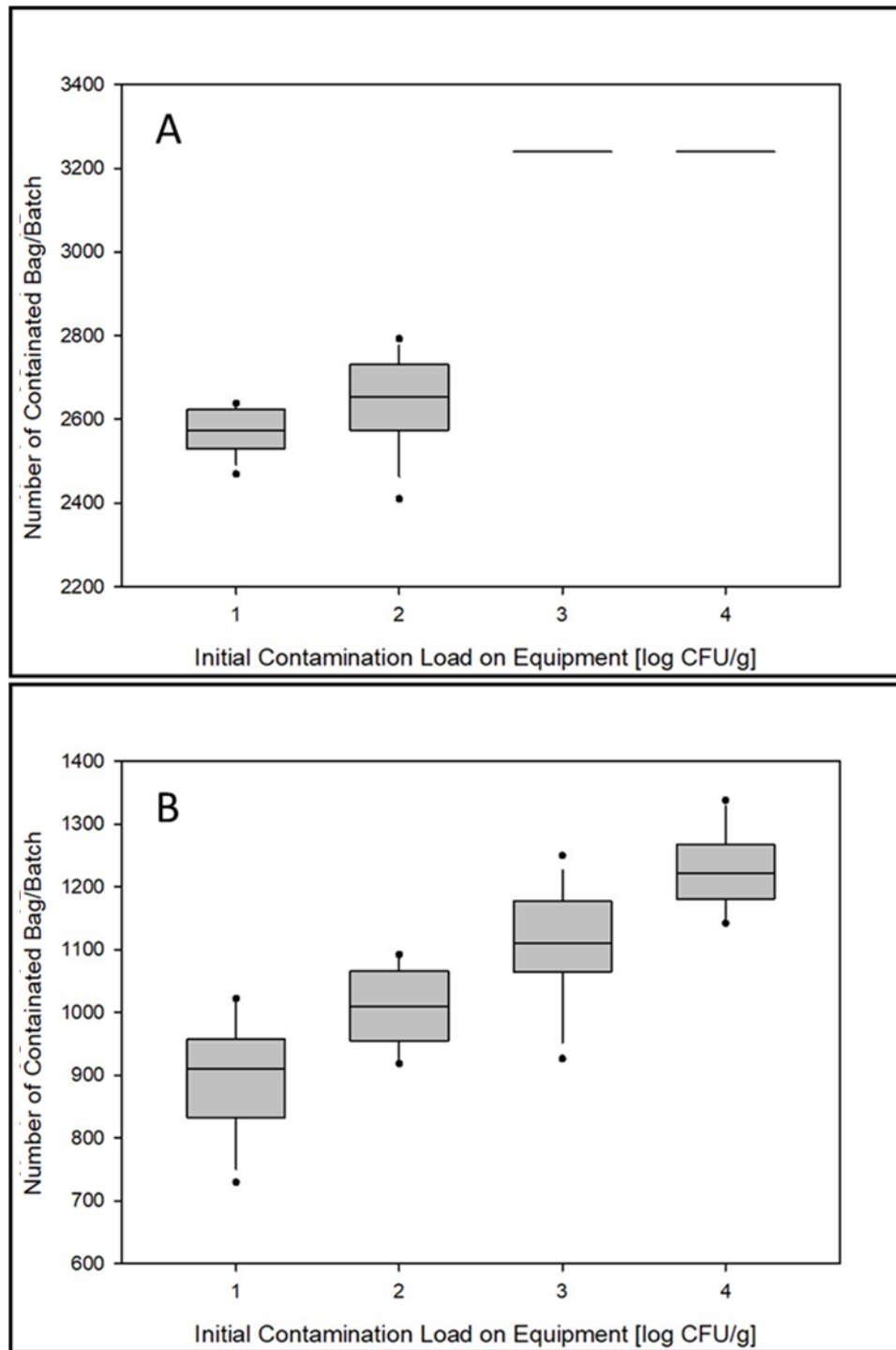CFU/100 g).

Figure 5.32: Effect of *E. coli* O157:H7 contamination level on equipment L6-L7 on the number of contaminated bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit). (A) no chlorination and (B) with chlorination treatments. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor

5.2.7 Effect Contamination Probability on Equipment (Scenario 6)

Figure 5.33 shows the average contamination levels in the shredded lettuces when the probability of 3 log CFU/g of *E. coli* O157:H7 contamination varies from 0.3 to 1 on the surface of equipment L1 - L4. The level of contamination increases linearly with increased probability when no FC is used in the flume tank (Figure 5.33-A). Figure 5.33-B shows that the mean contamination level did not vary as much when FC was applied to wash the shredded lettuces.

Figures 5.34 and 5.35 show an increase in the contamination level distribution in L1 to L4 with increased probability of contamination for the sanitizer-free wash water case, but with no effect on L5 to L7 contamination. When chlorination is used in L5, the contamination level distribution at L6 and L7 is affected by the probability of contamination in L1 to L4.

The processed bags are 100% contaminated (data not shown) when no FC was used to wash the lettuce pieces, regardless the contamination limit used to select the bags. These results indicate that when a high contamination load of 3 log CFU/g is present, a probability of contamination as low as 30% on the L1-L4 equipment can contaminate all the bags produced in that shift. Table 5.7 shows that the probability of contamination in L1 to L4 affects ($p<0.05$) the number of contaminated bags when FC is used in the flume tank.

Table 5.8: Average number of contaminated shredded Romaine lettuces bags after 8 hours processing as function of probability of contamination on equipment L1-L4 with sanitizer in L5.

	< 0.01 CFU/g	< 0.04 CFU/g
Contamination level [log CFU/g]	**Average Number of Contaminated Bags $\pm$ std**	
0.3	$_x3,614 \pm 91^a$	$_x2,561 \pm 104^a$
0.5	$_x4,160 \pm 104^a$	$_x3,068 \pm 91^b$
0.7	$_x4,407 \pm 52^b$	$_x3,315 \pm 78^c$
1	$_x4,745 \pm 65^c$	$_x3,627 \pm 65^d$

Different letters within the same $_{xy}$row and same column[a,b,c,d] for each component indicate significant difference ($p < 0.05$) according to one-way ANOVA and t-test test ($\alpha = 0.05$). L1 = worker, L2 = trim table, L3 = shredder, L4 = step conveyor.

Figure 5.33: Effect of probability of *E. coli* O157:H7 contamination on equipment (L1 - L4) on the average level of contamination on shredded lettuces after 8-hour processing time. (A) no chlorination and (B) with chlorination treatment. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor.

