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Optimizing supercritical carbon dioxide in the inactivation of bacteria in clinical solid waste by using response surface methodology

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ABSTRACT

Clinical solid waste (CSW) poses a challenge to health care facilities because of the presence of pathogenic microorganisms, leading to concerns in the effective sterilization of the CSW for safe handling and elimination of infectious disease transmission. In the present study, supercritical carbon dioxide (SC-CO_2) was applied to inactivate gram-positive *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, and gram-negative *Escherichia coli* in CSW. The effects of SC-CO_2 sterilization parameters such as pressure, temperature, and time were investigated and optimized by response surface methodology (RSM). Results showed that the data were adequately fitted into the second-order polynomial model. The linear quadratic terms and interaction between pressure and temperature had significant effects on the inactivation of *S. aureus*, *E. coli*, *E. faecalis*, and *B. subtilis* in CSW. Optimum conditions for the complete inactivation of bacteria within the experimental range of the studied variables were 20 MPa, 60 °C, and 60 min. The SC-CO_2-treated bacterial cells, observed under a scanning electron microscope, showed morphological changes, including cell breakage and dislodged cell walls, which could have caused the inactivation. This espouses the inference that SC-CO_2 exerts strong inactivating effects on the bacteria present in CSW, and has the potential to be used in CSW management for the safe handling and recycling-reuse of CSW materials.

1. Introduction

The management of clinical solid waste (CSW) continues to be a major challenge in health care facilities. Inadequate CSW management creates significant health hazards and environmental pollution. CSW is considered biohazardous because of the presence of pathogenic microorganisms (Park et al., 2009; Hossain et al., 2013a). Cautious and stringent handling and disposal methods of the CSW are needed to minimize health hazards and environmental pollution (Abd El-Salam, 2010).

Some studies argue that sterilization of CSW at its source would be an effective approach to minimize the hazards, generation, and disposal cost (Hossain et al., 2011; Marinkovic et al., 2008; Sawalem et al., 2009). Consequently, the handling and segregation of sterilized CSW material would be carried out without any risk of infection. Medical tools and equipment made of metal or plastic components, plastic materials, paper, cardboard, etc., retrieved from sterilized CSW materials can be reused and recycled. Thus, the adoption of an effective sterilization technique in CSW management would reduce infectious exposure and disposal cost. Hospitals and patients would have the benefit of saving treatment cost and a safer environment.

Sterilization of the CSW before their recycling-reuse is challenging because major portions of CSW materials are made of heat-sensitive plastics or biopolymers. Available sterilization technologies in medical care settings, including steam autoclave and ethylene oxide are not suitable for CSW sterilization. Steam autoclaves can destroy heat-sensitive materials owing to high temperature (White et al., 2006). Ethylene oxide sterilization can chemically destroy plastics and polymer materials. Therefore, it is important to explore a sterilization technology that can operate at low temperature (Sawalem et al., 2009).

Supercritical carbon dioxide (SC-CO_2) is an effective sterilization method having notable benefits over existing sterilization techniques. The fluid carbon dioxide at the supercritical state

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(31.1 °C, 7.4 MPa) is nontoxic and nonflammable. SC-CO_2 is effective against microorganisms because it destroys target microorganisms both physically and chemically (Hossain et al., 2013b; Jimenez et al., 2008; Kim et al., 2009). It is a gentle and viable sterilization method that can sterilize heat-sensitive plastics and polymers without damaging or lowering their quality (Dillow et al., 1999; Ellis et al., 2010; White et al., 2006; Zhang et al., 2006a). This technology has been effectively used to sterilize biomedical devices against bacteria (Dillow et al., 1999), viruses (Fages et al., 1998), and endospores (Zhang et al., 2006b), although in some cases water or chemical additives have been used to achieve terminal sterilization (White et al., 2006; Zhang et al., 2006b). The use of SC-CO_2 sterilization in CSW management is of considerable interest not only from the safety and health perspectives, but also for the sustainable use of the recycled CSW materials.

In our previous study, we used SC-CO_2 to inactivate gram-positive *Staphylococcus aureus* and gram-negative *Serratia marcescens* in CSW (Hossain et al., 2013b). We reported that SC-CO_2 inactivates bacteria in CSW through cell wall distortion and destruction of the cytoplasmic materials. Further studies on the SC-CO_2 sterilization technology are vital for the application and understanding of the inactivation of bacteria in CSW. In the present study, we used SC-CO_2 to inactivate *S. aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, and *Escherichia coli* in CSW by varying the SC-CO_2 pressure, temperature, and treatment time. *S. aureus* is a gram-positive coccus that causes life-threatening infectious diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis (Tang and Stratton, 2010). *E. faecalis*, a nonmotile, facultative anaerobic microbe, is a nosocomial and opportunistic human pathogen that causes endocarditis and bacteremia, urinary tract infections, meningitis, and other life-threatening infections (Arciola et al., 2007). *B. subtilis* is a gram-positive and spore-forming bacterium that is also heat-resistant. *E. coli* is a gram-negative and rod-shaped bacterium, and some *E. coli* strains can cause severe foodborne diseases. The optimum experimental sterilization parameters of the SC-CO_2 were ascertained using response surface methodology (RSM). RSM is a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several input variables, with the objective of optimizing the response (Box and Wilson, 1992). In the current study, central composite design (CCD) was applied to investigate the effect of SC-CO_2 pressure, temperature, and treatment time on the log reduction of bacteria (log CFU g^{-1}) in CSW as a measure of sterilization effectiveness.

