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Comparative Assessment of *Salmonella* Inactivation and pH Changes in Poultry Carcasses at Composting and Mesophilic Temperatures

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Abstract

Controlling pathogens in poultry carcasses is a serious issue. During catastrophic outbreaks in poultry, an enormous amount of dead birds are depopulated causing potential risk to environment. Mitigating the risk of pathogen dissemination caused by poultry carcasses requires identifying the safe methods for poultry carcass disposal. Composting of birds is often a preferred method for disposal assuming that the increased temperature (>55 ^{0}C) of composting piles will be likely to kill

pathogens attached with poultry carcasses. However, the temperature of composting piles is often uncertain, which depends on many factors including weather and feedstock. It is not unusual that the composting pile temperature is well below the assumed composting temperature (≈ 55-65 ^{0}C). To improve the understanding of *Salmonella* inactivation in poultry carcasses, we have assessed the effect of composting (≈ 62.5 ^{0}C) and mesophilic temperatures (≈ 38 ^{0}C) on *Salmonella* inactivation in ground poultry carcasses. Further, we evaluated the change in pH levels over the time in both temperatures. Results showed that at ≈ 62.5 ^{0}C, *Salmonella* levels reached to non-detectable levels in less than 5 hours under mixing and non-mixing conditions. However, *Salmonella* survival was prolonged during mesophilic temperature (≈ 38 ^{0}C) for more than 100 hours. At higher ≈ 62.5^{0}C, *Salmonella* inactivation was similar in both mixed and non-mixed condition, while at ≈ 38 ^{0}C *Salmonella* inactivation was increased in non-mixed condition. The pH values were comparable in both mixed and non-mixed conditions. At 38 ^{0}C, pH was slightly increased (changed from 6.0 to ≈ 7.5), while at 62.5 ^{o}C pH values remained steady ≈ 6.2.

Keywords. Composting, poultry carcass, *Salmonella*, temperature, pH change

Introduction

The disposal of dead poultry is an increasing concern, particularly during the catastrophic outbreaks, which requires the disposal of huge amount of poultry carcasses (Brglez and Hahn 2008, Gwyther, Williams et al. 2011). Poultry carcasses are considered to be a reservoir of many pathogens including *Salmonella*. Outbreak of salmonellosis caused by *Salmonella* is considered as the most common food-borne bacterial disease, which poses potential threat to biosecurity (Forshell and Wierup 2006). Each year more than 155,000 deaths and greater than 90 million incidences are caused by *Salmonella* (Majowicz, Musto et al. 2010, Chen, Wang et al. 2013). In addition to human

health issue, *Salmonella* is also a major pathogen of concern in the meat and poultry industry. The contamination of *salmonella* in poultry carcass and associated food risk is well documented elsewhere (Forshell and Wierup 2006).

To mitigate the risk of *Salmonella*, composting of poultry carcasses is a widely used method to dispose dead birds in developing as well as developed countries (Ritz 2008, Collins Jr 2009). During composting, temperature and moisture content of the composting piles enhances the decomposition poultry carcasses (Wilkinson 2007, Sivakumar, Kumar et al. 2008). Elevated temperature profile of compost piles accelerates pathogen inactivation (Kim, Diao et al. 2012). Despite the common understanding of pathogen inactivation in elevated temperature during composting processes, additional researches describing the pathogen inactivation at compost as well as mesophilic temperatures are needed. In order to optimize the pathogen inactivation during composting process, it is important to understand how a range of temperature influences pathogen survival. The United States Environmental Protection Agency (US EPA) has derived the guideline for pathogen inactivation indicating that the temperature of the pile must be maintained over 55 ^{o}C for more than 3 days (Wichuk and McCartney 2007). Currently, the efficacies of the methods used for treating poultry in *Salmonella* inactivation is not well understood, however (Mead, Lammerding et al. 2010). The goal of this study is to enhance the understanding of *salmonella* inactivation in ground poultry carcasses at composting (62.5 ^{o}C) and mesophilic (38^{o}C) temperatures. The primary objectives of the study are to: 1) assess the impacts of temperatures in *Salmonella* inactivation of ground poultry carcasses in mixed and non-mixed conditions; 2) evaluate the changes in pH levels of ground poultry carcasses during the digestion processes.