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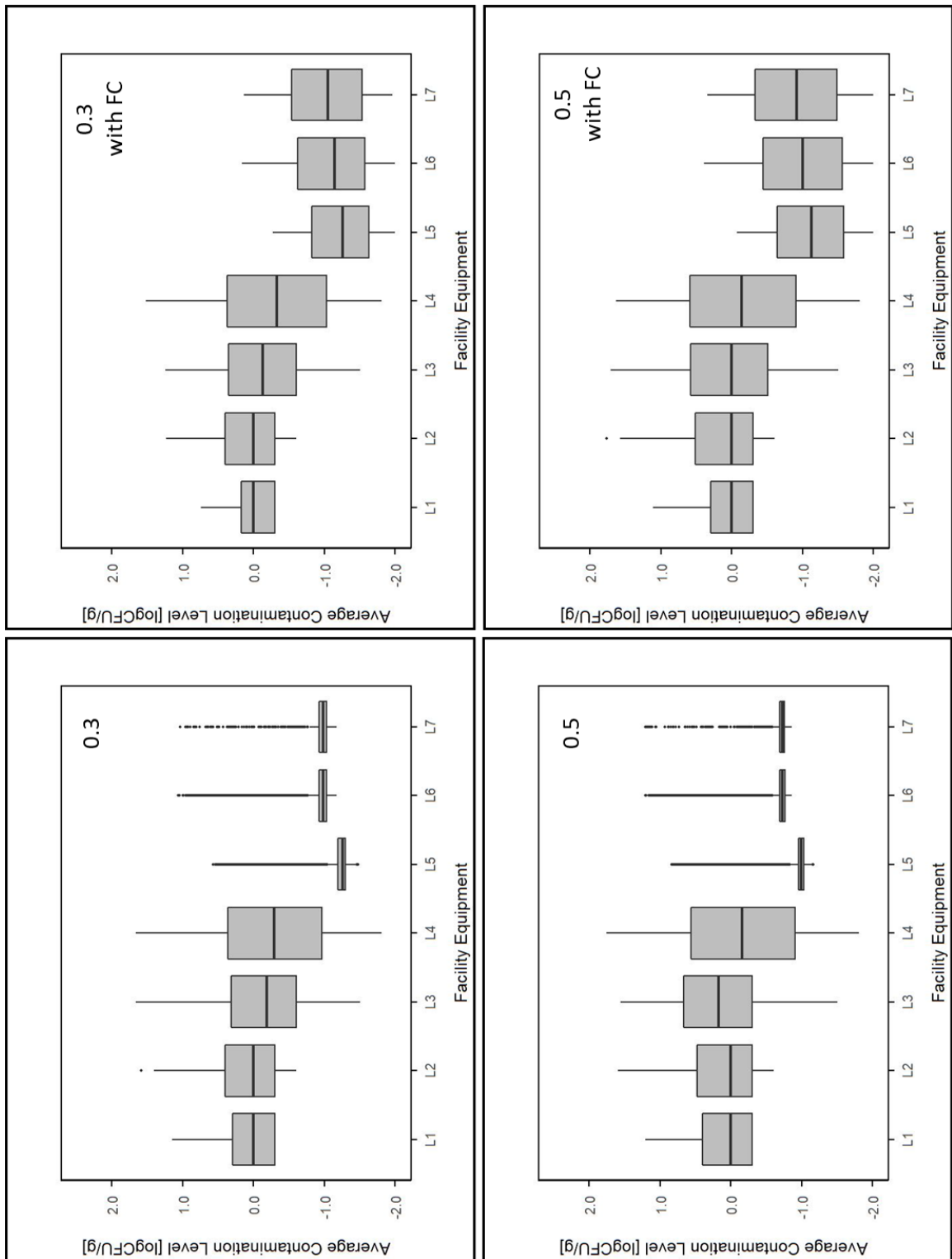
Figure 5.34: Effect of *E. coli* O157:H7 contamination probability (0.3-0.5) on equipment L1-L4 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.
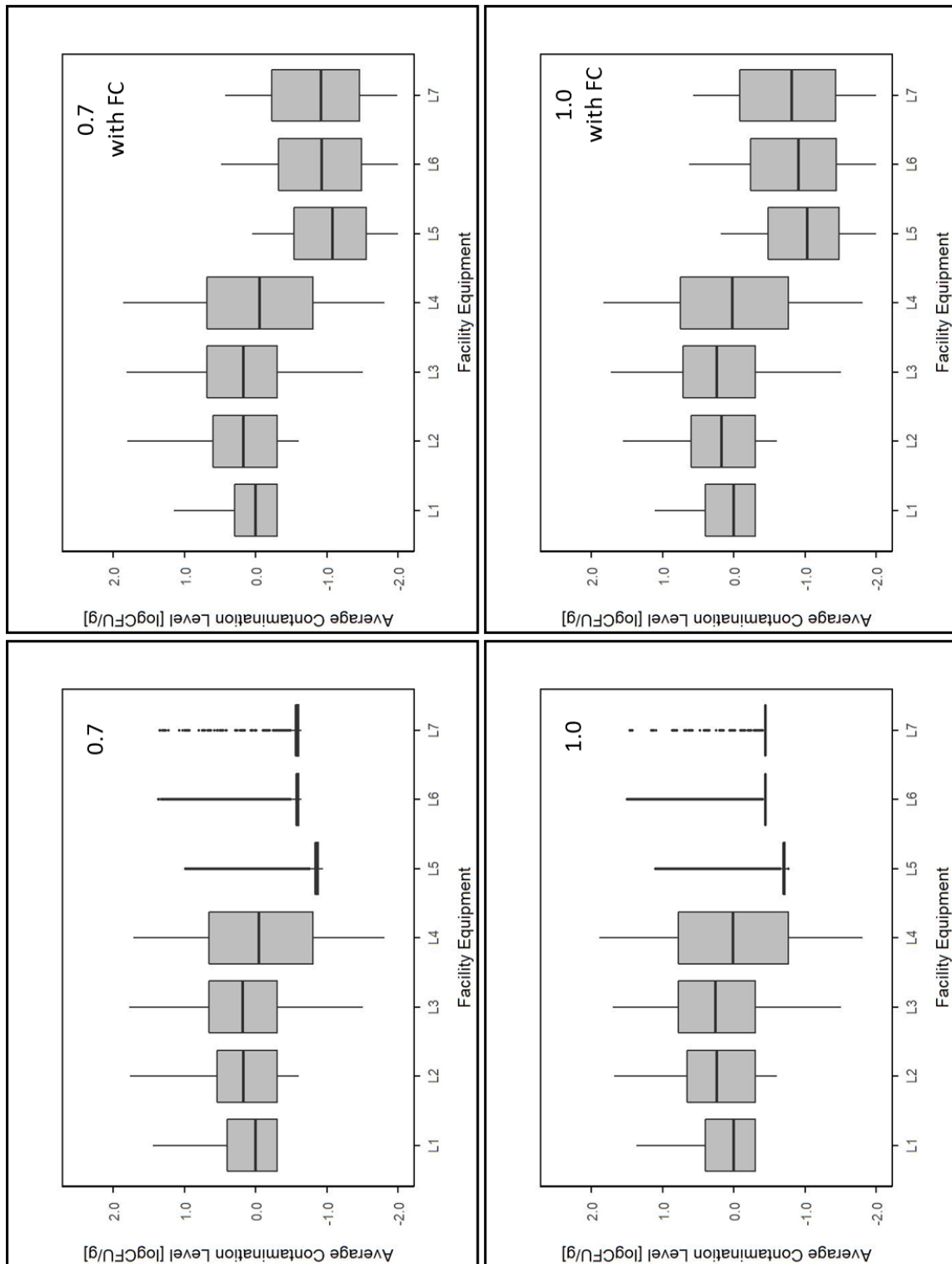
Figure 5.35: Effect of *E. coli* O157:H7 contamination probability (0.7-1.0) on equipment L1-L4 on average contamination level for 8-hour processing, where L0 =lettuce source, L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor, L5 = flume tank, L6 = shaker-dewatering; and L7 = centrifuge. Based on safety limit < 1 CFU/100 g.

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Figure 5.36 shows that by adding FC to the wash water, the average number of contamination bags increases with increased probability of contamination in L1 to L4. Only 10% of the bags became contaminated (above 1 CFU/100g) when FC was used, for all the probabilities considered in this study. We should remember that for Scenario 6 the incoming lettuce heads were not contaminated, but the contamination occurred in the equipment surfaces. In this situation, FC helps reduce the number of contaminated bags substantially.

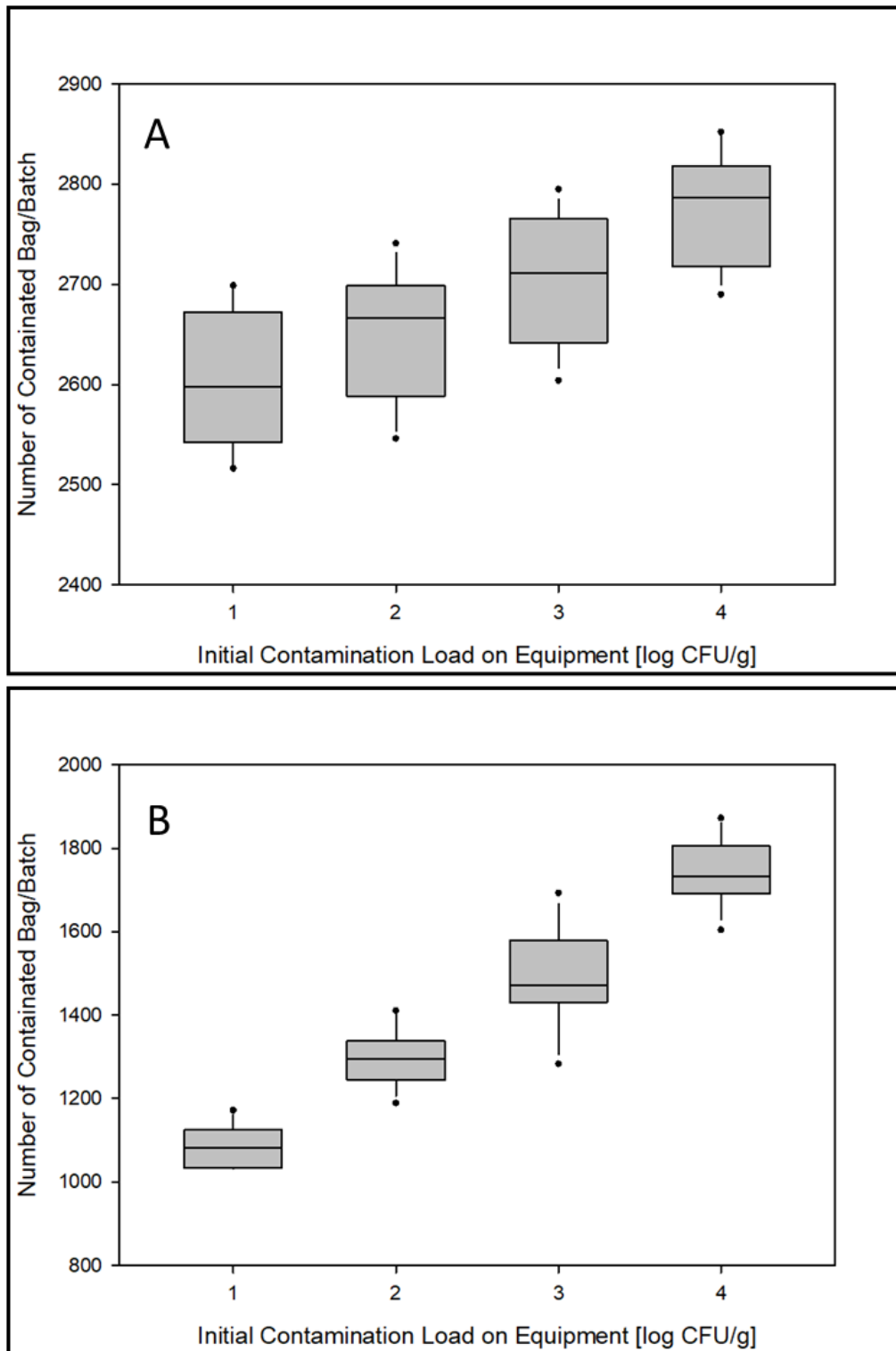**Figure 5.36:** Effect of probability of *E. coli* O157:H7 contamination on equipment (L1-L4) on the number of contaminated bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit). Lettuce treated with chlorination. L1 = worker, L2 = trimming table, L3 = shredder, L4 = step conveyor.

Figure 5.37 shows the average level contamination in the shredded lettuces when the probability of 3 log CFU/g of *E. coli* O157:H7 contamination varies from 0.3 to 1 on the surface of equipment L6 – L7. The level of contamination does not change with increased probability whether FC is used in the flume tank (Figure 5.37-A and 5.37-B). Contamination in the

equipment after the flume tank can increase the contamination levels because there is no other treatment applied to decontaminate the produce in the processing line.

   Table 5.8 shows that the average number of contaminated bags is affected ($p<0.05$) by the probability of *E. coli* contamination in equipment L6-L7. When the lettuces are washed without FC, the number of contaminated bags can range from 28% to 34% depending on the probability of contamination in the equipment. By adding FC to the wash water, the number of contaminated bags stayed the same.

