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Sterilization of solid materials by various methods and their comparative analysis

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Abstract

This study presents a comparative analysis of different methods for the sterilization of solid materials, including ozonation, ultraviolet (UV) irradiation, and barboting with a herbal alcohol extract ("Steroyl") in carbon dioxide. The results confirm that effective sterilization can be achieved using these methods; however, their efficiency and impact on treated materials vary significantly. Complete (100%) sterilization was achieved within 48 hours using the "Steroyl" extract, while "Steroyl Medium" and "Steroyl Low" did not provide full sterility. The barboting method with "Steroyl" proved particularly effective for manuscript restoration, as it did not damage paper substrates or inks. Various materials, including manuscripts, wool, leather, wheat, packaging materials, and cardboard, were examined. Ozonation showed aggressive effects on manuscripts, dyed textiles, and wood, while UV irradiation negatively affected inks and textiles and demonstrated limited effectiveness on shaded surfaces. In contrast, treatment with "Steroyl" produced positive sterilization results across all tested materials without altering their texture or color. These findings provide a basis for further investigation of sterilization processes and support the selection of appropriate methods for different materials based on specific technological requirements and preservation goals.

Keywords : Sterilization; Ozon; Ultraviolet radiation; Herbal Alcohol Extract; Manuscripts

1. Introduction

Sterilization of solid materials is a highly relevant topic worldwide due to the large volume of biological waste that cannot be directly recycled. In such cases, preliminary treatment through sterilization is required to ensure safe recycling and subsequent waste management [1,2]. Numerous sterilization methods are currently in use, including ozone treatment, ultraviolet (UV) irradiation, autoclaving, and heating [3,4]. These methods are applied according to specific requirements, as different materials demand targeted processing conditions, which may limit the applicability of certain techniques [5,6,7].

Autoclaving is one of the most widely used and effective sterilization methods. It is commonly applied to microbiological materials, laboratory equipment, and thermoresistant items. Although this method is highly efficient, it is unsuitable for thermolabile materials, as the steam temperature in autoclaves reaches at least 121 °C or higher [8,9].

Ozone treatment represents a universal sterilization method, as it can be applied to both thermoresistant and thermolabile materials. Being a gaseous substance, ozone has high penetration ability, resulting in an increased level of

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sterility. However, its disadvantages include potential carcinogenic effects on the human body in poorly ventilated environments. In addition, liquid materials cannot be effectively sterilized using ozone [10,11].

Ultraviolet irradiation is also widely used for the treatment of solid materials, particularly in microbiological applications. UV lamps are installed in areas with a high risk of contamination, such as laminar flow cabinets, operating rooms, and laboratories. Although a high degree of sterility can be achieved, the presence of irradiation shadows represents a major limitation, reducing the overall effectiveness of the process [12,13].

Heating is primarily used for sterilizing metal instruments in medical practice. This method is highly effective; however, similar to autoclaving, it cannot be applied to thermolabile equipment [14,15].

The aim of this study was to investigate the sterilization of solid materials using various methods. It should be noted that solid materials require different sterilization conditions depending on their physical and chemical properties. For example, in the case of contaminated manuscripts, which represent a global preservation problem, many commonly used sterilization methods are inadmissible. This limitation is related to the composition of the paper, ink, and various surface incrustations. Currently, no standardized method has been established for manuscript sterilization.

Therefore, this study proposes the treatment of solid materials, particularly manuscripts, using chemically neutral agents in an inert gaseous state. According to available data, material obtained by barboting carbon dioxide with herbal alcohol extracts possessing antiseptic properties may serve as a chemically neutral sterilization agent. In this study, the extract “Steroyl” was selected for this purpose.

Manuscripts were treated using “Steroyl” through CO_2 barboting. Although this method requires relatively long processing time, it offers significant advantages. The gaseous phase ensures high penetration into the material, while the gas itself does not contain toxic compounds harmful to humans and is less chemically aggressive than ozone.

Based on the obtained results, it can be concluded that ozone treatment and UV irradiation remain suitable for specific applications. For example, grain processing is effectively performed using ozone due to its high penetration capacity. Proper ventilation after treatment minimizes potential health risks associated with ozone exposure. In contrast, ultraviolet irradiation is less effective for grain sterilization, as it requires longer processing time and cannot ensure complete exposure of all particles, even under intensive mixing conditions. However, UV irradiation remains suitable for space sterilization.

These considerations represent the main requirements for solid material sterilization. Accordingly, this study provides conceptual approaches that enable researchers and technologists to select appropriate sterilization methods based on material characteristics and technological requirements.

