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Efficacy of activated persulfate in inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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ABSTRACT

Concerns have been on the rise regarding the use of chlorine-based sanitizers for fresh produce sanitation due to the production of toxic disinfection by-products (DBPs). This study was undertaken to evaluate the efficacy of activated persulfate in inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in pure culture. The objectives were to study the effect of persulfate to activator ratios and determine the major contributing radical in pathogen inactivation. A five-strain cocktail of each pathogen was treated with sodium persulfate activated by ferrous sulfate or sodium hydroxide for 60 s or 120 s. Non-selective agars supplemented with sodium pyruvate were used for pathogen enumeration. The steady-state concentrations of free radicals were quantified using HPLC-DAD. Radical scavengers (tert-butanol, isopropanol, and benzoquinone) were used to determine the major contributing radical in pathogen inactivation. The results showed more than 7 log CFU/mL reductions can be achieved in 120 s for both pathogens at appropriate activation conditions. For ferrous activation, the persulfate to ferrous ratio played an important role in the overall inactivation efficacy. The maximum pathogen reduction (7.77 log CFU/mL for *E. coli* O157:H7 and 7.25 log CFU/mL for *L. monocytogenes*) was achieved at persulfate to ferrous molar ratio of 1:0.33 when the initial persulfate concentration was set at 40 mmol/L. Further increase or decrease of ferrous ratio always leads to lower pathogen reductions. For alkaline activation, the inactivation efficacy increased with more initial sodium hydroxide. The maximum reduction was achieved at 40 mmol/L persulfate with 30 mmol/L sodium hydroxide for *E. coli* O157:H7 (6.21 log CFU/mL reduction) and at 500 mmol/L persulfate with 350 mmol/L sodium hydroxide for *L. monocytogenes* (8.64 log CFU/mL reduction). Also, persulfate activated by sodium hydroxide always achieved significantly ($P < 0.05$) higher microbial reductions than sodium hydroxide or persulfate alone. *L. monocytogenes* was generally more resistant against the activated persulfate treatment compared with *E. coli* O157:H7, which might be due to the different cell envelop structures between Gram-positive and Gram-negative bacteria. Hydroxyl radical was demonstrated to be the major radical to inactivate both pathogens in ferrous activation while superoxide radical was demonstrated to be the major radical to inactivate both pathogens in alkaline activation.

1. Introduction

Fresh fruits and vegetables are essential sources of numerous nutrients, such as vitamins and minerals (Asmita et al., 2016). The fresh produce industry has become one of the most important parts of global food market (Joshi et al., 2013). However, the trend of increasing consumption of fresh produce coincided with the increasing cases of foodborne illnesses and outbreaks (Afari et al., 2015). This is mainly because fresh produce are suitable habitats for pathogenic microorganisms due to the high moisture and natural openings (Carlin, 2007) as well as the fact that fresh produce are usually consumed raw (Yeni et al., 2016). Chlorine based-sanitizers, such as sodium hypochlorite, are the most commonly used disinfectant chemicals in the fresh produce industry because of the low cost and high efficacy in pathogen inactivation (Feliziani et al., 2016). However, the production of toxic disinfection by-products (DBPs), such as trihalomethanes, haloacetic acids, and nitrogenous DBPs, during chlorine sanitation has drawn attentions as these DBPs pose potential threats to consumers' health (Joshi et al., 2013; Legay et al., 2010). For example, one epidemiological study has suggested pregnant women exposed to DBPs have an elevated risk of delivering babies with cardiovascular defects (Wright et al., 2017). A recent study also found sodium hypochlorite washed lettuce contained significantly more toxic DBPs than unwashed controls (Lee and Huang, 2017). Alternative sanitizers that are effective in

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pathogen removal with less or no toxic DBPs production are therefore needed to ensure produce safety.