Table 5.9: Average number of contaminated shredded Romaine lettuces bags after 8 hours processing as function of probability of contamination on equipment L6-L7.

Contamination level [log CFU/g]	< 0.01 CFU/g	< 0.04 CFU/g
	Average Number of Contaminated Bags $\pm$ std	
No Free Chlorine		
0.3	$_x11{,}986 \pm 273^a$	$_x11{,}986 \pm 273^a$
0.5	$_{x,}13{,}026 \pm 260^b$	$_x13{,}026 \pm 260^b$
0.7	$_x13{,}624 \pm 143^c$	$_x13{,}624 \pm 143^c$
1	$_x14{,}482 \pm 312^d$	$_x14{,}482 \pm 312^d$
With Free Chlorine		
0.3	$_x12{,}181 \pm 143^a$	$_x12.181 \pm 143^a$
0.5	$_x13{,}117 \pm 325^a$	$_x13{,}104 \pm 325^b$
0.7	$_x13{,}728 \pm 52^b$	$_x13{,}728 \pm 52^c$
1	$_x14{,}378 \pm 182^c$	$_x14{,}378 \pm 182^d$

Different letters within the same $_{xy}$row and same column[a,b,c,d] for each component indicate significant difference ($p <$ 0.05) according to one-way ANOVA and t-test test ($\alpha = 0.05$). L1 = worker, L2 = trim table, L3 = shredder, L4 = step conveyor.
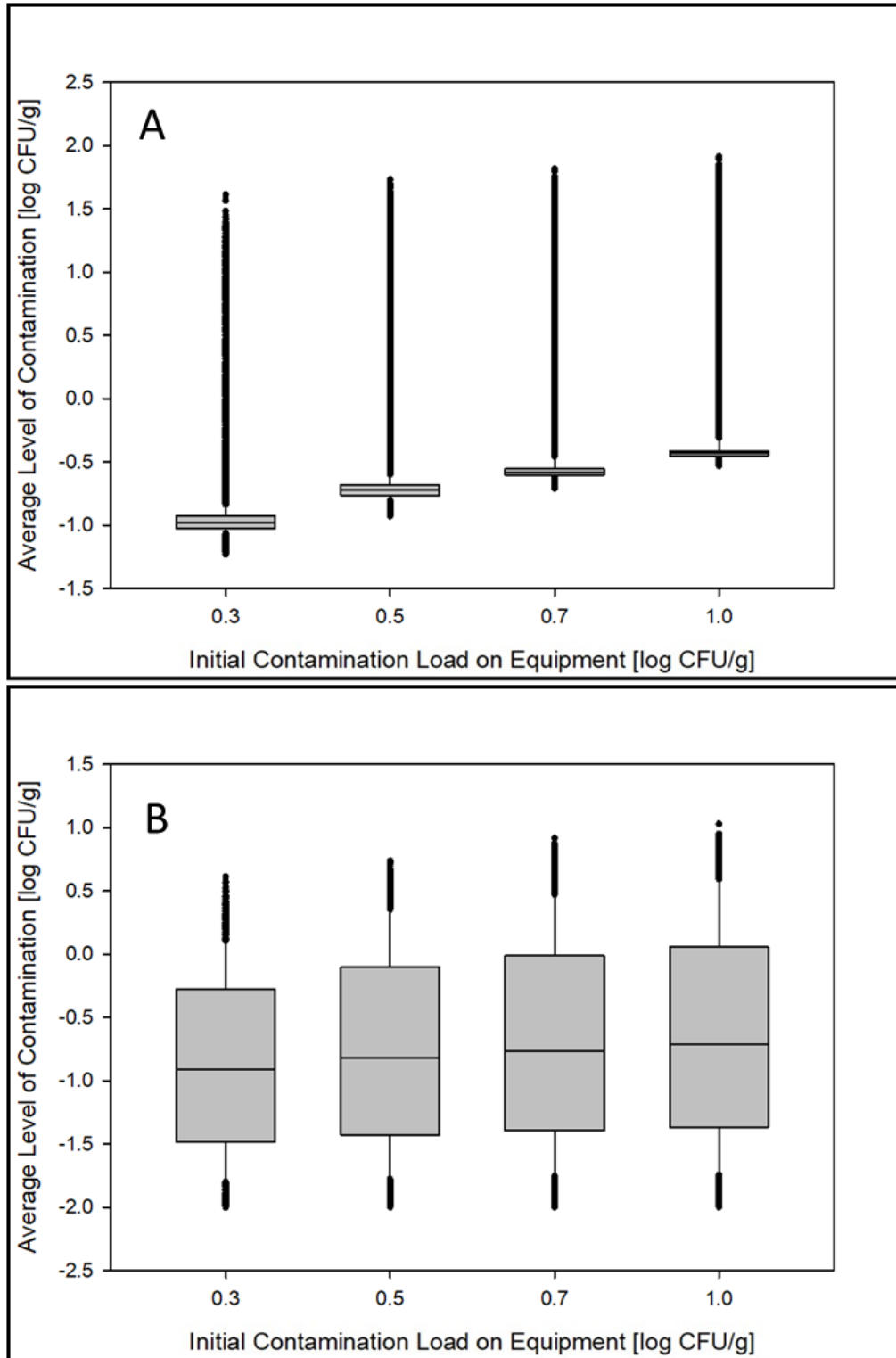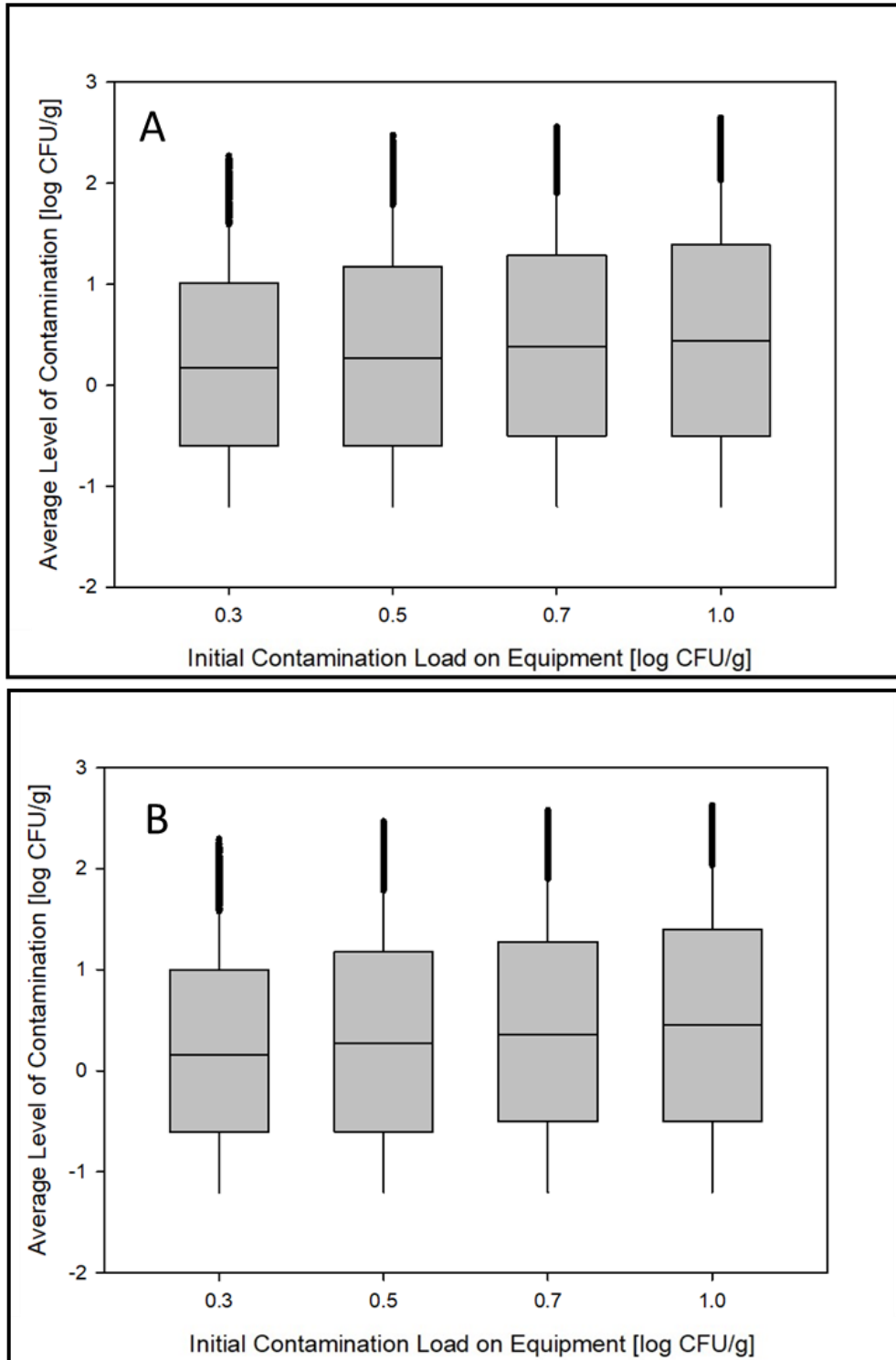
Figure 5.37: Effect of probability of *E. coli* O157:H7 contamination in equipment (L6 - L7) on the average level of contamination on shredded lettuces after 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit). (A) no chlorination and (B) with chlorination treatment. L6 = shaker, L7 = centrifuge.

The number of contaminated bags per batch increases linearly with increased probability of contamination in L6-L7 with or without FC (Figure 5.38-A and 5.38-B). The average percentage of contaminated bags per batch ranges from 28 to 34%t for both cases (with or without applied FC). These results indicate that when the uncontaminated lettuces pass through the system (assuming that L1-L4 are not contaminated), the level of contamination in equipment L6 and L7 is reduced. As the first batch of uncontaminated lettuces moves through the system, the contaminated equipment transfers *E. coli* O157:H7 to the uncontaminated lettuces, which then become highly cross-contaminated compared to the following batches. With time, the level of contamination in the equipment is reduced resulting in less numbers of bags being unsafe.

