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Supercritical CO_2 sterilization: An effective treatment to reprocess FFP3 face masks and to reduce waste during COVID-19 pandemic☆

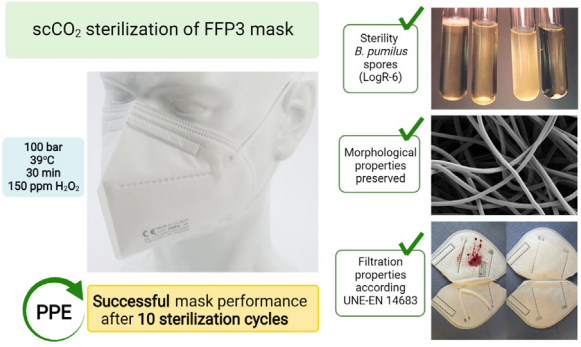
Víctor Santos-Rosales [a], Clara López-Iglesias [a,b], Ana Sampedro-Viana [a], Carmen Alvarez-Lorenzo [a], Samaneh Ghazanfari [c,d], Beatriz Magariños [e], Carlos A. García-González [a,*]

[a] Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma (GI-1645), Faculty of Pharmacy, iMATUS and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Institute of Pharmacy (Pharmaceutical Chemistry), Freie Universität Berlin, Berlin, Germany
[c] Aachen-Maastricht Institute for Biobased Materials (AMIBM), Faculty of Science and Engineering, Maastricht University, 6167 RD Geleen, the Netherlands
[d] Department of Biohybrid and Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, 52074 Aachen, Germany
[e] Departamento de Microbiología y Parasitología, Facultad de Biología, CIBUS, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

HIGHLIGHTS

- Shortages of PPE could be reduced by a safe and effective sterilization method.
- A 30-min sterilization with $scCO_2$ is effective and preserves functional integrity of PPE.
- This process can be potentially used in hospital environments in a context of urgency.
- Performance of PPEs was not remarkably compromised even after 10 sterilization cycles.
- Reuse of PPE could be achieved using $scCO_2$ resulting in a reduction of waste.

GRAPHICAL ABSTRACT



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ABSTRACT

The outbreak of COVID-19 pandemic unveiled an unprecedented scarcity of personal protective equipment (PPE) available in sanitary premises and for the population worldwide. This situation fostered the development of new strategies to reuse PPE that would ensure sterility and, simultaneously, preserve the filtering properties of the materials. In addition, the reuse of PPEs by reprocessing could reduce the environmental impact of the massive single-use and disposal of these materials. Conventional sterilization techniques such as steam or dry heat, ethylene oxide, and gamma irradiation may alter the functional properties of the PPEs and/or leave toxic residues. Supercritical CO_2 ($scCO_2$)-based sterilization is herein proposed as a safe, sustainable, and rapid sterilization method for contaminated face masks while preserving their performance. The functional (bacterial filtration efficiency, breathability, splash resistance, straps elasticity) properties of the processed FFP3 face masks were evaluated after 1 and 10 cycles of sterilization. Log-6 sterilization reduction levels were obtained for face masks contaminated with *Bacillus pumilus* endospores at mild operating conditions (CO_2 at 39 °C and 100 bar for 30 min) and with low contents of H_2O_2 (150 ppm). Physicochemical properties of the FFP3 face masks remained unchanged after reprocessing and differences in efficacy were not observed neither in the filtration tests, following UNE-EN 14683, nor in the integrity of FFP3 filtration after the sterilization process. The herein presented method based on $scCO_2$ technology is the first reported protocol achieving the reprocessing of FFP3 masks up to 10 cycles while preserving their functional properties.

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* Corresponding author.
E-mail address: carlos.garcia@usc.es (C.A. García-González).

