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Escherichia coli inactivation by pressurized CO_2 treatment methods at room temperature: Critical issues

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ABSTRACT

This study aims to increase the inactivation efficiency of CO_2 against *Escherichia coli* under mild conditions to facilitate the application of pressurized CO_2 technology in water disinfection. Based on an aerating-cycling apparatus, three different treatment methods (continuous aeration, continuous reflux, and simultaneous aeration and reflux) were compared for the same temperature, pressure (0.3–0.7 MPa), initial concentration, and exposure time (25 min). The simultaneous aeration and reflux treatment (combined method) was shown to be the best method under optimum conditions, which were determined to be 0.7 MPa, room temperature, and an exposure time of 10 min. This treatment achieved 5.1-log reduction after 25 min of treatment at the pressure of 0.3 MPa and 5.73-log reduction after 10 min at 0.7 MPa. Log reductions of 4.4 and 5.0 occurred at the end of continuous aeration and continuous reflux treatments at 0.7 MPa, respectively. Scanning electron microscopy (SEM) images suggested that cells were ruptured after the simultaneous aeration and reflux treatment and the continuous reflux treatment. The increase of the solubilization rate of CO_2 due to intense hydraulic conditions led to a rapid inactivation effect. It was found that the reduction of intracellular pH caused by CO_2 led to a more lethal bactericidal effect.

Introduction

The safety of drinking water is a particularly important and newsworthy topic. If water is improperly disinfected, it can have serious consequences. Chlorination has played an important role in water disinfection during the last century. Owing to its strong sterilization ability, ease of use and low cost, chlorine has been widely used in water and wastewater disinfection (Ma et al., 2013). However, the existence of carcinogenic disinfection by-products (DBPs) (Chu et al., 2011) and chlorine-resistant microorganisms limits the technology. Regarding other technologies, ozone treatment generates carcinogenic bromate and ultraviolet treatment not only has high cost but also a weak continuous disinfection effect. To overcome these shortcomings, it is imperative to find alternatives that demonstrate high efficiency, low cost, and few by-products.

Pressurized carbon dioxide (CO_2) has been widely used in food, medicine, and cosmetics due to high effectiveness, absence of toxic residue, and low residence time (Zhang et al., 2006). After Fraser (1951) noticed that *Escherichia coli* (*E. coli*) can be deactivated by a rapid release of pressurized CO_2, which

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bursts the cells, many studies have demonstrated that carbon dioxide has an inhibition effect on various microorganisms (Cheng et al., 2013; Kobayashi et al., 2013; Soares et al., 2013), and in various mediums (Casas et al., 2012; Li et al., 2012; Xu et al., 2011). However, when pressurized, it could achieve a better inactivation effect on pathogens. High-pressure carbon dioxide (HPCD) treatment and supercritical carbon dioxide (SC-CO_2) treatment have historically been the most widely studied methods. In addition, rising scientific interest has focused on combining these methods with synergistic techniques to enhance their inactivation effect, such as combining HPCD with pulsed electric fields (PEF) (Pataro et al., 2014; Spilimbergo et al., 2003) or SC-CO_2 with high power ultrasound (Ortuño et al., 2013).

After Kamihira et al. (1987) first published a comparison of the sterilization effects of gaseous, liquid, and supercritical CO_2 on four different microorganisms, the feasibility of CO_2 as a sterilization technique has gradually gained popularity. Over 100 literature reports on this technology's application have been published in the field of food preservation, but fewer in the field of drinking water or wastewater treatment. Moreover, many studies have stated that an increase in pressure, temperature, and exposure time would enhance the antimicrobial effects of pressurized CO_2 (Ballestra et al., 1996; Hong and Pyun, 1999; Oule et al., 2006), but that the pressure was too high to use in practical settings. Furthermore, the inactivation mechanism was not yet fully understood. Kobayashi et al. (2006, 2007, 2009) were the first to create a microbubble CO_2 system that could efficiently inactivate *E. coli* and other coliforms within 13.3 min. However, the pressure of this method was 10 MPa and the temperature ranged from 35 to 55°C, which were still too high. Cheng et al. (2011) found that a high level of dissolved CO_2 would effectively improve the inactivation effect. However, these studies did not reveal the effects of hydraulic conditions and the solubility of CO_2 on the inactivation of microorganisms. What's more, it is necessary to seek alternative methods able to decrease the pressure, temperature, and time required.