2. Materials and Methods

2.1. Culture Media

2.1.1. Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar contains digests of animal tissues (peptones), which provide a rich source of amino acids and nitrogenous compounds for the growth of fungi and yeasts. Dextrose serves as the primary energy and carbon source, while agar acts as the solidifying agent. To inhibit bacterial growth, chloramphenicol and/or tetracycline were added as broad-spectrum antimicrobial agents, and gentamicin was included to further suppress gram-negative bacteria. The pH of the medium was adjusted to approximately 5.6 to promote fungal growth, particularly of dermatophytes, and to partially inhibit bacterial contamination. The combination of high dextrose concentration and low pH favored fungal development and reduced bacterial interference.

For preparation, 65 g of the medium was suspended in one liter of distilled water, heated with frequent agitation, and boiled for one minute to ensure complete dissolution. The medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to 45–50°C, and poured into sterile Petri dishes or tubes for slants [16].

2.1.2. Potato Dextrose Agar (PDA)

Potato Dextrose Agar is a commonly used basal medium composed of potato infusion and dextrose, which supports fungal growth. It is recommended by the American Public Health Association (APHA) and the Food and Drug

Administration (FDA) for the enumeration of yeasts and molds in food and dairy products. PDA serves as a general-purpose medium for cultivating yeasts and molds and may be supplemented with selective agents such as chloramphenicol, tartaric acid, and chlortetracycline to inhibit bacterial growth. It is also suitable for cultivating clinically significant fungi and maintaining stock cultures of certain dermatophytes.

For preparation, 39 g of the medium was suspended in 1000 ml of distilled water and heated to boiling until completely dissolved. Sterilization was performed by autoclaving at 121°C (15 lbs pressure) for 15 minutes. The medium was mixed thoroughly before dispensing. For experiments requiring a pH of 3.5, the medium was acidified with sterile 10% tartaric acid. Approximately 1 ml of acid was added to 100 ml of sterile, cooled medium, and the medium was not reheated after acidification [17].

2.1.3. Czapek-Dox Agar (CZA)

Czapek medium, also known as Czapek's agar or Czapek-Dox agar, is widely used for the cultivation of fungi and other microorganisms. It is recommended for qualitative procedures involving saprophytic fungi, soil bacteria, and related organisms. Originally developed by Czapek in 1902 and later modified by Dox in 1910, the formulation used in this study was prepared according to Thom and Church. The medium contains sucrose as the sole carbon source and nitrate as the only inorganic nitrogen source.

For preparation, 49.01 g of the medium was suspended in 1000 ml of distilled water and heated to boiling until fully dissolved. Sterilization was carried out by autoclaving at 121°C (15 lbs pressure) for 15 minutes. After cooling to 45–50°C, the medium was mixed thoroughly and poured into sterile Petri plates.

Test materials were inoculated by thinly spreading the samples on the surface of the agar medium. Plates were incubated aerobically at 25–30°C for 1–2 weeks and examined at regular intervals for microbial growth. Each distinct colony type was subcultured onto appropriate media for isolation and subsequently identified using standard laboratory procedures [18].

Herbal alcohol extracts – "Steroyl", "Steroyl Medium" and "Steroyl Low" was provided by the company "Biologica".

3. Results and Discussions

For this study, strains of microorganisms were isolated from infested materials (books) at the Technical University of Georgia, as well as from the university's strain collection and infested grain crops (wheat and barley), totaling 18 strains. The identified fungal strains included: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Penicillium expansum*, *Chaetomium globosum*, *Trichoderma viride*, *Trichoderma harzianum*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Chaetomium elatum*, *Chaetomium funicola*, *Penicillium commune*, and *Penicillium citrinum*.

All fungal strains were initially cultivated on Sabouraud Dextrose Agar in Petri dishes. Subsequently, the cultures were re-inoculated onto three different media: Sabouraud Agar (medium I) [16], Potato Dextrose Agar (medium II) [17], and Czapek-Dox Agar (medium III) [18]. Since the study focused on fungal organisms, bacterial presence was considered a minor factor.

Samples from all cultures were placed on Petri dishes containing the three media types and subjected to sterilization in a sterile study incubator (Cube) using three different methods: ozonation, ultraviolet (UV) irradiation, and barboting of the herbal alcohol extract "Steroyl" in CO_2. It was observed that barboting the plant-based antiseptic material with CO_2 resulted in effective sterilization, confirming the efficacy of this gaseous mixture.