Material and Methods

To test *Salmonella* inactivation in ground poultry carcasses, specific pathogen free (SPF) birds were obtained from Charles River Laboratories Inc., New York, USA (www.criver.com)). The poultry carcasses were disintegrated into small pieces with the sterile knife. Subsequently, the pieces were blended using a blender (Ninja model BL800).The ground birds were diluted with 4.5-fold deionized water. Initial sample of SPF poultry was plated on Difco Xylose Lysine Deoxycholate (XLD) (Becton, Dickinson and Company, Sparks, MD, USA) agar plates to confirm the absence of *Salmonella*. The ground poultry carcasses were mixed with *Salmonella Typhimurium* LT2 culture, which was grown in Difco LB (Luria-Bertani) Broth Miller growth media for 24 hours. Subsequently the pellets of *Salmonella* were dissolved in the ground carcasses. The inoculated ground poultry carcasses were fed into two reactors (800 mL), which were subjected to mixed (continuous at 50 rpm) and non-mixed conditions. The digestion process was executed in controlled temperature conditions. A10L isotemp water bath (Thermo-Fisher Sci.) shown as Figure was used to maintain the desire temperature.

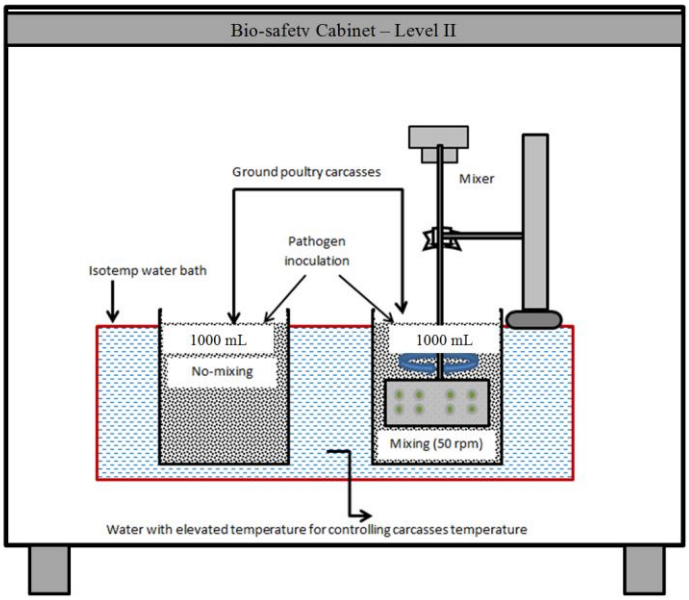

Figure. 1. Sketch of eexperiment setup

Samples were collected and processed with regular interval for *Salmonella* enumeration and pH assessment. *Salmonella* was quantified using the standard FDA Bacteriological Analytical Manual procedure (BAM) (USFDA, 2015), and a hand held pH meter was used for pH measurement.

Results and Discussion

The transmission electronmicrograph shows the ground poultry carcasses (Figure 2) indicating the

