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Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid

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ABSTRACT

A methodology to i) assess the feasibility of water disinfection in fresh-cut leafy greens wash water and ii) to compare the disinfectant efficiency of water disinfectants was defined and applied for a combination of peracetic acid (PAA) and lactic acid (LA) and comparison with free chlorine was made. Standardized process water, a watery suspension of iceberg lettuce, was used for the experiments. First, the combination of PAA + LA was evaluated for water recycling. In this case disinfectant was added to standardized process water inoculated with *Escherichia coli* (*E. coli*) O157 (6 log CFU/mL). Regression models were constructed based on the batch inactivation data and validated in industrial process water obtained from fresh-cut leafy green processing plants. The UV_{254}(F) was the best indicator for PAA decay and as such for the *E. coli* O157 inactivation with PAA + LA. The disinfection efficiency of PAA + LA increased with decreasing pH. Furthermore, PAA + LA efficacy was assessed as a process water disinfectant to be used within the washing tank, using a dynamic washing process with continuous influx of *E. coli* O157 and organic matter in the washing tank. The process water contamination in the dynamic process was adequately estimated by the developed model that assumed that knowledge of the disinfectant residual was sufficient to estimate the microbial contamination, regardless the physicochemical load. Based on the obtained results, PAA + LA seems to be better suited than chlorine for disinfecting process wash water with a high organic load but a higher disinfectant residual is necessary due to the slower *E. coli* O157 inactivation kinetics when compared to chlorine.

1. Introduction

Pathogens have been associated with fresh produce, with leafy vegetables estimated to have the highest risk among them, and with the bacterial pathogens *Escherichia coli* (*E. coli*) O157 and *Salmonella* spp. as the most prevalent pathogens on leafy vegetables (Olaimat and Holley, 2012; Tomas-Callejas et al., 2012). Washing fresh-cut produce removes, next to soil and exudates, a part of the produce-associated microorganisms and transfers them to the water. Therefore, pathogen cross-contamination via water can occur when washing fresh produce and the risk of cross-contamination is not removed by using large quantities of water (Holvoet et al., 2012; López-Gálvez et al., 2009). Washing in disinfectant solutions can be done to enhance the removal of microorganisms from the produce, although the main motivation is to avoid cross-contamination via water. In general, chemical oxidants, including peracetic acid (PAA), are much more effective for inactivation of bacterial pathogens in wash water than for removal of these pathogens from fresh produce (Gil et al., 2009; Sapers, 2001). In addition, once cross-contamination has occurred, rewashing the newly infected lettuce in disinfectant solutions proves unable to completely remove the newly attached *E. coli* O157, even shortly after the contamination event (López-Gálvez et al., 2009, 2010; Luo et al., 2011). Therefore, the primary purpose of washing produce in disinfectant solutions seems to be avoiding cross-contamination via wash water. Furthermore, microbial contamination of produce should be avoided as much as possible by respecting good agricultural and manufacturing practices during the production and processing of fresh produce (Holvoet et al., 2012, 2013; Keskinen et al., 2009; López-Gálvez et al., 2010; Sapers, 2001).

PAA has been suggested as an alternative wash water disinfectant for chlorine. It has been intensively studied for use in wastewater disinfection due to its stability in the presence of organic matter and because it does not produce harmful disinfection by-products (DBPs) (Santoro et al., 2007; Stampi et al., 2001). These properties make it attractive for use in fruit and vegetable washing processes, it has been studied as

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disinfectant for washing a variety of fruits and vegetables (González-Aguilar et al., 2012), and it has been commercialized in combination with lactic acid (LA) for washing salads as Fresh Rinse® (Ho et al., 2011).

Water disinfection in fresh-cut industry is carried out in washing tanks (immersion washers), where fresh-cut vegetables are washed, under agitation applied by water, air, sound or mechanical devices (Pao et al., 2012). Alternatively, non-immersion washers that wash produce by spraying or rinsing can be applied but the latter is not the focus of this article. Disinfection processes in this context can be divided as: i) process wash water disinfection in the washing tank and ii) process water recycling outside the washing tank. Process water recycling is defined as inactivation of microorganisms in process water outside the processing line before reuse in the washing process. Process wash water disinfection concerns the inactivation of incoming microorganisms by keeping a disinfectant residual in the washing tank.

The performance of water disinfection in fresh(−cut) produce washing operations will depend on the disinfectant residual (and therefore the disinfectant dose, disinfectant demand and water refreshing rate), the resistance of the target microorganism and the physicochemical conditions of the wash water (organic matter, pH, T) (Van Haute et al., 2015). Mathematical models can be applied to understand the relation between the disinfection efficiency and the influential variables. This knowledge can be used to allow a more calculated approach to the decision making process of implementing a water disinfection technique in fresh(−cut) washing operations.

