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Mathematical modeling of *Enterococcus faecalis*, *Escherichia coli*, and *Bacillus sphaericus* inactivation in infectious clinical solid waste by using steam autoclaving and supercritical fluid carbon dioxide sterilization

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HIGHLIGHTS

- SC-CO_2 is an effective sterilization method for treating clinical solid waste.
- SC-CO_2 inactivates bacteria by physicochemical disruption of cellular materials.
- SC-CO_2 sterilized waste materials could recycle after eliminating infectious exposure.

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ABSTRACT

In the present study, steam autoclaving and supercritical carbon dioxide (SC-CO_2) were utilized to inactivate vegetative cells of *Enterococcus faecalis*, *Escherichia coli*, and *Bacillus sphaericus* in clinical solid waste. The success of steam-based bacterial sterilization depends on temperature, treatment time, and the bacterial species present. Autoclave sterilization was found to be most effective at 121 °C for 60 min and 131 °C for 30 min. Complete inactivation of bacteria in clinical solid waste subjected to SC-CO_2 sterilization was obtained after 30–120 min at a treatment range of 10–40 MPa and 35–80 °C. The bacterial inactivation curves, which were generated using a modified Gompertz model to describe the relationship between survival rate and treatment time, was divided into three distinct phases. Scanning electron microscopy, bacterial protein, and enzymatic activity analyses showed that steam autoclaving physically inactivated bacteria by denaturing cellular enzymes, thereby inhibiting their activities. In contrast, SC-CO_2 inactivated bacteria both physically and chemically. The reduction of proteins and enzymatic activity in SC-CO_2-treated bacterial cells suggested that these cellular components were destroyed by the SC-CO_2. The absence of re-growth after SC-CO_2 sterilization and its promising bacterial inactivation efficiency suggested that it was an effective method for the treatment of infectious clinical solid waste. Therefore, SC-CO_2 sterilization could be utilized in clinical solid waste management to eliminate infectious exposure and to improve the hygienic recycling and reuse of clinical solid waste materials.

1. Introduction

Effective sterilization has become a matter of increasing importance in clinical solid waste (CSW) management because of concerns for hospital hygiene and environmental pollution [1–4]. CSW that is perceived to be infectious requires particular steps to minimize occupational hazards and environmental contamination [4–6]. The potential presence of pathogenic microorganisms in CSW poses infectious risks that can affect human health and the environment. CSW typically contains nosocomial and opportunistic human pathogens, including bacteria, viruses, fungi, and prions

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[4,5,7]. Personnel involved in the treatment of clinical waste are exposed to infectious agents through several routes, including skin penetration, skin contact, or aerogenically [5,8,9].

Technologies currently used (i.e., incineration, steam autoclave) to dispose CSW are not environmentally friendly and fail to cope with clinical waste in a safe manner [6,10,11]. Incineration, the most commonly used method to dispose of CSW, is viewed as an unsuitable approach because of the potential release of a wide range of pollutants as well as the high financial and occupational start-up costs for incineration facilities [3,6,10,11]. Considerable effort has been made to develop an acceptable alternative to incineration technology for the safe disposal of CSW, eliminating human health and environmental concerns. In recent years, environmentalists and policy agencies have been challenged to identify suitable technology to sterilize CSW at its source before it is recycled and reused [6,12,13].

Steam-based autoclaving, used widely in healthcare facilities to decontaminate highly infectious lab waste, is considered the most reliable sterilization method and is a process that is easily controlled. Steam autoclaves have proven to be effective for sterilization of reusable medical equipment in hospitals. Parameters used for steam autoclaving have been optimized for the sterilization of medical devices, while similar parameters have been applied to autoclave sterilization of clinical waste based on the assumption that this method would be effective in this context [14]. However, the factors affecting sterilization efficiency of clinical waste have not been thoroughly studied, thus there is little evidence to support that assumption.

Since 1987, when supercritical fluid carbon dioxide (SC-CO_2) was first shown to inactivate *Escherichia coli* [15], low temperature and moderately pressurized CO_2 has emerged as an alternative non-thermal sterilization option. Pressurized CO_2 beyond its critical point (7.4 MPa and 31.1 °C), referred to as SC-CO_2, is an effective sterilization method that offers notable benefits over existing sterilization technologies [15–18]. The effectiveness of SC-CO_2 results from the physicochemical properties of fluid CO_2, including its high dissolving power, low viscosity, and high diffusivity [19,20]. Fluid CO_2 in a supercritical state is neither a gas nor liquid and affects target microorganisms both physically and chemically [18,21]. Although SC-CO_2 has been shown to be an effective sterilizing agent in various fields, few studies have been conducted to evaluate SC-CO_2-mediated sterilization of CSW. In a recent study, SC-CO_2 was used to inactivate gram-positive *Staphylococcus aureus* and gram-negative *Serratia marcescens* bacteria present in CSW [22]. SC-CO_2 reportedly inactivates bacteria in CSW by distorting their cell walls and destroying cytoplasmic materials. Further studies to evaluate SC-CO_2 sterilization technology are vital to gain a better understanding of this method so that it can be applied to the inactivation of bacteria in CSW.