There have been studies that investigated alternative and novel disinfectants for produce disinfection, such as ozonated water, chlorine dioxide, and electrolyzed oxidizing water. However, all these techniques have their limitations making them unsuitable for widespread application. Ozonated water has been approved as GRAS by FDA as an effective disinfectant against bacteria, fungi, protozoa, and microbial spores (Joshi et al., 2013). However, ozone is very unstable and can be toxic in gaseous phase by causing irritations to eyes and the respiratory system (Artes et al., 2009). Chlorine dioxide has also been considered as an alternative due to the high efficacy in pathogen inactivation and low reactivity with organic matters (Joshi et al., 2013), but the explosive potential of chlorine dioxide is a safety concern. Electrolyzed oxidizing water has been extensively studied and reported by numerous scientific publications (Gil et al., 2009; Huang et al., 2008). Although electrolyzed oxidizing water is considered advantageous against bleach solutions, the major active compounds are free chlorine that would still present toxic DBPs problems.

Activated persulfate, a relative new advanced oxidation process (AOP) that has been extensively studied by environmental scientists, shows a great promise to be used for fresh produce sanitation. Persulfate usually exists in the form of salts, such as sodium persulfate (Tsitonaki et al., 2010). Persulfate is a thermodynamically strong oxidant but not reactive in chemical degradation through direct reaction (Devi et al., 2016). However, persulfate can be activated by activators, such as transition metals, alkaline reagent, and UV light, to produce reactive free radicals (Eq. (1)) (Matzek and Carter, 2016). These intermediate free radicals are highly oxidative and can degrade many recalcitrant organic chemicals, such as perfluorooctanoic acid (Yin et al., 2016), benzotriazole (Xiong et al., 2014), trichloroethylene (Liang et al., 2009), and ibuprofen (Paul et al., 2014). Although the efficacy of activated persulfate in degrading organic chemicals has been well studied, its effect on pathogen inactivation has not been fully explored. To our best knowledge, there are only two published papers that have studied the efficacy of activated persulfate in pathogen inactivation. Sun et al. (2016) found UV-254 nm activated persulfate can effectively inactivate pathogenic *Escherichia coli* while Wordofa et al. (2017) revealed ferrous activated persulfate could reduce the viability of *E. coli* O157:H7.

$$S_2O_8^{2-} + \text{activator} \rightarrow SO_4^{-}\cdot + (SO_4^{-}\cdot \text{ or } SO_4^{2-}) \quad (1)$$

The overall objective of the present work was to systematically evaluate the efficacy of ferrous and alkaline activated persulfate to inactivate *E. coli* O157:H7 and *Listeria monocytogenes* in cell suspensions and identify the appropriate activation conditions. Specific objectives include: 1) to determine the effect of persulfate to activator ratios in pathogen inactivation; 2) to quantify free radical concentrations during persulfate activation; 3) to determine the major contributing radical in pathogen inactivation for each activation method.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from either Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Fair Lawn, NJ, USA). The chemicals from Sigma-Aldrich include sodium persulfate, sodium thiosulfate, sodium chloride, sodium phosphate dibasic, anhydrous magnesium sulfate, benzoquinone (BQ), anisole and nitrobenzene (NB). The chemicals from Fisher Scientific include ferrous sulfate, ferric sulfate hydrate, sodium hydroxide solution (10 mol/L), sodium phosphate monobasic monohydrate, acetonitrile, isopropanol alcohol (IPA), and tert-butanol alcohol (TBA). All chemicals were at least reagent grade.

