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Sterilizing *Bacillus pumilus* spores using supercritical carbon dioxide

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Abstract

Supercritical carbon dioxide (SC CO_2) has been evaluated as a new sterilization technology. Results are presented on killing of *B. pumilus* spores using SC CO_2 containing trace levels of additives. Complete killing was achieved with 200 part per million (ppm) hydrogen peroxide in SC CO_2 at 60 °C, 27.5 MPa. Addition of water to SC CO_2 resulted in greater than three-log killing, but this is insufficient to claim sterilization. Neither ethanol nor isopropanol when added to SC CO_2 affected killing.

Keywords: Sterilization; Spores; Supercritical carbon dioxide

1. Introduction

Rapid development of novel surgical or implantable devices and biomaterials presents a challenge to existing medical sterilization technologies (Moisan et al., 2001). SC CO_2 is receiving interest as a potential new sterilization technology because of drawbacks of known sterilization methods. Standard sterilization methods include steam autoclaving, gamma-irradiation, and ethylene oxide (Matthews et al., 2001; Dempsey and Thirucote, 1989). Steam autoclaving damages heat-sensitive materials (Dempsey and Thirucote, 1989) and may deposit an oxide layer onto metallic surfaces (Lausmaa et al., 1985). Also, autoclaving may wet the surface, damaging electronic devices and hydrolyzing polymers. Dry sterilization is preferred for these applications. Gamma irradiation reduces shear and tensile strength, elastic modulus, and transparency of medical polymers by breaking polymer chains and generating active free radicals (Dillow et al., 1999; Premnath et al., 1996). Ethylene oxide not only degrades certain polymers, but also is flammable and toxic (Dempsey and Thirucote, 1989).

This work concerns the effectiveness of CO_2-based technology in killing *B. pumilus* spores. *B. pumilus* ATCC 27142 was selected because it is one of three species of spores commonly used to validate commercial sterilizers, and because no data exist on the effects of SC CO_2 on this organism (Spilimbergo and Bertucco, 2003).

Supercritical CO_2 is of interest as a potential sterilization medium because it is nontoxic, nonflammable, chemically inert, and physiologically safe, and because the critical temperature (31 °C) is low enough to qualify

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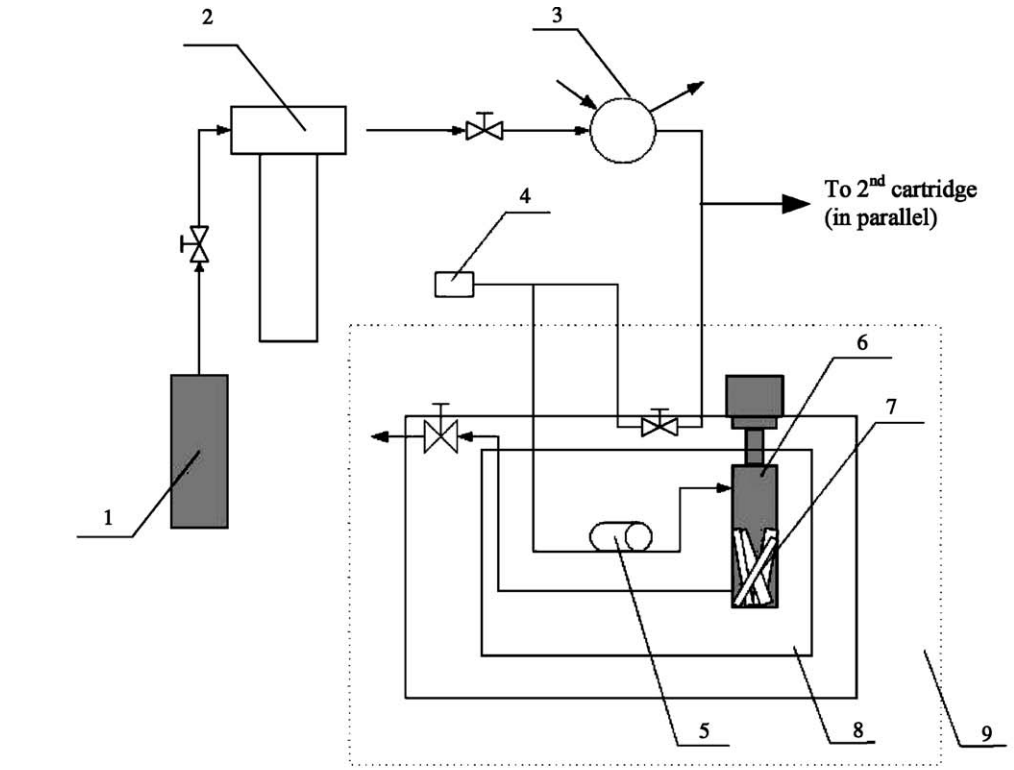
as a 'cold sterilization' technology (Elvassore et al., 2000; Enomoto et al., 1997b; Hong et al., 1999). The chief hazard is asphyxiation. CO_2 exists in the supercritical state at a temperature higher than its critical temperature (T_c=31.1 °C) and at a pressure higher than its critical pressure (P_c=7.38 MPa). SC CO_2 has a liquid-like density ($0.9–1.0\times10^3$ kg m^{-3}) (Span and Wagner, 1996), gas-like diffusivity ($10^{-7}–10^{-8}$ m^2 s^{-1}) and



1. CO_2 cylinder
2. ISCO D260 pump
3. Six-port liquid injection valve
4. Overpressure disk
5. CO_2 pre-heater
6. Cartridge
7. Spore strips
8. Heat block
9. ISCO SFX 2-10 Supercritical fluid extractor

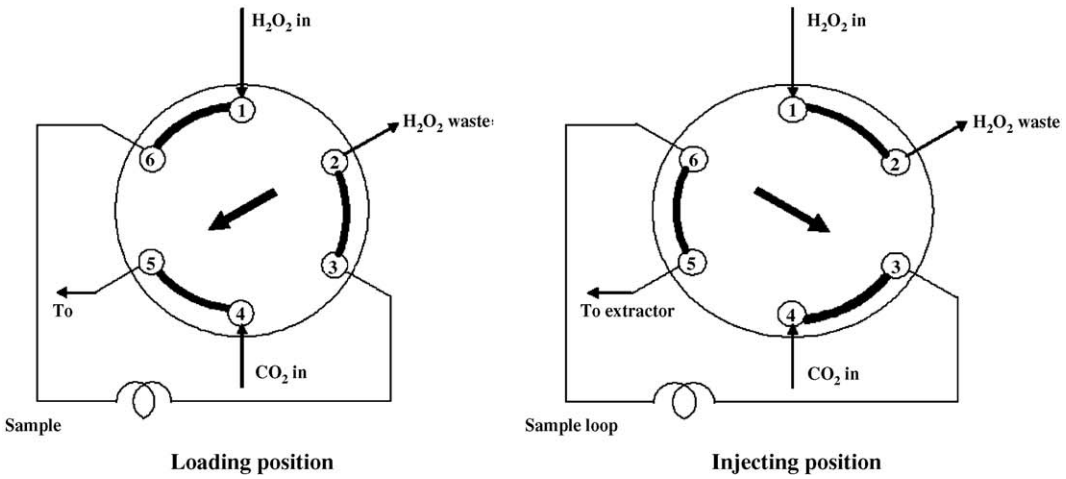


Fig. 1. Schematic of high-pressure CO_2 apparatus.