Evaluation of a New Ozone Apparatus, the BOX-O₃, for the
Bacteriological Disinfection of Medical Waste.

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Summary

The BOX-O₃ is a new, alternative apparatus that uses ozone for the disinfection of biomedical waste. This study evaluated the ability of the apparatus to disinfect contaminated medical and laboratory waste that included a variety of bacterial strains. Microbiological measurement was done on laboratory waste contaminated with a variety of bacterial strains, medical waste alone, and medical waste contaminated with Bacillus subtilis collected from the infectious disease ward of Kyushu University Hospital. The strains tested for were Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecium, and Candida albicans. A 100 mL (5 test tubes containing 20 mL each) bacterial suspension consisting of 10⁸ to 10⁹ colony forming units (CFU)/mL was added to waste in a container. The mean bacterial load of the waste was measured immediately after shredding and ozone treatment. Three samples were recovered from three different places at random in the bulk ground. The samples were diluted to 1:10 and 1:100, then cultured at 37°C for 48 hr in a blood agar medium. For the measurement of primary bacterial load, shredding alone was done without the boost additive (hydrogen peroxide and acetic acid) and ozone treatment. All assays were done in triplicate. In the laboratory waste contaminated with a variety of bacterial strains, all bacterial populations were decreased by at least 4 log₁₀ immediately after ozone treatment. The primary mean bacterial load of the untreated medical waste alone was 3.8 log₁₀ CFU/g, and only normal flora was observed. The primary mean bacterial load of the untreated medical waste contaminated with Bacillus subtilis was lower than that of the untreated laboratory waste contaminated with Bacillus subtilis. In the medical waste contaminated with or without Bacillus subtilis, no bacterial populations were detectable immediately after ozone treatment. The BOX-O₃ safely and efficiently treated infectious medical waste collected from hospital ward, indicating that the BOX-O₃ is an effective alternative technology for the treatment of infectious waste and for controlling hospital infection.

Key words: medical waste, alternative technology, ozone treatment

Introduction

Concern is growing about the chemical pollution of soil, air and water as well as the infection risk inherent in the sorting, storing and transportation of medical waste before treatment¹⁻⁶. Furthermore, regulation of incinerators has been tightened, causing the number of incinerators at medical facilities have rapidly decreased. Under these circumstances, alternative technologies for the treatment of medical waste that can be substituted for incinerators have received considerable attention⁷,⁸.

The BOX-O₃ is a new, alternative apparatus that uses ozone to disinfect biomedical waste⁹. It allows on-site shredding and decontamination of medical waste soon after it is produced.

This study evaluated the ability of the apparatus to disinfect contaminated medical and laboratory waste using a variety of bacterial strains.

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The BOX-O₃ is a new, alternative apparatus that uses ozone to disinfect biomedical waste⁹. It allows on-site shredding and decontamination of medical waste soon after it is produced.

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Material and Methods

Apparatus

The BOX-O₃ was developed by the BOX-O₃ International Company (Gümlingen, Switzerland). Figure 1 shows the general configuration of the BOX-O₃. The dimensions are 224 cm high, 60 cm wide, and 90 cm deep. The machine can be placed in any area equipped with electricity and compressed air. The BOX-O₃ consists of two parts, the center of the device contains a loading door and rotating lock, and a grinder and waste tank are in the lower part of the device. The ozone generator, accumulation tank, and the ozone neutralizer are in the upper part of the device. Gaseous ozone is produced continuously by an ozone generator supplied with the compressed air. The interior of the treatment unit is maintained at a constant partial vacuum with air expelled through a dry jacket, thereby eliminating the possible risk of ozone dispersal into the room in which the decontamination process is executed.

Treatment cycle

The BOX-O₃ treatment unit is controlled by a dual failsafe microprocessor with constant monitoring of the device’s basic functions and of the correct execution of the various phases of the cycle. Immediately after use, medical waste products are deposited in the BOX-O₃ container, a heavy-duty paper bag or a disposable carton, identified with a bar code. The rotating lock is opened and the container is introduced through the loading door after a personal bar code card has been validated. Next, the boost additive, consisting of 90 mL of 12% hydrogen peroxide and 80 mL of 80% acetic acid, is inserted. After this process, the container is automatically shredded together with its contents for 5 minutes. It is finely ground to a consistency at which the original parts are no longer recognizable or usable. Depending on the type of waste, the volume following shredding is reduced by 4 to 5 times the original volume. During the shredding, ozone is generated and accumulated up to a relative pressure of 1.2 bars and a maximum ozone concentration of 40 μg/L inside the ozone accumulation tank. The ground waste is then subjected to repeated cycles of vacuum/ozone injection for an amount of time necessary to complete the bacteriological decontamination process. At this point, the treated waste products can be unloaded into plastic bags and disposed as non-hazardous waste. The decontaminated end product can then follow the normal disposal path of urban waste, and is suitable for processing with any other standard waste product.