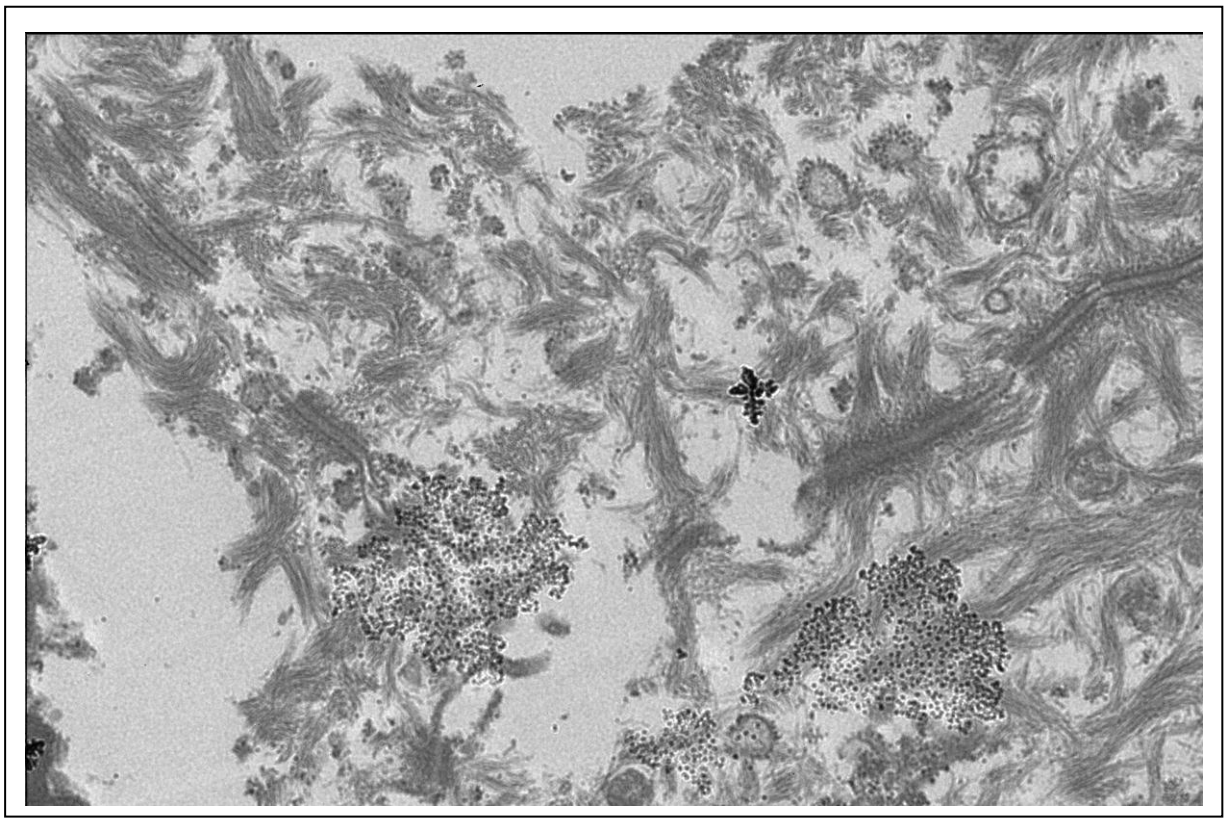

Figure 2. Transmission electron micrograph of ground poultry carcass.

poutry dissue tissues with spherical structures similar to bacteria/*Salmonella*. The change in *Salmonella* levels at 38 ^{0}C under mixed and non-mixed conditions is shown in Figure 3. Initial *Salmonella* concentration was about 10^8 CFU/mL. As shown in the figure, during the first 80 hours *Salmonella* inactivation was slow (*Salmonella* levels reduced from 9 to ≈ 8 orders of maginuted).

However, beyond 80 hours, a sudden collapse in *Salmonella* levels was observed. Under mixed condition, *Salmonella* concentration decreased to undetectable level in about 120 hours while it took 100 hours in non-mixed condition. Persistance of *Salmonella* at 62.5^{o}C is shown in Figure. 4. The initial *Salmonella* level was about 8 orders of magnitude. In ≈ 5 hours, *Salmonlla* levels