At defined time intervals, Petri dishes were removed from the incubator, sterilized externally with lids, and incubated at 25 °C for 2–3 days. Following incubation, visual assessment of fungal growth was conducted.

Due to the large number of samples (54 cultures grown on three media each, totaling 162 samples), only principal results are presented in this work. These results represent the evaluation of the research material after 2–3 days of incubation and highlight the effectiveness of the sterilization methods under study.

Figure 1 Eighteen researchable fungal strains inoculated in Petri dishes on three types of culture media (Sabouraud, Potato Dextrose Agar, Czapek), resulting in a total of 162 samples

Figure 2 Incomplete sterilization results. The figure corresponds to the time interval indicated in Table 1, showing visual assessment of the inoculated strains that exhibited growth (negative result) after the initial sterilization treatment

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Figure 3 Complete sterilization in dedicated time, positive result

The degree of sterilization was checked as follows :

All cultures were placed on sterilized Petri dishes containing the appropriate media, from which they were then inoculated onto the study Petri dishes following the same procedure as shown in Figure 1.

Table 1 Sterilization results with different methods and incubation times

Sterilization method / Incubation time	Ozone O_3	Irradiation UV	Antiseptic Steroyl	Antiseptic Steroyl medium	Antiseptic Steroyl Low
2h	-	-	-	-	-
4 h	+	-	-	-	-
8 h	X	-	-	-	-
12 h	X	+	-	-	-
16 h	X	X	-	-	-
20 h	X	X	-	-	-
24 h	X	X	-	-	-
28 h	X	X	-	-	-
32 h	X	X	-	-	-
36 h	X	X	-	-	-
40 h	X	X	-	-	-
44 h	X	X	-	-	-
48 h	X	X	+	-	-

Different microorganisms grow on different media. Positive result (+), Negative result (-), Experiment discontinued due to positive result (X).

As shown in Table 1, after 2 hours of incubation, microbial growth was observed in all samples, regardless of the sterilization method. After 4 hours, growth was no longer observed in samples treated with ozone (see Fig. 3), whereas growth continued in samples treated with ultraviolet (UV) irradiation and "Steroyl" (see Fig. 2). At this point, ozonation was stopped and the exposure time was fixed.

For UV irradiation, sterilization was achieved after 10 hours of incubation, as no further growth was observed (see Fig. 3). In the case of "Steroyl," complete (100%) sterilization was observed only after 48 hours of incubation (see Fig. 3). Trials with "Steroyl Medium" and "Steroyl Low" were discontinued due to ineffective sterilization times.

These results indicate that each method has a specific scope of application:

- **Ozone sterilization** achieves maximum efficiency after 4 hours, making it suitable for heavily contaminated or decomposed materials, such as medical and food instruments, garbage bags, and materials with rot or damp odors. However, ozone is harmful to humans, so ventilation is essential. Its use on sensitive materials like manuscripts is limited due to potential discoloration of inks.
- **UV irradiation** is effective for surface and space sterilization, provided the light source is properly positioned to ensure direct contact with all surfaces. In this context, UV is highly efficient and safer for delicate materials compared to ozone.
- **"Steroyl" treatment** delivered via CO_2 barboting proved to be the most suitable for sensitive materials, including manuscripts, as it did not affect the substrate or inks. The main limitation is the longer processing time, which requires at least 48 hours. Importantly, the CO_2 barboting method was effective in cases where other methods failed.

Based on the observations, ozone is the preferred choice for rapid sterilization of robust materials such as grains, due to its high penetration and fast action. Conversely, for delicate materials like manuscripts, UV irradiation or CO_2-assisted "Steroyl" treatment is recommended, with "Steroyl" providing the safest and most inert approach.

These results highlight that the choice of sterilization method must consider both the material type and the desired speed and effectiveness of sterilization.

4. Conclusion

The present study demonstrated that solid materials can be effectively sterilized using ozone, ultraviolet irradiation, and plant-based alcohol extracts. All three methods are applicable for the sterilization of solid materials under different operational conditions. These differences enable users to select the most appropriate sterilization technique according to the specific requirements of the material being treated.

the results confirmed that a mixture of antiseptic herbal alcohol extracts, when supplied through CO_2 barboting, can be used efficiently for sterilization purposes. This method proved particularly suitable for the treatment of contaminated manuscripts, as it does not adversely affect either the substrate material or inks of different origins. Therefore, CO_2-assisted delivery of herbal alcohol extracts represents a promising and gentle alternative for the preservation and sterilization of sensitive solid materials.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest

Statement of informed consent

Informed consent was obtained from all individual participants included in the study

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