Microbiological studies

To evaluate the ability of the BOX-O₃ to disinfect infectious medical waste, microbiological test was done on laboratory waste contaminated with a variety of bacterial strains from culture collection, medical waste alone, and medical waste contaminated with *Bacillus subtilis* collected from the infectious disease ward of Kyushu-university hospital (Fukuoka, Japan). The strains used were *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Enterococcus faecium* ATCC27853, and *Candida albicans JCM2085/ATCC2698*. *Bacillus subtilis* is commonly used in Japan for evaluating the ability of an alternative technology to disinfect infectious medical waste²⁷. A 100 mL (5 test tubes containing 20 mL each) bacterial suspension consisting of 10⁶ to 10⁷ colony forming units (CFU/mL) was added to waste in a container. The mean bacterial load of the waste was measured immediately after shredding and ozone treatment. Three samples of 10–20 g were recovered at random from three different places in the bulk ground, then transferred to...
sterile beakers. A sample of 5 g from the sterile beaker was transferred to a sterile polypropylene container containing 100 mL phosphate-buffer saline and 20 g of glass beads, using an aseptic technique, then shaken intensively for 45 seconds. The sample were diluted to 1 : 10 and 1 : 100 by the addition of phosphate-buffer-saline, then 200 µL of these diluted samples were cultured at 37°C for 48 hr in a blood agar medium. For the measurement of the primary bacterial load, shredding alone was done without the boost additive and ozone treatment. Assays were done in triplicate. To verify reproducibility, the same experiment was repeated once a week for three weeks.

Results

In the present study, an accident such as wounds and infection by needles and sharp objects was not happened as those were collected separately in special plastic safety boxes and the tested medical wastes including safety boxes were deposited in the BOX-O₃ container. At the end of the cycle, the remaining scrap in the waste tank was unrecognizable without danger, and the contents were safely unloaded into plastic bags and disposed of.

Microbiological studies

Table 1 shows the primary mean bacterial load of the untreated laboratory waste contaminated with a variety of bacterial strains. In the present study, the detection limit was <2.0 log₁₀. Immediately after ozone treatment, all bacterial populations were reduced below the detection limit and decreased by at least 4 log₁₀. In testing of the sporicidal effect of BOX-O₃, a minimum reduction of 4 log₁₀ was observed in all assays performed.

Table 2 shows a comparison of the primary mean bacterial load of the untreated laboratory waste with and without Bacillus subtilis contamination. The primary mean bacterial load of the untreated medical waste alone was 3.8 log₁₀ CFU/g, with only normal flora observed (Bacillus sp., Corynebacterium sp.). The primary mean bacterial load of the untreated medical waste contaminated with Bacillus subtilis (mean ± S.E.; 4.5 ± 0.1 log₁₀ CFU/g) was lower than that of the untreated laboratory waste contaminated with Bacillus subtilis (mean ± S.E.; 6.6 ± 0.1 log₁₀ CFU/g) (Table 1, 2), although 100 mL of Bacillus subtilis suspension at 2.45 x 10⁸ CFU/mL was added. No bacterial populations were detected immediately after ozone treatment in either test.

Discussion

The BOX-O₃ is a new apparatus especially designed for on-site treatment of infectious medical waste. The BOX-O₃ requires only limited space and is designed to be placed within a hospital ward. This safe and easy to use process can be employed by the nursing staff for assuring the briefest delays in the decontamination of biohazardous wastes. The BOX-O₃ would appeal to medical, laboratory, and research and development institute personnel, as well as to public and private health service providers concerned about improved hygiene.

Ozone is a powerful oxidizing agent that assures the complete and total disinfection of waste. Ozone inactivates microorganisms through its oxidizing action, with any residual ozone spontaneously decomposing into non-toxic oxygen. Various applications for ozone have been documented in the field of hygiene, especially in the field of the microbiological disinfection.
The BOX-O3 is suitable for the treatment of infectious waste, in particular for the disposal of potentially infected waste products such as syringes, needles, non-woven fabrics, and drip sets. Body parts, toxic chemicals and radioactive substances can not be treated.

Microbiological inactivation is defined as a bacterial population reduction of 99.99% in the Japanese guidelines for evaluation of the ability of a new apparatus to be used for disinfecting infectious waste\(^7\). In the present study, a reduction of 99.99% for *Bacillus subtilis* was achieved. The primary bacterial load of the untreated medical waste was low, and no pathogenic bacteria were observed. Furthermore, the primary bacterial load of the untreated medical waste contaminated with *Bacillus subtilis* (Table 1, 2), although 100 mL of *Bacillus subtilis* suspension at 2.45 × 10\(^8\) CFU/mL was added.

Since there were many used antibiotics bottles with a small quantity of liquid residues, used cotton and gauze soaked in disinfectant in the medical waste from the infectious ward, these may have decreased the bacterial load of the tested medical waste.

The main advantage of the BOX-O3 is the ability to treat contaminated medical waste inside a hospital ward soon after it is collected. With on-site treatment, infection and environmental pollution by medical waste can be avoided because there is no need to be stored and transported as the infectious waste. Most infectious waste must be carefully handled and must be incinerated in special incinerators. However, after treatment with the BOX-O3, waste can be eliminated as non-hazardous waste in conventional incinerators. The total cost of this treatment is much lower than the cost of incineration of infectious waste\(^14\).

In conclusion, the BOX-O3 safely and efficiently treated infectious medical waste collected from hospital ward, indicating that the BOX-O3 is an effective alternative technology for the treatment of infectious waste and for controlling hospital infection.

References