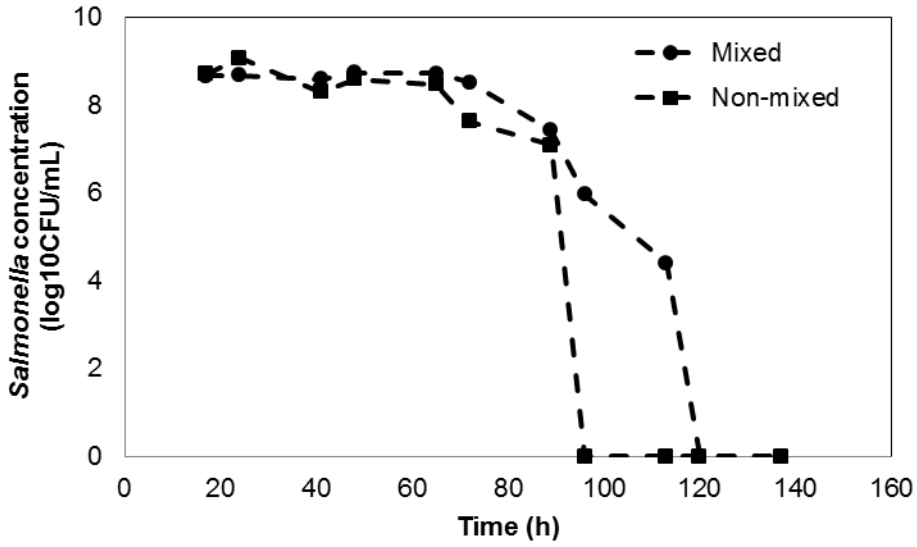

Figure 3. *Salmonella* persistance during composting at 38 ºC under mixed and non-mixed conidtions.

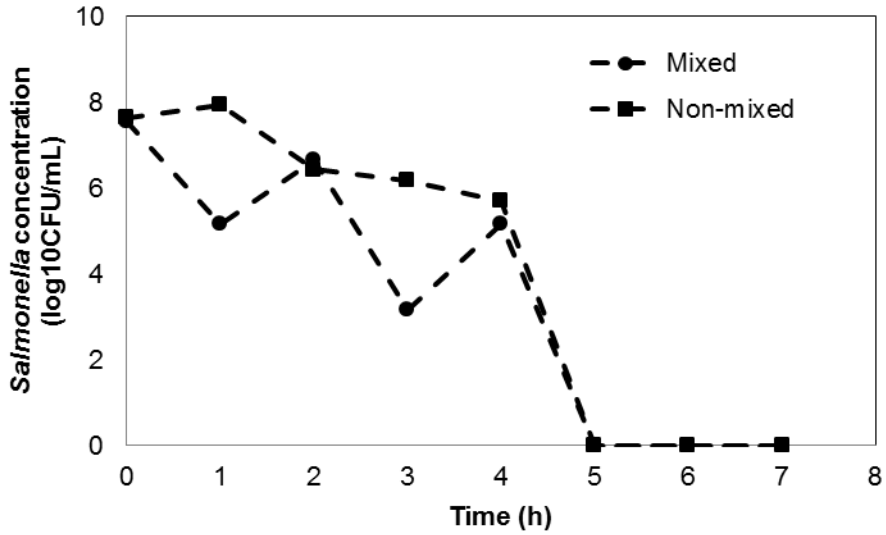

Figure 4. *Salmonella* persistance during composting at 62.5 ºC

in both mixed and non-mixed conditions were decreased to undectable levels. Compared with the result of 38 ^{o}C, composting at 62.5 ^{o}C was more effective to inactivate *Salmonella* levels indicating the considerable effects of temperature during the composting of poultry carcasses in pathogen inactivaiton. As shown in the Figures, the effects of mixing in pathogen inactivaiton was minimal at 62.5 $^{\circ}$C. Under 38 $^{\circ}$C, the inactivation of *Salmonella* in non-mixing condition was faster than that of the mixing condition. Further experiments are needed to quantify the role of mixing in pathogen inactivation durting ground poultry carcass composting as well as whole bird carcass composting. The details of pH variation in poultry carcasses at 38 $^{\circ}$C and 62.5 $^{\circ}$C are shown in Figures 5 & 6. At 38 $^{\circ}$C, the intial pH was about 6, indicating the slightly acidic environment at first. The pH values in both mixed and non-mixed conditions conditions were increased to ≈ 7 in about 48 hours. The similar trends indicate no considerable impacats of mixing and non-mixing on pH changes. At 62.5 ^{o}C, initial pH was ≈ 6 and it kept relatively stable during 7 hours of experiment. Similar to 38 $^{\circ}$C, both mixed and non-mixed showed similar pH (≈ 6.2) values, hence no effects of mixing.