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1. Introduction

The pandemic caused by SARS-CoV-2 virus unveiled the limited contingency of the healthcare systems towards outbreak situations worldwide. Namely, the demand for personal protective equipment (PPE) dramatically surpassed the global production capacity of manufacturers (Rowan and Laffey, 2020). Subsequently, the general scarcity of different PPEs, particularly face masks, resulted in healthcare professionals being at the frontline of the COVID-19 outbreak without the suitable protective "shields" to mitigate the risk of becoming infected by the virus (Rowan and Laffey, 2021; Heinzerling et al., 2020). In the advent of the COVID-19 outbreak situation, the number of clinicians infected by SARS-CoV-2 represented ca. 15–20% of the total number of cases in several developed countries like Spain (Torrente et al., 2021). A dramatic increase in PPE production capacity by the manufacturer lines has momentarily alleviated the unmet demand, but the quest for providing tools in hospitals and other sanitary premises to prepare healthcare workers and individuals for future PPE scarcity episodes is still unsolved.

Different disinfection and sterilization methods have been tested with PPEs as reprocessing attempts to reuse them, including technologies already available at the hospital environment. These methods can be broadly categorized into heat (dry and moist), chemical (ethylene oxide) or radiation (gamma, X-ray and UV) treatments (Cadnum et al., 2020; Bennet et al., 2021; Wilson and Nayak, 2019). The limited number of possible sterilization cycles (e.g., the use of vaporized hydrogen peroxide sterilization causes changes in masks after the fourth cycle (RIVM, n.d.); alterations in masks are observed after the tenth cycle using UV-irradiation (Liao, 2020)), the modifications of the filtration efficacy after reprocessing (e.g., UV-exposure led to a certain increase in particle penetration (Lindsley et al., 2015); the use of steam altered the filtration efficiency after 5 cycles (Liao, 2020); gamma-ray caused significant reduction in the filtering capacity (de Man et al., 2020)) and the problems of adjustment of the respirators to the face (irradiation, heat (Viscusi et al., 2011), or hydrogen peroxide vapor (Commissioner, 2021)) are among the severe obstacles of implementing a PPE reprocessing strategy. Moreover, some technologies (e.g., UV-light) can only be applied to the surface of materials due to their low penetration capacity that hinders an efficient disinfection activity within the multi-layered filters of the filtering facepiece respirators (N95, FFP2 and FFP3) and the medical or surgical face masks (RIVM, n.d.; Rubio-Romero et al., 2020; Farke, 2020; Lore et al., 2012). Overall, a suitable sterilization technique to establish standardized protocols for PPE reprocessing is required with emergency not only from the healthcare but also environmental perspective. Due to the COVID-19 pandemic, the general and forced wear of PPE led to a high risk of improper disposal and it contributed to the ca. 8 million tons of pandemic-related plastics reported as of August 2021 (Peng et al., 2021). This dramatic increase in wastes becomes a new threat to the global ecosystem (Saliu et al., 2021; Hartanto and Mayasari, 2021). In general terms, the effective reuse by reprocessing of masks could reduce the overall consumption and therefore limit the contribution of PPEs to the world's plastic pollution (Kutralam-Muniasamy et al., 2022; De-la-Torre et al., 2022).

Sterilization with supercritical CO_2 ($scCO_2$) has emerged as a green alternative to conventional sterilization processes, being particularly effective for the processing of sensitive materials, such as novel polymeric scaffolds or synthetic tissues (Ribeiro et al., 2019). Namely, $scCO_2$ sterilization operates at mild conditions and has excellent penetration capacity in complex porous materials for biomedical applications (Ribeiro et al., 2019; Santos-Rosales et al., 2021). Moreover, it does not leave toxic residues that could cause skin irritation, so its use has been proposed in the disinfection of medical textiles and fabrics (Aslanidou et al., 2016; Cinquemani et al., 2007; Calvo and Casas, 2018). In this context, $scCO_2$ sterilization has been shown useful for the sterilization of nuclear, biological, and chemical suits for military purposes without altering the properties of the materials or their functionality (Calvo and Casas, 2018). In a recent investigation (Bennet et al., 2021), $scCO_2$ was used to inactivate spores and viruses in different environmental conditions on N95 and cloth masks with encouraging results.

