ACID ELECTROLYZED WATER DISINFECTION OF PSEUDOMONAS FLUORESCENS ADHESIVE BIOFILMS, DETACHED BIOFILMS AND PLANKTONIC CELLS

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I. INTRODUCTION

Bacteria in adhesive biofilms (AB), including the detached biofilms (DB) triggered by environmental changes, are more resistant to disinfectants than the planktonic cells (PC) [1]. Regular disinfectants may not be effective and safe enough in disinfecting cells within biofilms. Acid electrolyzed water (AEW) has been seen as a promising sanitizer in food industry and other fields [2]. Although biofilms are hazardous, studies on the application of AEW for disinfecting biofilms are still lacking [3]. *Pseudomonas fluorescens* was selected as an experimental organism because it represents one of the most important spoilage bacteria in food products [4]. The Weibull model was applied to describe the inactivation kinetics of disinfectants on food and food-contact surfaces [5]. Therefore, the aim of the present study was to assess the disinfectant efficacy of AEW against *P. fluorescens* using the Weibull method.

II. MATERIALS AND METHODS

Pseudomonas fluorescens (NCM 90) was collected from spoiled chicken carcasses and conveyor belt surfaces in a slaughter plant. The biofilm formation was determined according to Wang *et al*. with some modification [6]. Cultures were incubated in trypticase soy broth at 20 °C for 5 days. The AEW (20, 40 and 60 mg/L) were prepared as discribed by Duan *et al*. [7]. For AB, the rinsed stainless steel coupons were immersed in sterile 0.9% NaCl or AEW. Subsequently, the neutralizing agent (PBS containing 0.8% $Na_2S_2O_3$) was added to interrupt the bactericidal effects. Then AB were scraped and serial dilutions in 0.9% NaCl. For DB, the cells were scraped from the stainless steel coupons and exposed to AEW or 0.9% NaCl. For PC, the cell concentrations were adjusted to 8-9 log CFU/mL with 0.9% NaCl and exposed to AEW or 0.9% NaCl. Then the cells were immediately transferred to neutralizing solution.The cell counts of all sample types were determined by trypticase soy agar after 24 hours at 28 °C.

The Weibull model was used to fit the data of survival curves using the equation: Log N/N0=-at^b. N, N0 are the cell number of bacteria counts at time t and initial, while a and b are the scale and shape factors. To evaluate the membrane integrity (MI), the BacLight LIVE/DEAD membrane permeability kit (Invitrogen, Carlsbad, CA, USA) was used following the manufacturer guidelines. *P. fluorescens* cells were analyzed after 5, 15, and 30 min exposure to AEW for three sample types respectively. Curve Expert Version 1.4 was used for non-linear regression analysis of Weibull model. The reported results represent an average of each experiment assay and were analyzed by a one-way Duncan's test using SPSS (SPSS Inc., Chicago, USA) to assess whether significant differences ($P < 0.05$) existed.

III. RESULTS AND DISCUSSION

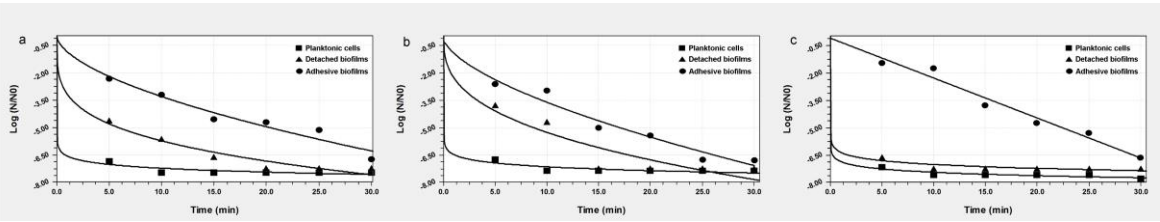

Fig. 1. Survival curves of *Pseudomonas fluorescens* treated with (a) 20 mg/L, (b) 40 mg/L and (c) 60 mg/L of acid electrolyzed water. The figure shows the mean values of six independent experiments fitted by the modified Weibull model.

In this model, the R^2 values were between 0.974 and 0.999, indicated that the agreement between the data and calculated values for Weibull model was a good fit through the variation of a and b parameters (data not shown). The thorough disinfection was acquired in 5, 15 and 30 min for PC, DB and AB, respectively (Fig. 1).PC exhibited lower resistance than DB and AB at the same concentration, as reflected by the higher scale (a) and lower shape (b) parameters. Measurement of MI represents one useful indicator of cell functioning. With the longer contact time and higher concentrations of AEW (Fig. 2), more membranes were compromised. At the final time points, the red/green ratio of the DB was higher than that of PC, followed by AB. The results showed that the extent of cell membrane damage was much smaller than that obtained by CFU counting. Thus, the red/green ratio of fluorescence is a conservative estimate of membrane damage in *P. fluorescens*. Overall, AEW can be an excellent alternative to regular disinfectants and can be applied to control *P. fluorescens* in food processing facilities as well as protecting foods from cross-contamination.

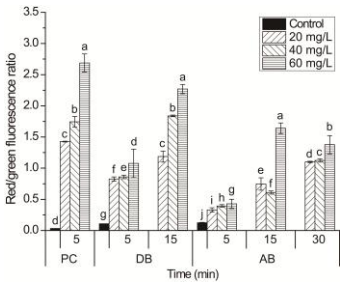

Fig. 2. Membrane integrity of *Pseudomonas fluorescens*. Bars represent the standard deviation (n = 3). Different letters on the top of data bars indicate significant differences ($P < 0.05$).PC, planktonic cells; DB, detached biofilms; AB, adhesive biofilms.

IV. CONCLUSION

The Weibull model was a flexible model to fit the data for PC, DB and AB treated with disinfectants. The effects on DB and PC were similar but were very different from that with AB. AEW decreased the bacterial membrane integrity, and these data support the effectiveness of AEW against *P. fluorescens*.

ACKNOWLEDGEMENTS

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References should be arranged numerically by order of appearance. References should be presented in the following format:

Paper:

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