

438g Mechanisms of Supercritical Carbon Dioxide Sterilization of Bacterial Spores

Jian Zhang, Michael A. Matthews, Nishita Dalal, Alvin Fox, Karen Fox, Jason Hemmer, Martine LaBerge, Michael Drews, and Michael Stump

Sterilization of implants and medical devices (such as endoscopes) is essential to prevent clinical infection. Commercial sterilization using the steam autoclave, ethylene oxide, or irradiation has serious limitations when sterilizing heat-sensitive polymers or substrates with complex internal structures. To meet the clinical needs associated with these situations we evaluated the viability of using SCCO₂ as a sterilant. Experimental results verified that with approximately 200 ppm of H₂O₂ (equivalent to 5 mL of 30% H₂O₂), SC CO₂ has successfully sterilized all three commercial sterilization indicators. However, the addition of 1 mL of de-ionized water was not as effective as 5 mL of H₂O₂.

The TEM images of SCCO₂/H₂O₂ treated *B. atrophaeus* spores revealed a weblike matrix around the spores. This matrix could only be observed with ruthenium red, a carbohydrate stain, thus indicating the matrix was evidence of a disrupted exosporium. DPA analysis showed significant amount of DPA leakage after SCCO₂/H₂O₂ treatment compared to the trace amount of DPA release from untreated and pure SCCO₂ treatment. This was an indication of perforation of the spore coat. However, SCCO₂ did not extract and remove DPA from spores because autoclaving the SCCO₂/H₂O₂ treated spores released 100% of the DPA content. Based on these studies, we infer that disruption and perforation of the outer layers of spore structures are the cause of spore deactivation.