$ScCO_2$ combines several sterilization mechanisms for cell inactivation operating in tandem, being the cytoplasm and extracellular acidifications with CO_2 the most relevant ones (Spilimbergo and Bertucco, 2003; García-González et al., 2015; Matthew et al., 2013). The inactivation process mainly consists of three steps: 1) Acidification of the extracellular space due to the reaction between CO_2 and H_2O forming bicarbonate and hydrogen ions. 2) Modification of the cell membrane composition due to the extraction of lipids from the membrane with $scCO_2$ (Lin et al., 1992). Additionally, the cell membrane structure is damaged by the acidification, increasing the permeability and further penetration of CO_2 (Kamihira et al., 1987; Garcia-Gonzalez et al., 2007). 3) Intracellular modifications due to cytoplasmatic pH lowering (Garcia-Gonzalez et al., 2007), which alter the activity of key enzymes and the cell metabolism pathways (Ishikawa et al., 1995; Hutkins and Nannen, 1993). The disbalance of the pH also modifies electrolytic concentrations (Ho-mu et al., 1993), resulting in the removal of vital constituents from the cell.

In this work, a supercritical sterilization protocol was developed for the sterilization of FFP3 face masks with the vision of being implemented at hospital environments. To achieve this, two main principles were set: the guarantee that the materials would preserve their safety and protection capacity after the sterilization treatment, and the development of a suitable sterilization protocol transferable to hospital sterilization units that effectively removes bioburden from the masks. Firstly, an effective sterilization protocol was developed against *B. pumilus* spores, a highly resistant microorganism towards $scCO_2$ sterilization methods (Santos-Rosales et al., 2021). A trade-off between mild pressure and temperature conditions, short processing times, and low amounts of chemical additives was established. Then, this protocol was applied to the FFP3 masks to evaluate the functional integrity (physicochemical properties, filtration efficacy, and mechanical resistance of straps) of the PPEs after 1 and 10 supercritical sterilization cycles.

2. Materials and methods

2.1. Materials

CO_2 (99.8% purity) was supplied by Nippon Gases (Madrid, Spain). Hydrogen peroxide (H_2O_2) 30% (v/v) was purchased from Sigma-Aldrich (Madrid, Spain). FFP3 masks with a five-layer filtering cloth were purchased from Galmask (Santiago de Compostela, Spain). *Bacillus pumilus* spore suspensions ($1.5–1.9 \times 10^6$ spore units/100 μL) were obtained from Excelsior Scientific Ltd. (Wisbech, UK) and used as bioindicator.

2.2. Supercritical CO_2 sterilization procedures

2.2.1. Sterilization of bacterial endospores and contaminated FFP3 masks

Sterilization tests of bacterial endospores were performed in a 600-mL NovaGenesis equipment provided with agitation (NovaSterilis Inc., Ithaca, NY, USA) using two different scenarios. On one hand, the sterilization of *B. pumilus* spore suspensions was performed in HPLC vials containing 100 μL ($1.5–1.9 \times 10^6$) spores. Samples were placed in the autoclave and subjected to the sterilization conditions. On the other hand, FFP3 masks were contaminated with *B. pumilus* spores to recreate a more realistic and complex scenario. Briefly, FFP3 masks were cut into 4 cm^2 pieces and 100 μL of spore suspension ($1.5–1.9 \times 10^6$ spores) were inoculated in the melt-blown polypropylene (PP) filter (Fig. 1). The cuts were confined in thermo-sealed pouches and put inside the autoclave. Varying volumes of a 30% (v/v) H_2O_2 solution were dosed at the bottom of the autoclave achieving the desired additive contents (100 to 600 ppm of H_2O_2), avoiding direct contact of the liquid with the samples. The high-pressure autoclave was conditioned to the sterilization parameters and finally the system was manually depressurized at a constant rate of 14 bar/min. A summary of all the tested parameters can be found in Table 1.