By comparing three different treatment methods, this study investigates the effects of CO_2 solubilization rate and solubility in aqueous solution on pathogen inactivation. The goal is to achieve a higher inactivation effect on pathogens within a shorter residence time at lower pressures and at room temperature than previous studies. In this study, *E. coli* was selected as a representative bacterium. Based on a pressurized aerating-cycling apparatus (Fig. 1), three different treatment methods were tested and compared. Nitrogen (N_2) and disodium hydrogen phosphate citrate buffer were used to help study the mechanisms of CO_2 disinfection in these treatments. It is hoped that the results of this study will facilitate the practical use of low-pressure CO_2 as an alternative disinfectant technique in drinking water and wastewater disinfection.

1. Materials and methods

1.1. Microorganism preparation and enumeration

E. coli (ATCC 1.3373), provided by the China General Microbiological Culture Collection Center, was cultivated using Nutrient Broth (peptone 10 g/L, sodium chloride 5 g/L, and beef extract 3 g/L). Flasks containing 100 mL Nutrient Broth were continuously shaken at 37°C for 16 to 18 hr at 150 r/min. The concentration of *E. coli* was enumerated as colony-forming units (CFUs) by injecting 1 mL of the suspension onto a nutrient agar medium, and then incubating at 37°C for 24 hr. The initial enumeration was 10^9 to 10^{10} CFU/mL.

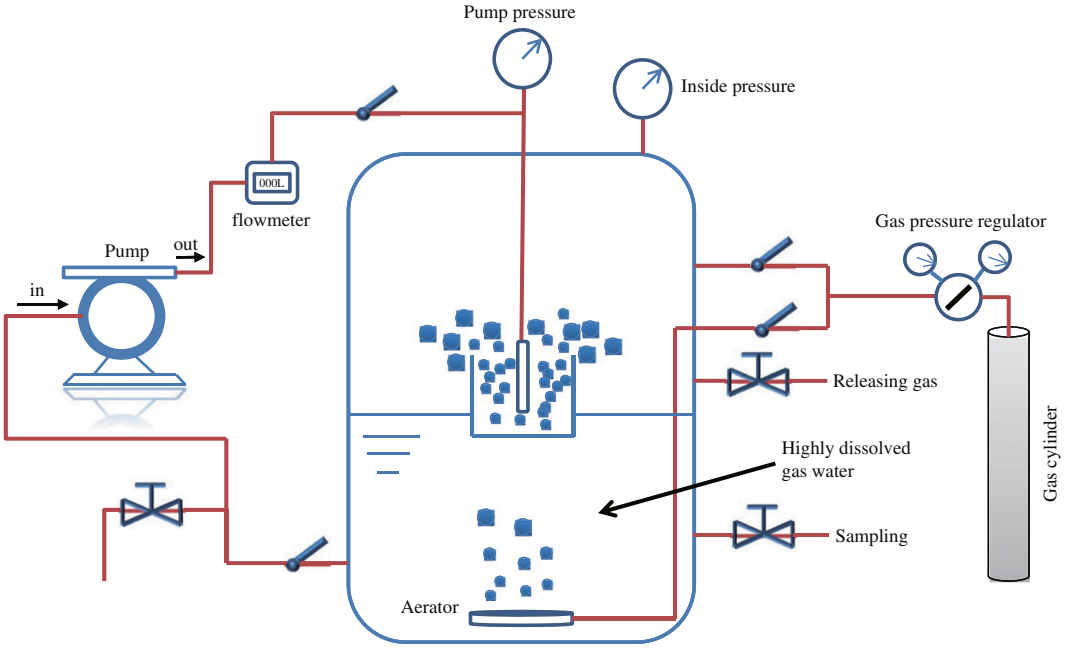

Fig. 1 – Schematic representation of study apparatus.