205 Control of planktonic bacterial cells and biofilms through magnetic hyperthermia

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Disinfection of surfaces is a challenging task aggravated by bacteria's capacity to form biofilms, which enables them to survive and resist a wide variety of antimicrobial agents and hostile conditions. Potential application of magnetic hyperthermia (MH) as a new disinfection method against biofilms has been recently proposed, however, studies comparing its performance and effectiveness on planktonic and biofilm cells from the same bacterial species remain unexplored. This work evaluated the effect of MH generated by iron oxide magnetic

nanoparticles (MNP) against planktonic and biofilm cells of *Pseudomonas fluorescens*, a major food spoilage microorganism. A *P. fluorescens* collection strain (ATCC 27663) was used and its biofilms allowed to form on silicone coupons during three days incubation in tryptic soy broth culture medium, at room temperature (20 ± 2ºC) and constant agitation of 120 rpm. Hyperthermia experiments were performed by applying an oscillating magnetic field of 873kHz and 100 Oe to several identical solutions of bacteria and MNP. To study cell viability as a function of temperature, magnetic heatings were performed at the same heating rate and up to different maxium temperatures. Bacterial survival was assessed through colony forming units count, while confocal laser scanning microscopy (CLS) was used to evaluate cellular membrane integrity of both bacterial life forms, as well as eventual effects of MH in biofilms' structure. Results showed a significant reduction (3 Log) of viable planktonic cells when a maximum temperature of 40ºC was reached, corresponding to only about 3 minutes of exposure to alternate magnetic field. A complete cellular eradication was achieved after only 8 minutes, when the maximum temperature was increased up to 55ºC. In contrast, a significantly lower reduction of cellular viability was accomplished for biofilms at the same temperatures, and no eradication was achieved even after 17 minutes of magnetic field exposure, reaching a maximum temperature of 60ºC. CLS images showed that MH inflicted cellular membrane damages both in planktonic and biofilms cells, and also suggested that the outer cell layers of biofilms were more damaged than inner ones, as denoted by the higher amount of injured cells observed in the external layers. Summarizing, this work confirms the potential of MH as a disinfection method and shows for the first time its efficacy against a food spoilage microorganism. More importantly, it presents the first insights about how different bacterial life forms are affected by MH, showing a significantly different effectiveness against planktonic cells and biofilms.
Keywords: Planktonic bacteria; Biofilm; Magnetic nanoparticles; Magnetic hyperthermia.