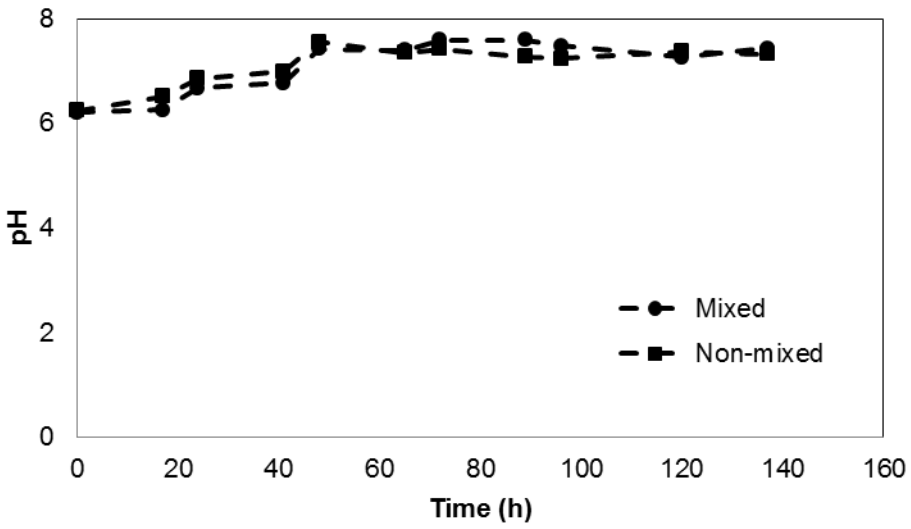

Figure 5. pH variation at 38 $^{\circ}$C under mixing and non-mixing conditions

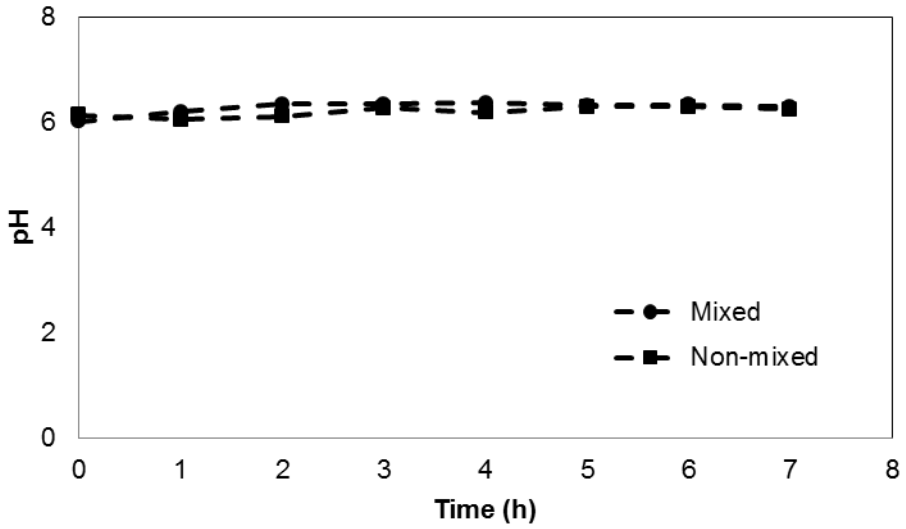

Figure 6. pH variation at 62.5 °C under mixing and non-mixing conditions

Conclusions

In this study, a comparative assessment of *Salmonella* inactivation in poultry carcasses at composting (62.5 °C) and mesophilic (38 °C) temperatures was determined. The effects fo mixing and non-mixing in the pathogen inactivaiton was tested. Results showed that the elevated temperature increased *Salmonella* inactivation considerably in poultry carcasses. At 62.5 °C, *Salmonella* levels reached to non-detectable levesl in 5 hours, while at 38 °C more than 100 hours were needed for eliminating the pathognes. While at 62.5 °C both mixing and non-mixing have similar effects in the pathogen inactivation, at 38 ºC, the inactivation was faster in the non mixed conditons. In mesophilic conditions, pH of ground poultry carcasses was increased, while at composting temperature pH remained steady. Aditional ffield scale studies focused on understading the effects of changing temperature and moisture contentent of composting piles in the inactivaiton of various pathogens such as *Salmonella* and *E. coli* are needed to improve the existing understainng of pathogen inactivaiton in poultry carcasses during composting process .

Acknowledgment

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