The difference in results between washing with or without FC solutions is small because it is assumed that water alone can reduce up to 90% of the microbial population from the lettuce surfaces, and only ~1% of the bacteria in the contaminated wash water can transfer to the uncontaminated lettuces (Jensen et al., 2017; Luo et al., 2018). Recall that chlorination can only eliminate up to 2% from the produce surface and it is assumed that there is no cross-contamination when FC is used in the flume tank.
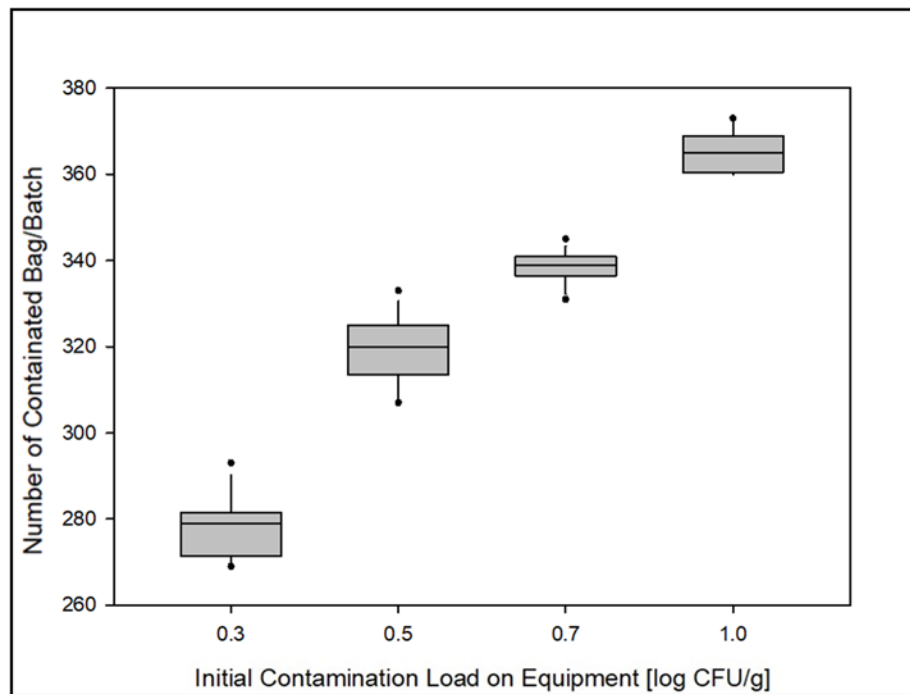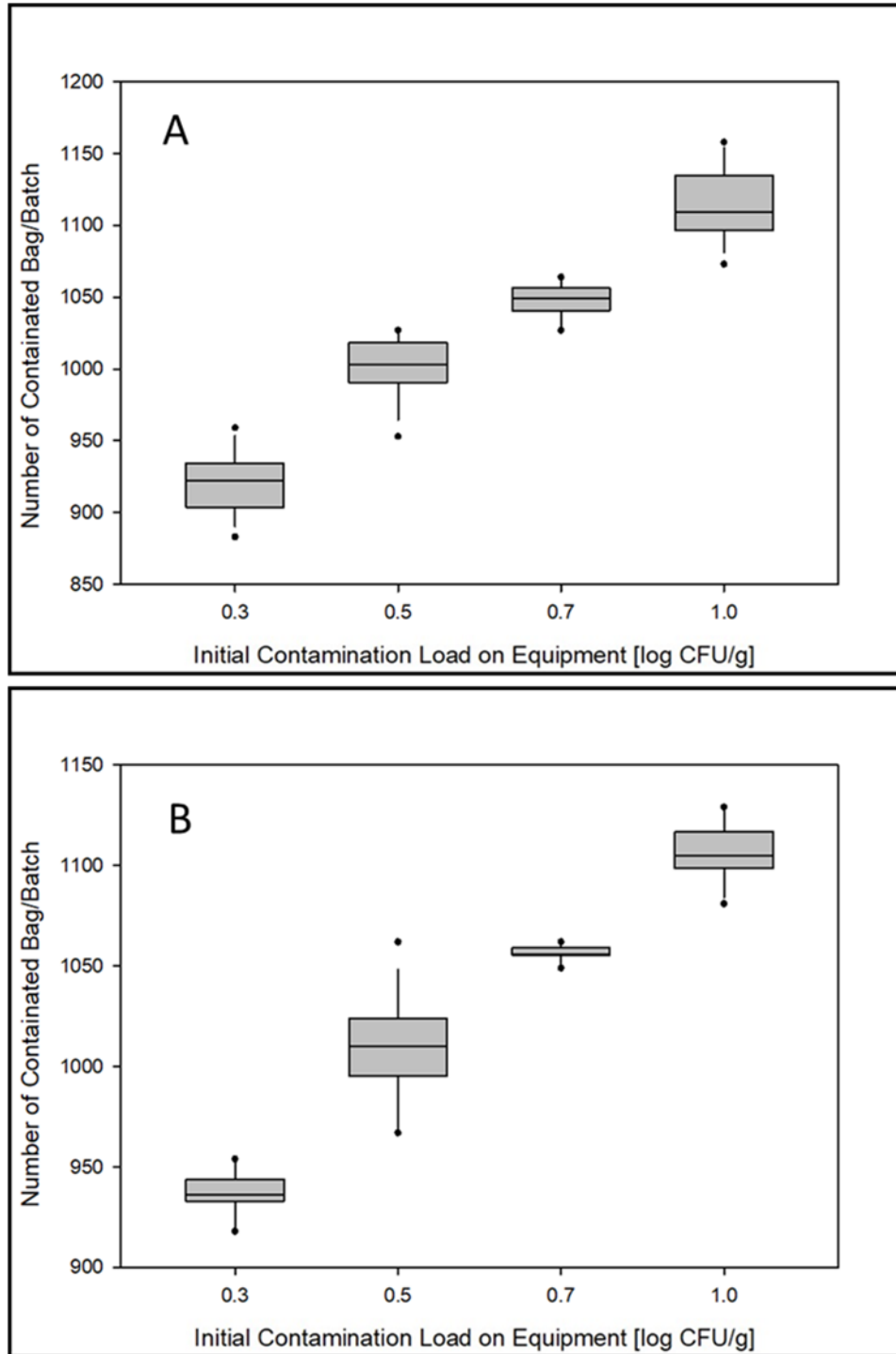
Figure 5.38: Effect of probability of *E. coli* O157:H7 contamination in equipment (L6-L7) on the number of contaminated bags per batch for 8-hour processing time (contaminated bags above 1 CFU/100 g safety limit). (A) no chlorination and (B) with chlorination treatments. L6 = shaker, L7 = centrifuge.

5.2.8 Effect of First Batch Contamination and Different Batch Sizes (Scenario 7)

Scenario 7 considers that only the first batch of lettuces is contaminated with *E. coli* O157:H7. The subsequent batches are therefore uncontaminated. The level of contamination is randomly distributed among the lettuces following a normal distribution between 0 and 6 log CFU/g. A total of 1,620 kg of lettuces are processed, considering different batch sizes: 180, 90, 60, 45, and 36 kg, which corresponds to 9, 18, 27, 36, and 45 batches, respectively.

Figure 5.39 shows the effect of batch sizes on the percentage of contaminated bags (> 1 CFU/100 g) processed with different FC concentrations. The percentage of contaminated bags decreases during the process, regardless of the applied FC concentration levels. For the lettuces treated with 21-10-6 mg/L FC concentration cycles, about 56% of the batches will be contaminated when the batch size is 180 kg/batch, 50% for a 90 kg/batch size, 44% for a 60 kg/batch, 39% for a 45 kg/batch, and 36% for a 36 kg/batch. For the medium and small concentrations, these values are not different (p>0.05) among the groups. The results become more variable as the batch size decreases (thus increasing the number of batches).

It is worth noticing that the first 5 batches of the 45-batch case are 100% contaminated compared (Fig. 5.39-H) to only 3 batches (Fig. 5.39-E) and 1 batch (Fig. 5.39-B) when the mass of lettuce per batch increases, for the same FC level. Higher cross-contamination happens with the smaller batch size (36 kg/batch) at the beginning of the process when the first batch is largely contaminated with the pathogen.