2.2. Bacterial cultures

Five strains of nalidixic acid-adapted *E. coli* O157:H7 and *L. monocytogenes* were obtained from USDA or University of Georgia Center for Food Safety (CFS). The *E. coli* O157:H7 strains were 1 (beef isolate), 4 (human isolate), 5 (human isolate), E009 (beef isolate), and 932 (human isolate). The *L. monocytogenes* strains were Scott A (human isolate), LCDC 81–861 (raw cabbage isolate), F8027 (celery isolate), H77–50 (hot dog isolate), and F8369 (corn isolate). All strains were stored at −70 °C and recovered in tryptic soy broth (TSB, Difco, Sparks, MD, USA) for *E. coli* O157:H7 or brain heart infusion broth (BHIB, Difco, Sparks, MD, USA) for *L. monocytogenes* at 37 °C for 24 h. Then, a loop of each cultured broth was transferred to TSB supplemented with 50 mg/L nalidixic acid (TSBNA) or BHIB supplemented with 50 mg/L nalidixic acid (BHIBNA) and incubated at 37 °C for 24 h. The resulting cultures were confirmed by streaking onto sorbitol MacConkey agar (SMAC, Criterion, Santa Maria, CA, USA) for *E. coli* O157:H7 or Palcam Listeria selective agar (LSA, Bioworld, Dublin, OH, USA) supplemented with Palcam Listeria selective supplement (EMD Millipore Corporation, Billerica, MA, USA) for *L. monocytogenes* and incubated at 37 °C for 24 and 48 h, respectively. The same cultures were used as stock cultures after confirmation. Working cultures were made by sub-culturing the stock cultures twice in TSBNA (*E. coli* O157:H7) or BHIBNA (*L. monocytogenes*), each at 37 °C for 24 h. After sub-culture, the bacterial cells in the broth were harvested by centrifuging at 3000 × *g* for 12 min. The cell pellets were washed with 20 mmol/L phosphate buffer saline (PBS, pH 7) once and re-suspended in PBS. Finally, equal volumes of each strain of each pathogen were mixed together to obtain a five-strain cocktail for the following experiment.

2.3. Activated persulfate treatment

One mL of prepared bacterial suspensions was added into sterile water containing pre-calculated amount of sodium persulfate in 150 mL sterile glass beakers. The beakers were placed on a magnetic stirrer plate and a magnetic bar spinning at high speed inside the beaker was used to keep the mixture homogenized. The treatment was initiated by adding specific amount of activators (200 mmol/L ferrous sulfate or 1 mol/L sodium hydroxide). The total volume of the mixture was 25 mL. At 60 s and 120 s, 1 mL was withdrawn from the mixture and transferred into 9 mL neutralizing buffer containing 5 g/L sodium thiosulfate and 20 mmol/L PBS for ferrous activation to neutralize the residual persulfate (Kambhu et al., 2012) or 100 mmol/L PBS for alkaline activation to neutralize the pH to quench the activation. Radical scavengers were also spiked into the activated persulfate mixtures to identify the major contributing radicals in pathogen inactivation. The radical scavengers used include IPA (scavengers for both hydroxyl and sulfate radicals, $k_{HO\cdot,\ IPA} = 1.9 \times 10^9\ L{\cdot}mol^{-1}\ s^{-1}$, $k_{SO_4^-\cdot}$, IPA $= 8.2 \times 10^7\ L{\cdot}mol^{-1}\ s^{-1}$), TBA (scavenger for hydroxyl radical only, $k_{HO\cdot,\ TBA} = 5.2 \times 10^8\ L{\cdot}mol^{-1}\ s^{-1}$, $k_{SO_4^-\cdot}$, TBA $< 10^6\ L{\cdot}mol^{-1}\ s^{-1}$), and BQ (scavenger for superoxide radical only, $k_{O_2^-\cdot}$, BQ $= 9.6 \times 10^8\ L{\cdot}mol^{-1}\ s^{-1}$) (Wu et al., 2014).

2.4. Microbiological analysis

The populations of each pathogen after the treatment were determined by plating 0.1 mL of 10-fold serial dilution of the neutralized 10 mL mixtures onto tryptic soy agar supplemented with 0.1% sodium pyruvate (TSASP) for *E. coli* O157:H7 and brain heart infusion agar supplemented with 0.1% sodium pyruvate (BHIASP) for *L. monocytogenes* in duplicate. To determine samples with low populations, 0.25 mL of non-diluted samples were also plated in quadruplicate. The TSASP plates were incubated at 37 °C for 24 h while the BHIASP plates were incubated at 37 °C for 48 h before enumeration. Enrichment was also conducted by transferring 1 mL of the neutralized mixture into 9 mL of TSB supplemented with 0.1% sodium pyruvate (*E. coli*