2. Materials and methods

2.1. Sample preparation

The CSW materials used in the present study were collected from Hospital Lam Wah Ee, a specialized health care facility in Penang Island, Malaysia. The collected samples were sterilized in an autoclave to ensure safe handling. The sterilized waste was dried at room temperature to reduce moisture content. Heat-resistant waste materials (i.e., hard plastic materials, broken glass, textile, metals, etc.) were manually removed from the sterilized waste. The waste materials were sorted and manually cut into smaller pieces of 0.5–1.0 in. The gravimetric composition of the CSW used in this study is presented in Table 1.

S. aureus, *E. faecalis*, *B. subtilis*, and *E. coli* were isolated from the CSW (Hossain et al., 2013a). The isolated bacteria were grown in selective media to obtain fresh cultures (i.e., blood agar media for gram-positive bacteria and MacConkey agar media for gram-negative bacteria). A single isolated colony from the culture was transferred to nutrient broth (NB) agar and incubated at 37 °C for 24 h. Twenty milliliters of bacterial solution was prepared using 5 mL of bacterial culture in nutrient broth, which was then added to 5 mL of sterile glycerol and 10 mL of sterile saline solution. The glycerol was used as a surfactant for homogeneous distribution of bacteria in the CSW sample. The bacterial mixture was then added dropwise to 0.25 kg of waste and mixed well using a glass rod. The CSW was then placed in a 1.2-L SC-CO_2 sterilization vessel.

Table 1
Gravimetric composition of clinical solid wastes used in the study.

Material	% by mass (dry basis)
Hard plastic	30
Broken glass	25
Fabric	15
Metal	20
Rubber	10
Total	100

2.2. SC-CO_2 treatment

A supercritical sterilization reactor system was used to sterilize the CSW as shown in Fig. 1. The sample was placed in the SC-CO_2 sterilization vessel, and the vessel was closed tightly. The inactivation of the bacteria was performed at varying pressure (5–40 MPa), temperature (35–80 °C), and treatment time (5–90 min). When the temperature of the SC-CO_2 system reached the set temperature, the connecting valve (V01) was opened and the decompression valves (V02 and V03) were closed. Liquid CO_2 (95% purity), contained in a siphoned cylinder, was pumped into the treatment vessel until the desired pressure was achieved. The connecting valve (V01) was open throughout the entire treatment. After the desired treatment period, the connecting valve (V01) was closed and the vessel was depressurized by slowly opening the decompression valve (V02 and V03). The depressurized SC-CO_2, which is not liquid or a gas, was release into the atmosphere as a pure CO_2 gas through a water bath, as shown in Fig. 1. The sterilized samples were collected for the enumeration of viable colonies. Triplicate experiments were conducted, and the results are expressed as means ± standard error.

2.3. Enumeration of viable cells

The number of viable cells in untreated and SC-CO_2-treated CSW was determined using the pour plate method. One gram of SC-CO_2-treated and untreated samples was added into 5 mL of sterilized distilled water. One milliliter of contaminate was taken for an eight-fold serial dilution, from which 0.1 mL was used for seeding on nutrient agar media. The culture plates were labeled and incubated at 37 °C for 48 h before counting. This procedure was carried out in triplicate, and the average bacterial colony count was determined. Results were expressed as the logarithm of surviving colony-forming units per gram of waste (log CFU g^{-1}). The initial concentrations of the bacteria were estimated to be 7.80 ± 0.1, 7.75 ± 0.05, 6.95 ± 0.05, and 7.72 ± 0.05 log CFU g^{-1} for *S. aureus*, *E. faecalis*, *B. subtilis*, and *E. coli*, respectively.

The number of bacterial colonies was determined from the number of individual colonies that formed on the surface of culture media. The number of colony-forming units per gram of waste was calculated using the following equation:

$$\text{CFU g}^{-1} = \frac{\text{Number of bacterial colonies}}{\text{Agar plating volume}} \times \frac{1}{\text{Dilution factor}} \times \frac{\text{Volume of dilute}}{\text{Mass of waste}} \quad (1)$$