Based on these results, it is preferable to process small batch sizes since fewer batches will be contaminated during the process.
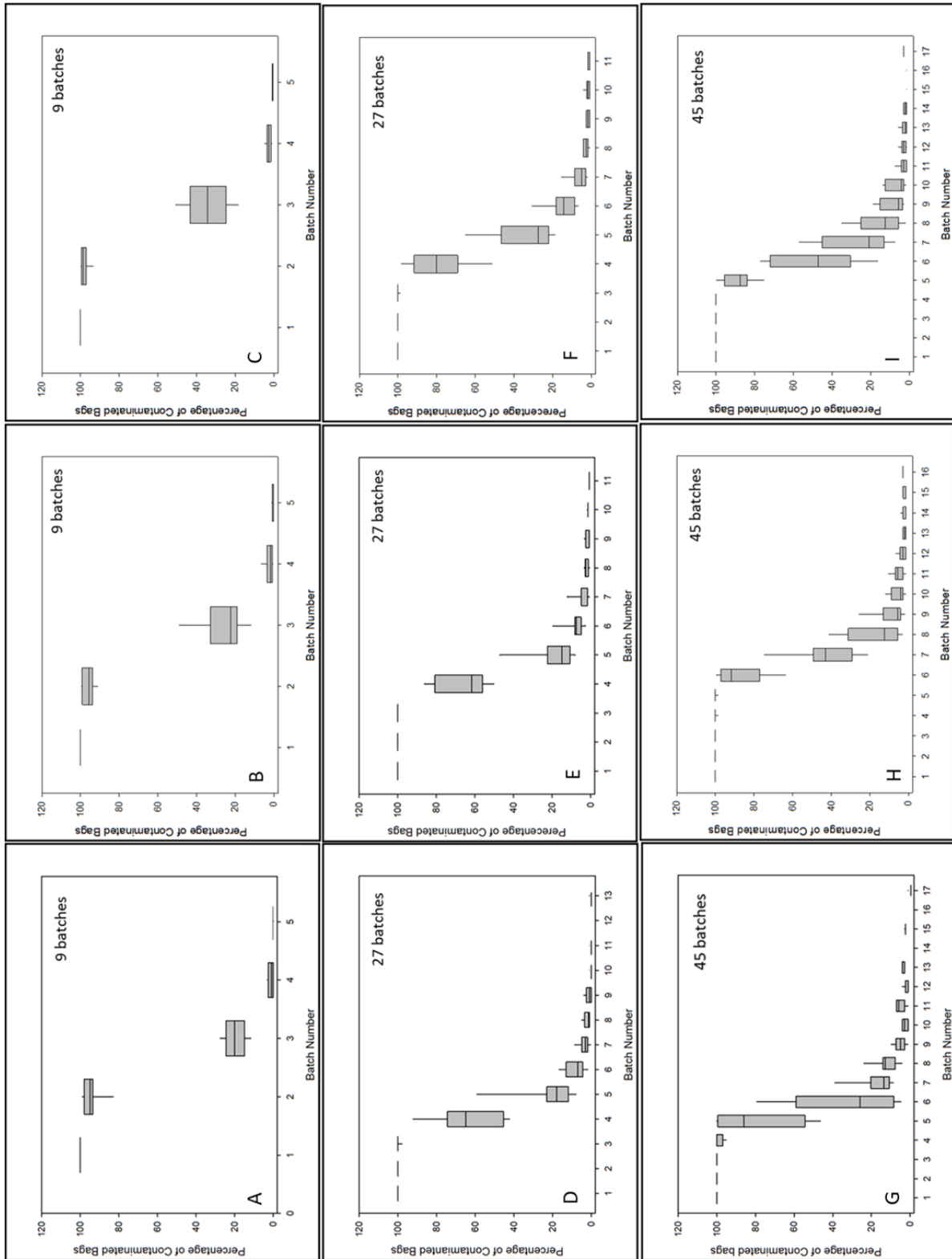
Figure 5.39: Effect of number of batches on the percentage of bags contaminated (> 1 CFU/100g) with E. *coli* O157:H7 treated with FC at different concentrations. A-C: 21-10-6 mg/L; D-F: 11-5-3 mg/L; G-I: 6-3-2 mg/L.

5.2.9 Effect of First Batch Contamination with Varying Initial Contamination and Probability of Contamination (Scenario 8)

In Scenario 8, the first batch of lettuces is contaminated with *E. coli* O157:H7 with levels ranging from 1 log CFU/g to 6 log CFU/g at different probability of contamination (0.05, 0.25, 0.5, 0.7, 1). The subsequent batches are therefore uncontaminated. A total of 1,620 kg of lettuces are processed, considering different batch sizes: 60 and 36 kg, which corresponds to 27 and 45 batches, respectively. The equipment (L1 to L7) were free from contamination.

Figure 5.40 shows that most of the bags in the first batch were contaminated (> 1 CFU/100 g). After processing 12 batches, only 2% of the bags were contaminated reducing to 0% of contaminated bags after Batch # 17. In total, 41% of the 27 batches are not contaminated with *E. coli* O157:H7.
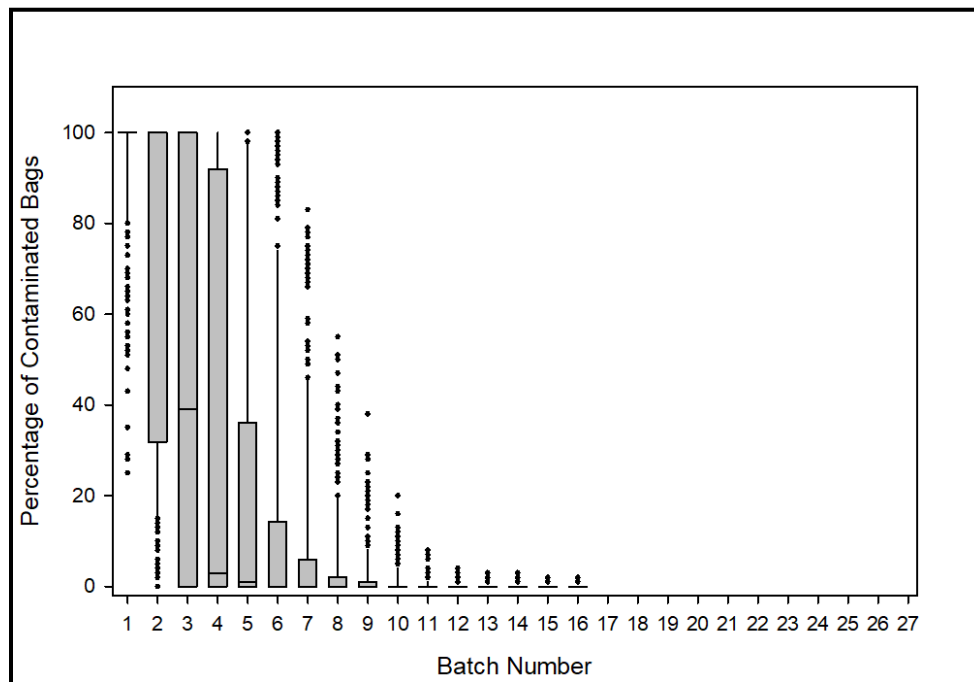
Figure 5.40: Effect of first batch contamination levels from 1-6 log CFU/g and probability of contamination (0.05-1) on the percentage of bags contaminated (> 1 CFU/100 g) with E. *coli* O157:H7 treated with FC concentration of 21-10-6 mg/L for Batch #1 to Batch #27.

Figure 5.41 shows that most of the bags in the first batch were contaminated (> 1 CFU/100 g). After processing 18 batches, only 3% of the bags are contaminated. There are no contaminated bags after Batch # 25. In total, 56% of the 45 batches are not contaminated with *E. coli* O157:H7. So, the smaller the batch size the less contamination.
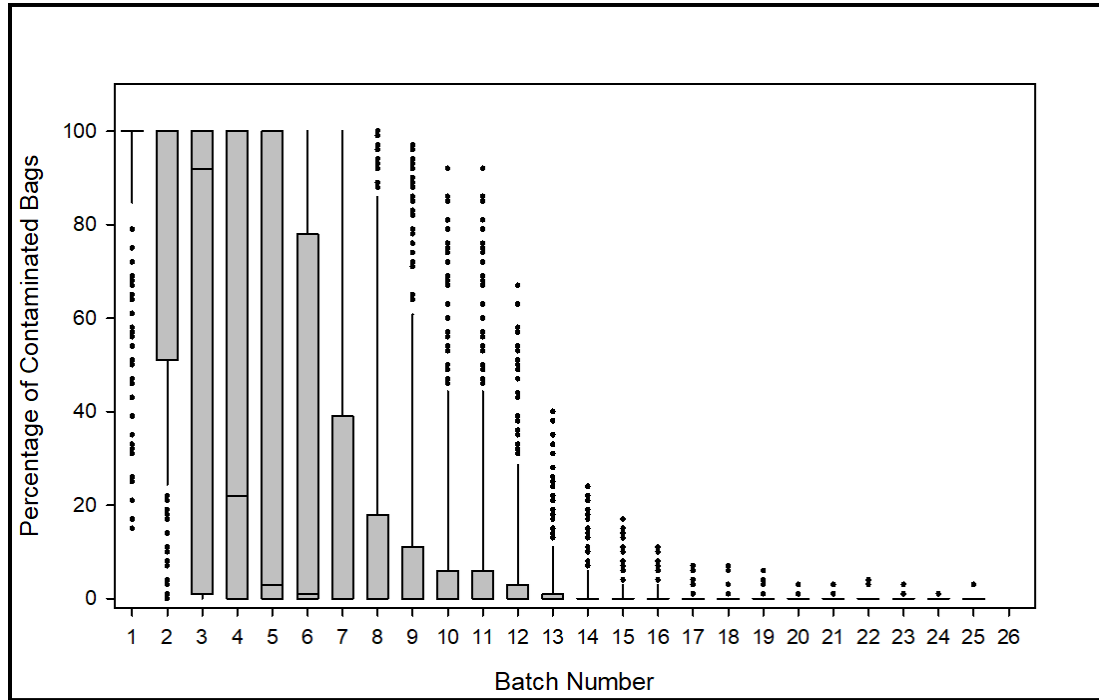
Figure 5.41: Effect of first batch contamination levels from 1-6 log CFU/g and probability of contamination (0.05-1) on the percentage of bags contaminated (> 1 CFU/100 g) with E. *coli* O157:H7 treated with FC concentration of 21-10-6 mg/L for Batch #1 to Batch #45 (showing only 25 batches).