To study and compare the performance of water disinfectants, an experimental setup that incorporates these factors and that can be applied to study different water disinfectants under similar conditions seems to yield a greater value for industry and governmental agencies than a collection of studies, each with unique and independent experimental setups. In a previous study models were used to understand the relation between chlorine disinfection efficiency and the physicochemical quality, the disinfectant residual and the water refreshing rate in fresh(−cut) produce washing operations (Van Haute et al., 2013). In this study PAA is researched according to a similar experimental setup, both to understand the behavior of PAA in these operations, as well as to compare it with free chlorine.

2. Materials and methods

2.1. Experimental setup modeling

For the process water recycling, inactivation models were calibrated in standardized process water (SPW) with controlled physicochemical parameters and inoculated with *E. coli* O157 (Fig. 1). Both statistical and kinetic models (based on PAA decay) were considered. PAA decay is the decrease in PAA concentration in the water, primarily due to reaction with water matrix constituents. Repetition of the experiments in industrial process water (IPW) was executed for generation of validation data in order to assess the validity of the constructed models. Three replicates of each experiment were performed.

For the process wash water disinfection, a dynamic leafy vegetables washing process was simulated (Fig. 1). The microbial contamination was introduced by continuous in- and outflow of inoculated SPW. The experiment was initiated with tap water, after which a chemical oxygen demand (COD) build-up occurred due to continuous introduction of SPW. Semi-mechanistic models were constructed based on *E. coli* O157 inactivation constants and experimental operational data (water refreshing rate, contamination inflow, disinfectant residual concentration). Models were validated with measured *E. coli* O157 wash water contamination values. The experiment consisted of one trial of 60 min.

2.2. Preparation of standardized process water (SPW)

Two types of standardized process water were produced. For the water recycling experiments, iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale market in Ghent (Belgium) and transported within 15 min to the laboratory, where it was kept at 4 °C before use. Water with high COD was prepared following the procedure described in López-Gálvez et al. (2012), and then was filtered through the filter of a stomacher bag (Seward, UK), in order to separate big solid particles. Afterwards, samples were taken to measure COD and water was kept at 4 °C before use (always the same day as the preparation). Finally, SPW with different levels of COD (500, 800, or 1500 mg/L) was prepared by mixing the adequate volume of high COD water with tap water. In the case of the process wash water disinfection experiments, SPW from spinach (*Spinacia olearacea* L.) was made as described by Gómez-López et al. (2014).

2.3. Collection of industrial process water (IPW)

Wash water from two fresh-cut produce companies was collected into sterile recipient containers and transported under refrigerated conditions to the laboratory, where it was stored at 4 °C for a maximum of 24 h. At company 1, tap water was used as the water source during washing of sugarloaf (*Cichorium intybus*), iceberg lettuce, endive and radicchio. Company 2 utilized borehole water for processing butterhead lettuce, iceberg lettuce, endive, and radicchio.

2.4. Bacterial inoculation

Two attenuated (non-verotoxin producing) nalidixic acid resistant *E. coli* O157 strains (LFMFP 662 and 679) were used. The strains were grown at 37 °C for 24 h in Brain Heart Infusion (Oxoid, United Kingdom) containing 50 μg/mL nalidixic acid (Sigma-Aldrich, Belgium). LFMFP 662 is a nalidixic acid-resistant version of the strain CECT 5947 provided by the Hibro Group from the University of Cordoba (Spain), while LFMFP 679 is a nalidixic acid-resistant version of the strain MB3885 provided by the Technology and Food Science Unit from ILVO (Belgium). A cocktail was made by combining volumes of individual strains. Cocktails were centrifuged at 4 °C, 1800 *g* for 10 min. The pellets were washed twice in phosphate buffer (pH 7), with intermittent centrifugation, and subsequently resuspended in phosphate buffer.

2.5. Disinfection treatments

Disinfectant solutions consisted of a combination of PAA (Chriox 5, Christeyns NV, Belgium) and LA (Purac Biochem, The Netherlands). PAA + LA solutions were used in a mass ratio of 1:40 in all experiments (Ho et al., 2011).

2.5.1. Process water recycling

The two different types of SPW and the IPW from two fresh-cut produce companies were inoculated with the *E. coli* O157 cocktail to a level of approximately 6 log CFU/mL just before the beginning of the treatment. The SPW was continuously stirred during the experiment. Disinfectant solution was added to obtain the desired PAA and LA concentrations, and samples for microbiological analysis were taken periodically. All water recycling experiments were performed in triplicate at 5 °C. To assess the influence of pH on *E. coli* O157 inactivation, inactivation in oxidant demand free buffer was executed in the same way as described for the water recycling experiments. The acid dissociation constant of PAA is 8.2 (Kitis, 2004). Buffer solutions at pH 6, pH 8.2 (consisting both of phosphate buffer 0.07 M) and pH 10.2 (carbonate buffer 0.1 M) were used to manipulate the acid dissociation of PAA to 1%, 50% and 99% respectively.

2.5.2. Process wash water disinfection

Disinfection experiments were performed using a pilot plant system that has been used as a standard dynamic system in previous studies (Gómez-López et al., 2014). Process wash water disinfection treatments were performed starting with clean potable water and applying a