

PHYSICAL TREATMENTS FOR ELIMINATION OF PATHOGENS

OBSERVATION OF INJURED *ESCHERICHIA COLI* POPULATION RESULTING FROM THE APPLICATION OF HIGH THROTTLING PRESSURE TREATMENTS

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Conventional culture techniques may underestimate the number of viable bacteria, especially when cells have been damaged by physical treatments. In this research, flow cytometry (FC) and epifluorescent microscopy (EM) were used to evaluate the potential for formation of injured cells after applying high pressure throttling (HPT). *Escherichia coli* cells suspended in Butterfield's phosphate buffer and skim milk were treated with HPT at pressures from 35 to 207 MPa. Treated cells were stained with SYTO 9 and propidium iodide (PI) (Live/Dead Baclight kit) to assess their membrane integrity, and with SYTO BC to detect total cell population present in the sample. Cells were counted using FC and EM. Mac Conkey agar (MC), Tryptone Soya agar (TSA) and a modification of the thin agar layer method (TAL_m) were used to determine injured and non-injured cells. Results obtained with culture media indicated a reduction in *E. coli* counts as pressure increased but no significant injured population was detected in both matrices. FC and EM observations indicated that the membrane integrity of a portion of the bacterial population was affected by HPT, producing different degrees of injured cells. The percentage of this heterogeneous population increased as pressure heightened. These results reassert the importance of studying the formation of injured bacteria and developing techniques to detect them in order to prevent their potential ability to repair themselves under favorable conditions.