5.3 Simulation of the Lettuce Storage Facility

The temperature fluctuation in a cold storage facility was simulated based on literature data for *E. coli* O157:H7 growth in fresh-cut Romaine lettuce in a retail storage (Zeng at al., 2014). Figure 5.42 shows the temperature fluctuation and microbial growth for 70 hours as presented by Zeng et al. (2014). Simulated retail storage led to *E. coli* O157:H7 population increases of 3.1 log CFU/g.
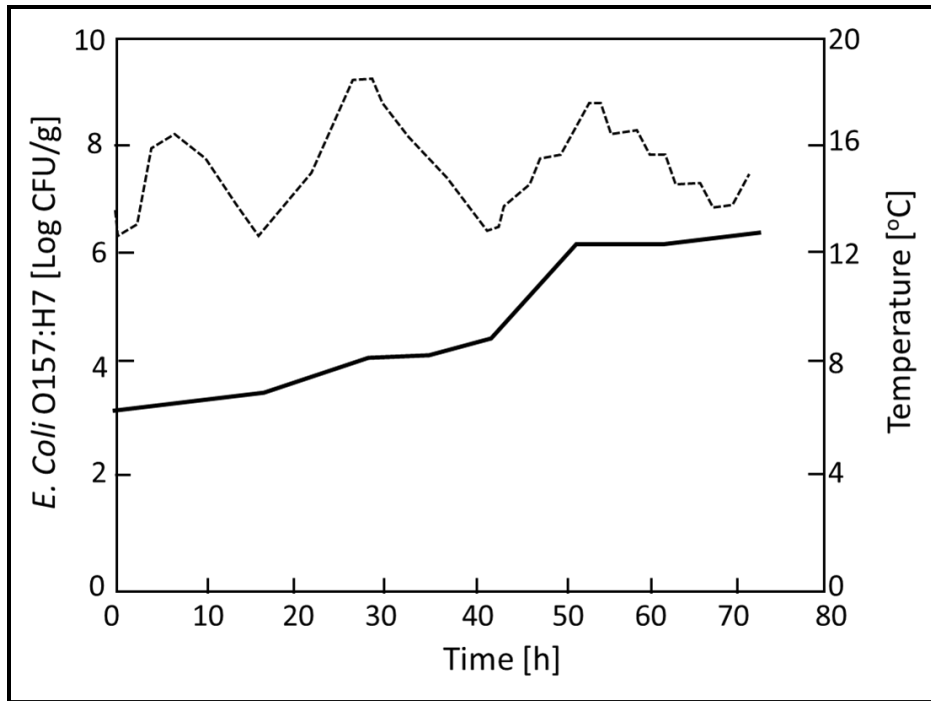
Figure 5.42: Experimental growth of *E. coli* O157:H7 in fresh-cut Romaine lettuce under selected temperature during retail storage (modified from Zeng et al., 2014).

Figure 5.43 shows the simulation of *E. coli* O157:H7 growth in in fresh-cut Romaine lettuce for temperatures alternating between (A) 16 ℃ and 19℃ and between (B) 8 ℃ and 12℃ in a 25-h cycle for 70 h. The simulation is based on the results obtained from Scenario 3, considering an initial contamination level on the lettuce heads of 1 log CFU/g with a probability of contamination of 10%. Chlorination was used during the process. The lettuces left the processing facility with an average contamination level of -2.21 log CFU/g and with 20% contaminated bag (above 1 CFU/100 g) or 0.2% (above 1 CFU/25 g).
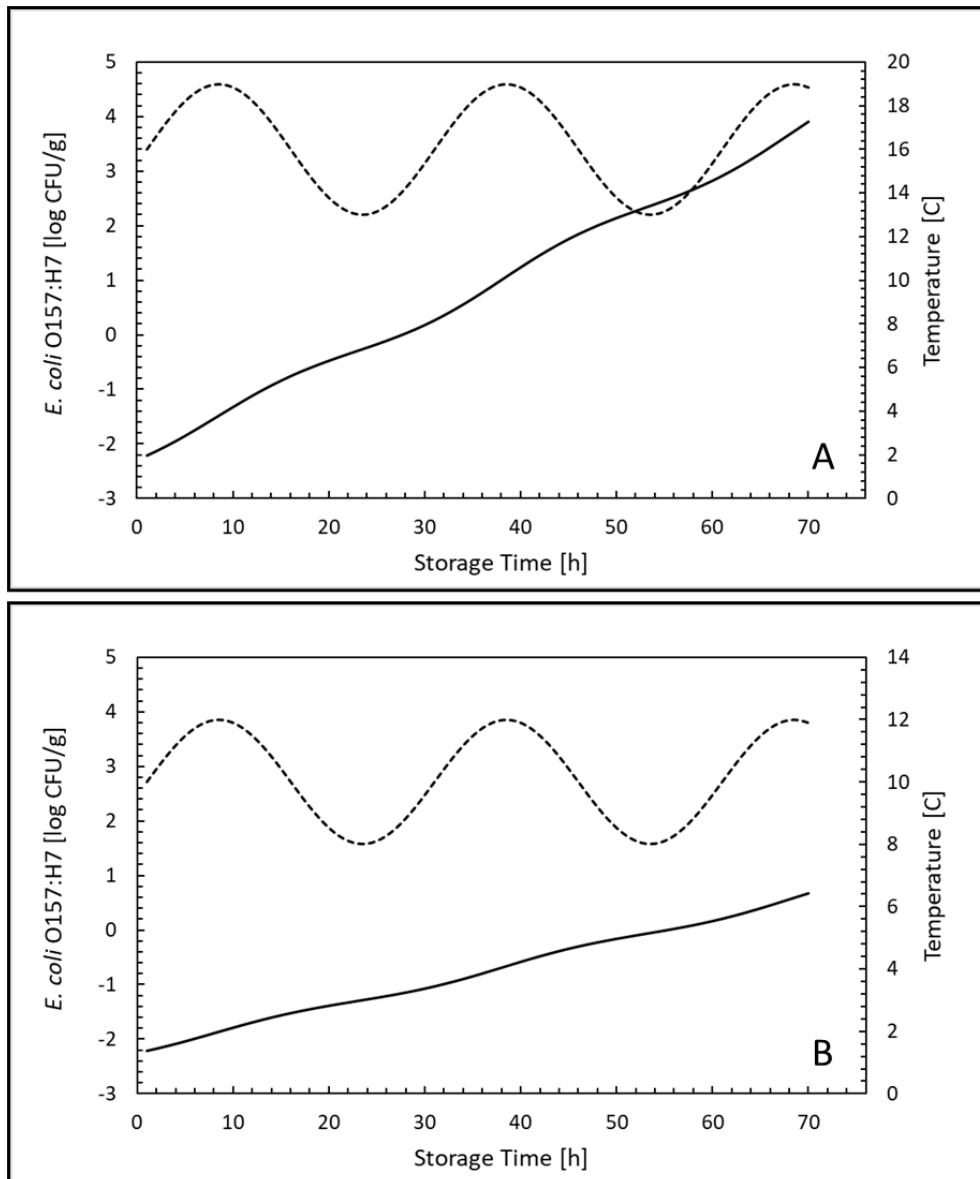
Figure 5.43: Simulation of E. coli O157:H7growth on shredded Romaine lettuce bags for 70 hours storage with input from Scenario 3 (Table 4.14). A: Temperature fluctuation between 16 and 19oC (A) and between 8 and 12oC (B).

In 70 hours, the temperature fluctuations in storage led to microbial population increases

of 6.1 log CFU/g for the higher temperature fluctuation (Figure 5.44-A) and 2.9 log CFU/g for

the lower temperature range (Figure 5.42-B). The average percentage of bags that becomes

contaminated under higher temperature fluctuation increased to 82% (Figure 5.44-A). For the

lower temperature fluctuation, the percentage contaminated bags increased to 75%.
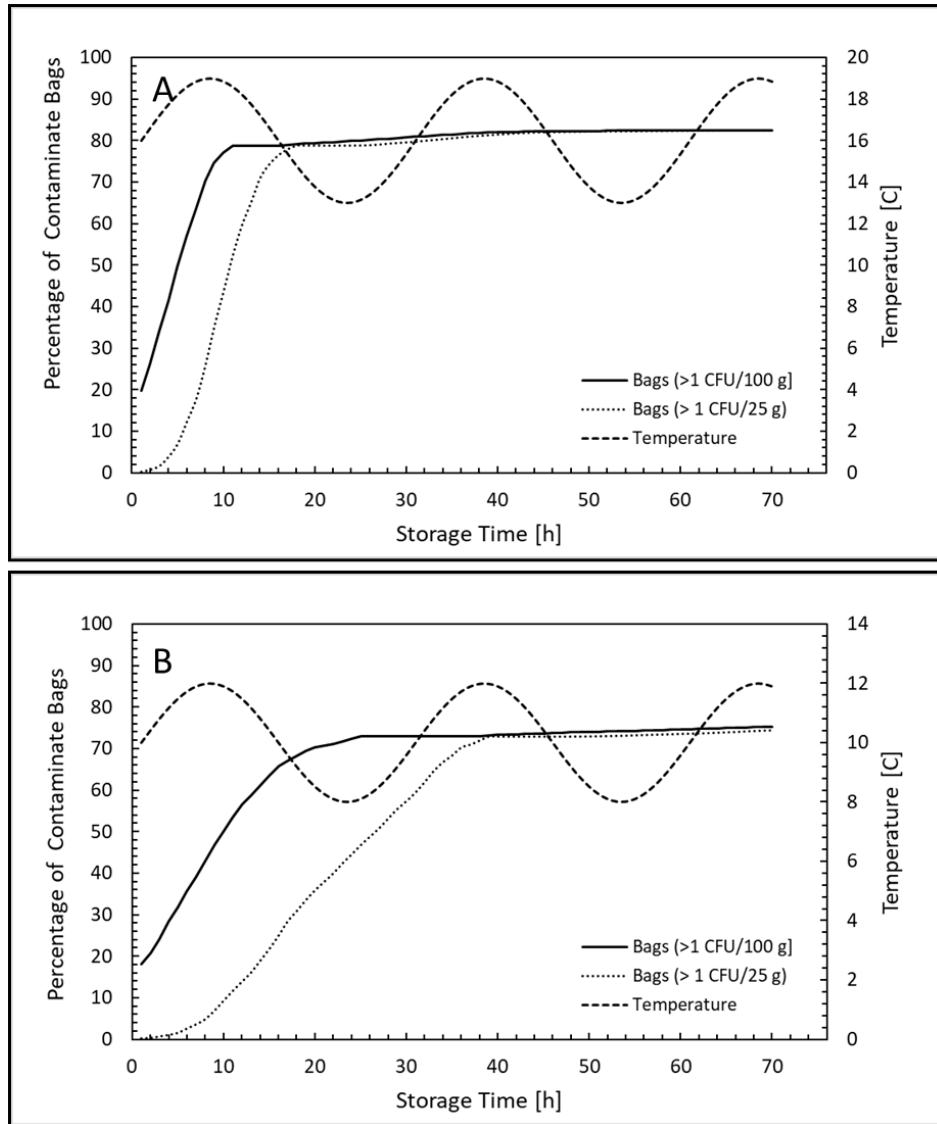
Figure 5.44: Simulation of *E. coli* O157:H7growth on shredded Romaine lettuce bags for 70 hours storage with input from Scenario 3 (Table 4.14). A: Temperature fluctuation between 16 and 19°C (A) and between 8 and 12°C (B).