Predictive modeling is a useful method to describe the behavior of microorganism growth under different physical conditions. The purpose of mathematically modeling bacterial inactivation methods is to assess the effects of various factors without having to perform numerous experiments [19,23,24]. In first-order kinetics, the logarithmic plot of the number of survival microorganisms versus time forms a straight line for isothermal condition. Bacteria inactivation typically shows a lag phase in which the survival rate is zero for a certain period before it accelerates, increasing the survival rate. Moreover, the survival rate increases to maximum level in the final phase such that an asymptote is reached. If the survival rate is defined as the logarithm of the number of bacterial survival ratio and it is plotted against time, the survival rate changes appear as a sigmoidal curve that shows a lag phase followed by exponential and stationary phases [24,25]. Therefore, bacterial inactivation modeling cannot be represented fully following a first-order kinetics model.

A number of mathematical theories have been applied to elucidate the sigmoidal tendencies of microbial survival rates. For example, the Gompertz equation and its modified forms have the ability to model both linear and asymmetrical sigmoidal microbial survival rate values [24,26–28]. In the present study, a modified Gompertz equation was used to examine the effect of SC-CO_2 on bacterial inactivation, wherein a first-order kinetics model was utilized to determine the inactivation rate for bacteria that had undergone steam autoclaving. The modified Gompertz equation was not applicable to bacteria exposed to steam autoclaving, as it was unable to predict the inflection point and, therefore the lag phase of bacterial inactivation in CSW.

The purpose of this study was to evaluate the feasibility of using SC-CO_2 as a sterilization method for CSW management. Steam autoclaving and SC-CO_2 treatment were used to inactivate *Enterococcus faecalis*, *E. coli*, and *Bacillus sphaericus* in CSW. *E. faecalis* is a nonmotile, facultative anaerobic microbe; it is a nosocomial and opportunistic human pathogen that causes endocarditis, bacteremia, urinary tract infections, meningitis, and other life-threatening infections in humans [29]. *B. sphaericus*, a gram-positive and spore-forming bacterium, is highly resistant to heat, radiation, and chemicals [30]. *E. coli* is a gram-negative and rod-shaped bacterium. Some *E. coli* strains can cause severe foodborne disease. Several analytical methods were utilized to elucidate the mechanisms of bacterial inactivation in CSW subjected to SC-CO_2 and steam autoclave treatment. Bacterial re-growth potential in CSW subjected to these two treatments was also evaluated. The findings of this study are useful for developing a safe handling and resource-recovery practice for CSW management.

2. Materials and methods

2.1. Microorganisms and culture conditions

The microorganisms used were *E. faecalis*, *E. coli*, and *B. sphaericus* isolated from CSW [7]. Stock cultures grown on nutrient agar were suspended in nutrient broth containing 10% glycerol. The stock cultures were then stored in −80 °C until use. One loopful from each frozen culture was streaked and cultured on nutrient agar for 24 h at 37 °C. Culture plates were stored at 4 °C and transferred monthly to generate stock cultures. In each experiment, the cultures obtained from the stocks were subcultured twice on nutrient agar at 37 °C for 16 h.

2.2. Sample preparation

The clinical solid waste materials used in this study were collected from Hospital Lam Wah Ee, one of specialized healthcare facilities in Penang Island, Malaysia. The collected samples were sterilized in an autoclave (121 °C for 15 min) to ensure the safe handling. Later, the sterilized waste was dried in air at room temperature until the moisture content reduced to about 5% (w/w.). Heat-resistant waste materials (i.e., hard plastic, broken glass, textiles, metals, etc.) were manually separated from the sterilized waste. Waste materials were then trimmed to the desired size by manually cutting the materials to 1.52–2.54 cm. Five milliliters of bacterial culture in nutrient broth was transferred into 15 mL of sterile saline solution containing 5 mL of sterile glycerol and the solution was mixed thoroughly. The mixture was then added to 250 g of waste in a drop-wise fashion and mixed vigorously using a glass rod.

2.3. Steam autoclave treatment

Bacteria in CSW were inactivated using a laboratory autoclave (Model: ES-215; Tomy Seiko Co., Ltd., Japan.). The sensitivity of