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Author: Parvin Mahdavi

Title: Antibiofilm effects of aqueous ozone, acetic acid and lactic acid on *salmonella typhimurium* and *staphylococcus aureus*

Summary: Today, biofilms are known as a major source of food contamination, and the main concern regarding biofilms is their high resistance to disinfectants and antimicrobials. *Salmonella typhimurium* and *Staphylococcus aureus* bacteria have a good ability to form biofilm, which are the most important pathogenic factors in livestock and humans. The purpose of this research was to investigate the anti-biofilm effects of ozone water, acetic acid (%2) and lactic acid (%2) individually and sequentially on the biofilm of *Salmonella Typhimurium* and *Staphylococcus aureus*.

After adjusting to 10^6 CFU/ml, the desired bacteria were cultured on 96-well microplates for 48 hours to form a biofilm. The intended treatments, which included ozone water (2ppm), acetic acid (%2), lactic acid (%2) and sequential treatment of ozone water and acid, were applied on bacterial biofilms and the amount of biofilm reduction was determined by two methods: crystal Violet and Viable count. The findings of this research showed that the highest percentage of biofilm reduction by the crystal violet method was related to the successive treatment of ozone water and acetic acid, which was able to reduce *Staphylococcus aureus* biofilms by %44/36. Also, ozone water and lactic acid treatment could reduce *Salmonella Typhimurium* biofilms by %57/26. In the Viable count method, the most effective treatment was the sequential treatment of ozone and lactic acid, which reduced *Staphylococcus aureus* and *Salmonella typhimurium* biofilms by 1/76 and 4/06 log, respectively. Our findings showed that successive treatment (ozone-acid water) was effective in reducing bacterial biofilm, so it can be used to control *Salmonella typhimurium* and *Staphylococcus aureus* biofilm to ensure food safety and promote public health.

S. aureus, *Sallmonella typhimurium*, Acetic acid, Lactic acid,,Ozone ,Biofilm **Key words:**