Figure 5.45 shows the relationship between the number of contaminated bags (> 1

CFU/100 g) and batch number. The number of contaminated bags per batch ranges between

1200 to 2642 during storage with temperature fluctuations ranging from 8ºC to 12ºC. The developed model can predict the pathogen growth pattern while providing the number of bags that are affected by the temperature fluctuation during storage.
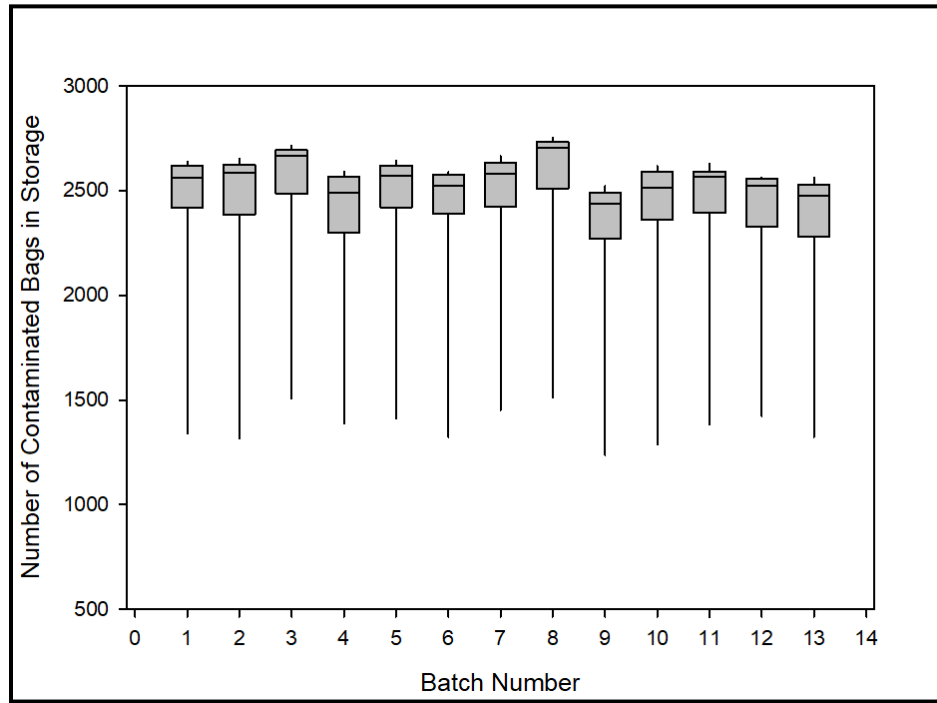
Figure 5.45: Number of contaminated (> 1 CFU/ 100 g) shredded Romaine lettuce bags stored for 70 hours with temperature fluctuations between 8 and 12°C.

Similar results were obtained for contaminated bags above 1 CFU/25 g (Figure 5.46). In this case the boxes are more spread, with contaminated bags varying as low as 6 to 2611 per batch during storage. For the higher temperature fluctuation range (13 to 19ºC) (not shown), the average number of contaminated bags varied from 7 to 2669 per batch during storage.
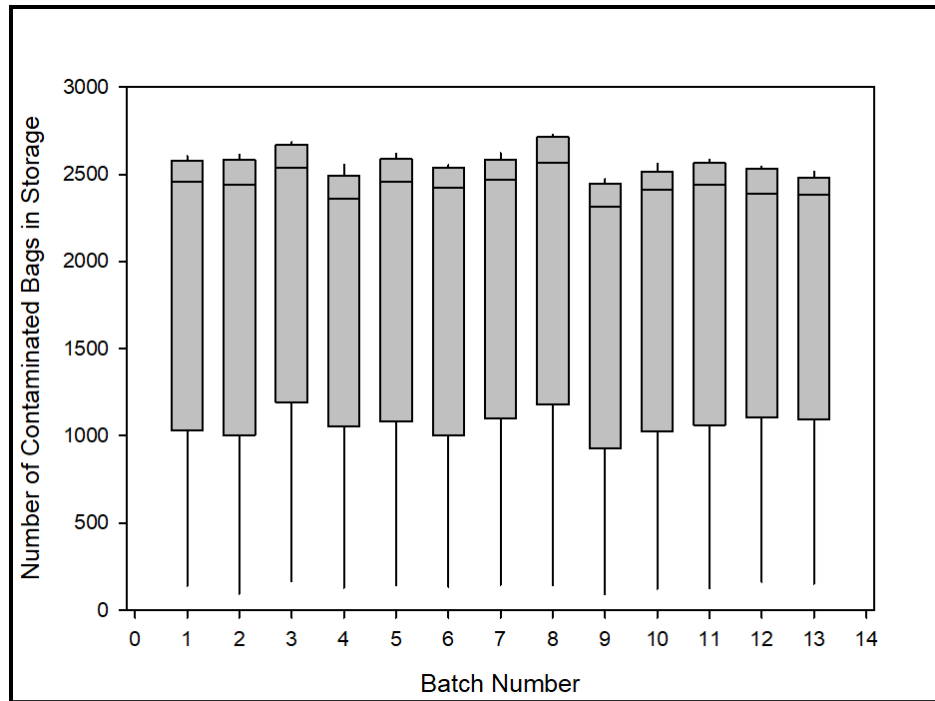
Figure 5.46: Number of contaminated (> 1 CFU/25 g) shredded lettuce bags per batch stored for 70 hours with temperature fluctuations between 8ºC and 12ºC.

Figure 5.47 is the whisker plot showing the number of contaminated (> 1 CFU/ 100g) bags per batch as a function of storage time with temperature fluctuations from 8ºC to 12ºC. The number of contaminated bags per batch increased non-linearly with time and varied from 631 ± 65 and 2642 ± 62, with 70% of the bags ended being contaminated after 16 hours under the simulated temperature abuse in storage. For contaminated bags above 1 CFU/25 g (not shown), it would take 33 hours for 70% of the processed bags to become contaminated. These results clearly show that temperature abuse in the facility can cause great losses for the leafy green producer. In this simulation, the number of contaminated bags after processing was either 0.2% (> 1 CFU/25 g) or 20% (> 1 CFU/100g) and in 3 days 80% of the bags can be tinted because of the high temperature fluctuations during storage. Storing bags of Romaine lettuce for 70 h significantly increases the growth probabilities for *E. coli* O157:H7 as demonstrated by Zeng et al. (2014).

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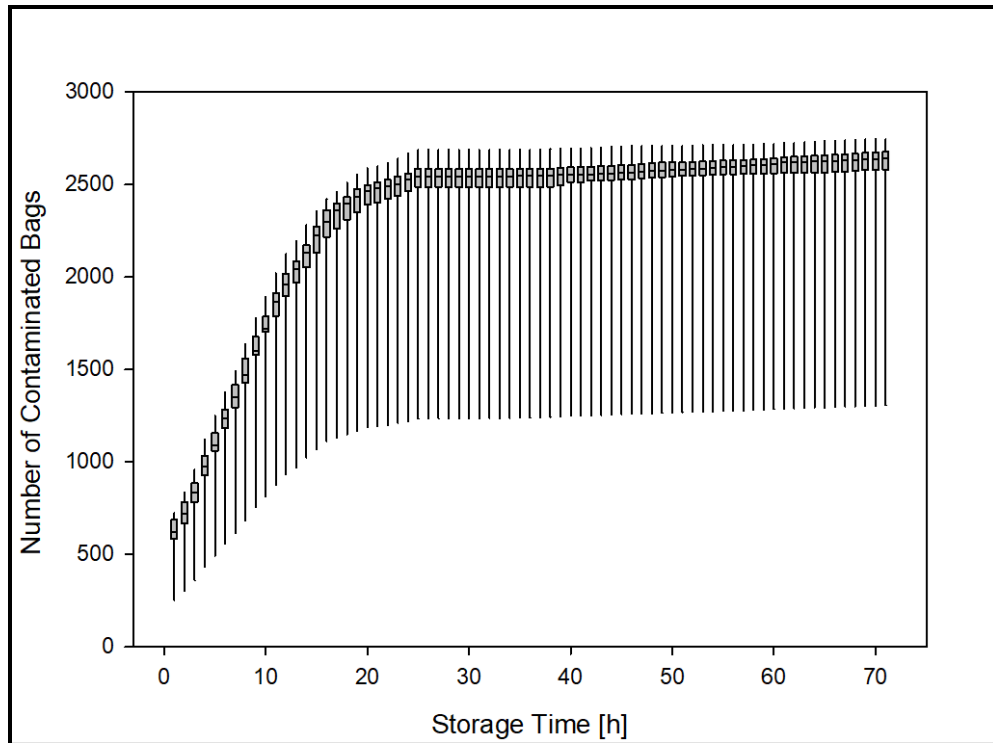
Figure 5.47: Number of contaminated (> 1 CFU/100 g) shredded lettuce bags per batch changes for 70 hours storage with temperature fluctuations between 8 and 12°C.

Temperature abuse of fresh-cut leafy greens is most likely to occur during retail storage, particularly when the refrigeration system is temporarily shut down during cleaning and sanitizing of the cold room. Storage duration typically varies between 1 and 3 days, depending on the size of the supermarket, supply chain distribution pattern, and consumer sales activity (Danyluk, and Schaffner, 2011). When combined with varying retail storage times, these short-term periods of temperature abuse can lead to significant microbial growth (Zeng et al., 2014).

