

Flow cytometry as a tool for assessing spore cell viability

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ABSTRACT

The presence of filamentous fungi can be a problem due to their capacity to produce mycotoxins. Several methods to inactivate spores have been studied; however, the evaluation of spores' viability is time consuming. Flow cytometry (FC) is a method to evaluate quickly and simultaneously several characteristics of cells, including its viability. To evaluate the efficacy of heat, gamma radiation and ozone in inactivating *Aspergillus parasiticus* spores, FC together with propidium iodide (PI) staining was used. Gamma irradiation was performed at doses between 0.5 and 10 kGy. Ozone treatment was done with aqueous ozone (10 or 20 mg/L) for 0 to 60 min exposure. The heat treatment (autoclaved by 20 min at 121 °C) was used as a control. FC was performed in a Sony EC800 and the method was adapted from Mesquita *et al.* (2013). Briefly, 40 µL of sample (untreated or treated with different sterilization methods) was added to 360 µL of PI solution (25 µg/mL) and incubated for 10 min in the dark. Thereafter, samples were mixed and analysed by flow cytometry. FC together with PI was effective to assess cellular viability of spores treated with heat and ozone solution. The inactivation of *A. parasiticus* was effective with ozone with short exposure time. The best results were obtained with ozone at 10 mg/L where an inactivation efficiency of 97% was observed. With gamma radiation, FC with PI fluorescence was not effective. Probably because with irradiation the cellular membrane is not degraded but the DNA is. Thus, PI cannot enter the cell and bind to the DNA chain explaining the absence of fluorescence.

Reference: Mesquita, N., et al. *International Biodeterioration & Biodegradation* 84 (2013): 250-257.

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