Lettuces can become contaminated at any point in the production chain, from farm to consumption, and therefore washing of produce has become a standard in commercial processing. Washing and sanitizing can reduce the number of microorganisms on the surface of leafy greens, but bacteria cells can internalize in stomata and survive the chlorination process.

Spoilage microorganisms can increase substantially during the cold chain after harvest, and pathogens can survive after washing (Rosberg et al., 2021).

Zeng et al. (2014) found that temperature abuse has the potential to increase the chance for *E. coli* O157:H7 and *Listeria monocytogenes* growth in bagged salad greens during transport, retail, and display. The authors observed increased growth of *E. coli* O157:H7 during summer and discussed the importance of continuous temperature monitoring through the supply chain to reduce the risk of outbreaks. The model developed in this study support such claims.

5.4 Discussion

Leafy greens, specifically fresh cut, are potential vehicles for foodborne pathogens such as *Escherichia coli O157:H7*, and are at high risk of causing foodborne illnesses, as it is generally consumed without cooking. In the last decade, the number of outbreaks related to leafy greens in the US have increased sharply from 69 in 2008 to 2953 in 2018 resulting in approximately 853,000 illness, 16,00 hospitalizations, and 1,174 deaths (CDC, 2020).

Cross-contamination of *E. coli* O157:H7 during post-harvest processing of fresh-cut leafy greens has become a major health concern worldwide. During commercial shredding, conveying, flume-washing, and drying leafy greens can become contaminated with *E. coli* and have been linked to many outbreaks in the US and Canada (CDC, 2020). To better understand the role of processing facility patterns on *E. coli* O157:H7 cross-contamination at romaine lettuce-processing equipment interface, we developed an agent-based model for a pilot plant processing facility for fresh-cut romaine lettuce (Buchhlolz et al., 2012a) using the open-source NetLogo. The model was designed to (1) track *E. coli* O157:H7 and lettuce movements in time, (2) evaluate microbial contamination in different piece of equipment (spatially explicit) and calculate the probability events of cross-contamination between agents (lettuces) and patches

(equipment), and (3) determine the number of fresh-cut contaminate processed bags and their level of contamination at the end of the processing line. An extension was also added to the main model to determine *E. coli* O157:H7 growth due to temperature abuse in a cold storage facility.

Several no spatial models on risk assessment were developed for cross-contamination of leafy greens during pre- and postharvest processing (Pang et al., 2017, Omac et al., 2017, Puerta-Gomes et al, 2013, Danyluk and Schaffner, 2011). Most of their results agree with our results that chlorination is responsible for the reduction of contamination, but it is not the best treatment to reduce the risk of contamination. Few models were developed to describe the dynamics of cross-contamination in leafy-greens post harvesting processing facility.

Perez-Rodriguez et al. (2011) developed a stochastic model in Excel to simulate the cross-contamination of lettuces in the same processing line described in this study (Buschholz et al., 2012 a, b) using transfer data from Bushholz et al. (2008). The packaging of processed lettuce was modelled assuming that *E. coli* O157:H7 was distributed homogeneously on the incoming contaminated lettuce and the cross-contaminated lettuce during processing. Their model did not track the contaminated product through the processing line and does not account for the spatial cross-contamination events between lettuces and processing equipment (Mokhtari et al., 2018). Contrary to our results, their model predicted that there is no significant relationship between the initial contamination levels on incoming lettuce heads (log CFU/g) and level of contamination in bags of processed fresh-cut lettuce (log CFU/bag). Like our simulator, their model showed that using free chlorine in the wash water inside the flume tank could reduce contamination in the final product.

A more advanced approach was used by Mokhtari et al. (2018) who developed a probabilistic mathematical model to describe the dynamics of cross contamination of *E. coli*

O157:H7 using agent-based modeling framework to track the individual pieces of lettuces throughout the processing line following the work of Perez-Rodriguez et al. (2011). They used the open-source language R version 3.3.2 (R Core Team) to develop the model. Like our model, they used data and the processing facility information from Buschholz et al. (2012 a, b) to validate their model. Their agents were individual lettuce heads that interacted explicitly with time and space. They did not explain how that interaction happens spatially and what was the role of the equipment in the agent-based model context. The flume tank washing step was modeled based on the mathematical approach developed by Munther et al. (2015). Lettuces in the flume tank moved as a bulk, unlike our approach that uses Markov chain-random walking approach to describe the movement of lettuces in the flume tank. They validated their model only on one set of data, ignoring the most important dynamic data. Ours results compare with their studies on effect of initial contamination level, FC concentrations, and initial level of contamination on the first incoming batch on the level of contamination of *E. coli* O157:H7 lettuce pieces.

Unlike our model, theirs model does not have a user-friendly graphical interface that helps visualization and understanding of the simulated process. Our model also includes temperature abuses during storage by integrating the processing modeling to the storage modeling.

CHAPTER VI

CONCLUSION

The field of computational cross-contamination of leafy-greens in processing facility has arisen as a new branch of food safety to understand pathogen transfer patterns, and to help in planning preventive measures. Therefore, a multi agent-based cross-contamination model of pathogens transfer in a post-harvest facility was developed in this study.

This work represents the development of a food-safety agent-based simulator (FS-ABS) to investigate the stochastic cross-contamination of *E. coli* O157:H7 on fresh-cut Romaine and iceberg lettuces in a processing plant environment. Using the simulation software NetLogo, the FS-ABS model focuses on leafy-greens, and on the impact of cross-contamination, free-chlorine concentration on wash-water pathogen decontamination, and cold room storage facility temperature fluctuation strategies on pathogen growth.

This work provided insights on applications of real-time cross-contamination data in fresh-cut leafy green processing operations. It analyzed the knowledge of cross-contamination information and its impact on processing performance by studying the effect of various mitigation strategies.

Results of a case study of a pilot plant facility for fresh-cut Romaine lettuce demonstrate that real-time track of product contamination level can improve the facility's performance in terms of produce safety at various points in the processing line. The findings enable decision support on which strategies should be implemented to mitigate transfer of pathogens to the produce in the facility. These decisions can reduce losses and in the event of an outbreak, real-time tracking could at least be used as a guide to help estimate the amount of product which may have become cross-contaminated during processing and would need to be recalled.

The sensitivity analysis reveals that selecting lettuce heads contamination level based on the detection limit can lead to processed produce being regarded as safe, resulting in large quantities of produce bags that are unsafe for consumption.

Processing facility configuration is important in explaining the dynamics of lettuce-equipment cross-contamination paths. Spatial and temporal variations in risk of pathogen transferring between agents were well captured by the model. The FS-ABS was designed as a set of modules that can be adapted to any situation and any type of pathogen.

The key factor affecting cross-contamination is the chlorination concentration dose rate. Other factors include the initial level of contamination of the incoming lettuce heads, the probability of contamination in incoming produce, level of contamination, as well as probability of contamination in the facility environment (equipment). Storage room temperature fluctuations showed the importance of real-time monitoring to avoid microorganism growth and thus prevent an increase in the number of contaminated bags. In summary:

- The level of free chlorine in the flume tank affects significantly ($p<0.05$) the level of contamination of the fresh-cut lettuce (contaminated from incoming lettuce head or equipment surface) and then the number of contaminated bags.

- Initial level of contamination on lettuce heads or on the equipment surfaces located at L1 to L4 affects significantly ($p<0.05$) the number of contamination bags processed.

- More bags are contaminated if the initial level of contamination is on the surface of equipment located after the flume tank (at L6 and L7) than on equipment located at locations L1 to L4.

- The number of contaminated bags is affected significantly ($p<0.05$) by the probability of contamination on the incoming lettuce heads or on the equipment located at L1 to L4.

- The probability of contamination on equipment located after the flume tank (L6 and L7) does not affect ($p>0.05$) the number of contaminated bags when chlorination or non-chlorination is used in the flume tank.

- The percentage of contaminated bags decreases when only the first incoming batch is contaminated during the process. The number of contaminated batches decreases with the batch size (kg of lettuce).

- Higher cross-contamination happens with the smaller batch size (36 kg/batch) at the beginning of the process when the first batch is largely contaminated with the pathogen.

- The number of contaminated bags in the storage facility can increase by 80% in 2 days because of temperature abuses.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE STUDY

Recommendations for future research on simulating the effect of *Escherichia coli* O157:H7 cross-contamination during post-harvest of fresh-cut lettuce processing and temperature fluctuation in a cold storage from an agent-based prospective are the following:

(1) Develop a 3-D simulation model of the processing facility to improve visualization, explanation, and understanding of the process.

(2) Cross-contamination between lettuces were considered indirectly, from contaminated surfaces or from contaminated water. Experimental data are needed to model the transfer of microorganism directly among lettuce pieces/heads.

(3) Implement fluid dynamics (aerosol and water flow) and thermodynamics (heat transfer) to calculate changes the environment that affect cross-contamination.

(4) Add biofilm formation in different surfaces/equipment that can affect cross-contamination.

(5) Instead of having lettuce heads moving at a constant rate, make some lettuce pieces stay in the equipment longer. It is very likely to have traces of lettuce pieces from the first batch of lettuces heads after 8 hours of production. These lingering lettuce pieces may become contaminated with bacteria thus increasing the potential for bacteria growth depending on ambient conditions. The temperature did not affect growth during the process because how fast the processing was in comparison to the time needed for growth to occur.

(6) Add freshwater recycling and mixing with tank water at some rate to affect COD (and therefore FC). The current COD function was derived from Luo et al. (2012) and is specific to that situation. COD will decrease if the dirty water is mixed with clean water. This will be useful if we have multiple tank systems or combinations.

(7) Develop experiments on cross-contamination of different pathogens (*Listeria*, *Salmonella*) from different surface materials, e.g., plastic, metals, etc.

(8) Better (realistic with more sensitive bacteria count technique) data are needed to validate the transfer between low amounts of bacteria in the water and product.

(9) Develop an algorithm/random technique to simulate eight hours of processing to account for changes in the processing line (become more dirt/infected) after each batch. Using replication with different random seeds is not making the process realistic.

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