2.3. Sterilization efficacy assessment and microbiological evaluation

2.3.1. Spore membrane integrity tests and sterility assessment

Live and treated spores were stained using the Live/Dead BacLight bacterial viability kit (Thermo Fisher Scientific, Waltham, MA, USA) according

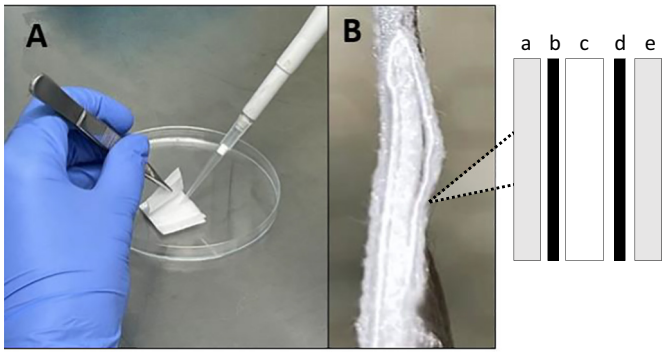

Fig. 1. (A) Image illustrating the contamination procedure of FFP3 mask pieces with a *B. pumilus* spore suspension in aseptic conditions. (B) Representation of the different filters of the mask (from outer to inner layers): Filter a (spunbond PP fabric (50 g/m^2)), Filter b (cotton fabric (40 g/m^2)), Filter c (melt-blown PP fiber (25 g/m^2)), Filter d (cotton fabric (40 g/m^2)), and Filter e (spunbond PP fabric (30 g/m^2)).

to the manufacturer protocol for the evaluation of the membrane integrity. Control samples (untreated spores) and sterilized samples of 1.9 × 10^6 spores of *B. pumilus* were incubated for 30 min in the dark with a 1:1 mixture of Syto™ 9 (green dye) and propidium iodine (PI, red dye) to stain nucleic acids of live and dead spores. Stained spores were observed under confocal microscopy (LEICA TCS-SP2, LEICA Microsystems Heidelberg GmbH, Mannheim, Germany).

In addition to the Live/Dead staining tests, the sterilization efficacy of the $scCO_2$ process was evaluated by turbidity measurements as follows. After processing, the spore suspensions from the HPLC vials were inoculated in 10 mL of tryptic soy broth (TSB) medium. After 24 h of incubation at 37 °C, tubes were visually examined for bacterial growth. Finally, 1 mL of the culture was seeded in tryptic soy agar (TSA) plates to verify the absence of growth. The sterilization of FFP3 mask pieces was evaluated in the same way.

2.4. Evaluation of the physicochemical and functional integrity of FFP3 mask components

2.4.1. Preliminary evaluation of FFP3 masks under stress conditions

Prior to the sterilization tests, the processing window of the mask parts was evaluated at 200 bar of pressure and 45 °C of temperature for a period of 2 h. The selected stress conditions of pressure and temperature correspond to more aggressive values than those defined as working conditions for the sterilization tests (Table 1). In addition, experiments were also performed under the previously mentioned processing parameters but in the presence of 1200 ppm of H_2O_2.

The tested FFP3 masks consisted of five sequential filters of different composition. Some of the filters of the mask were made from the same material with different fiber density. The following filter pattern was identified from the outer side to the internal layer in contact with the skin. (1) Filter *a*, made of spunbond (PP) fabric at a density of 50 g/m^2; (2) Filter *b*, cotton fabric at a density of 40 g/m^2; (3) Filter *c*, melt-blown PP at a density of 25 g/m^2; (4) Filter *d*, identical to filter *b*; (5) Filter *e*, spunbond PP at a density of 30 g/m^2. A representative image of the FFP3 mask configuration is depicted in Fig. 1.

Table 1
Supercritical sterilization conditions tested for the deactivation of bacterial endospores of *B. pumilus* (1.5–1.9 × 10^6 spores) with the corresponding logarithmic reduction (logR) of the process. In bold, optimized static conditions are shown.

Duration (min)	H_2O_2 (ppm)	LogR
120	600	6
60	600	6
30	600	6
30	300	6
30	200	6
30	**150**	**6**
15	150	–
30	100	–
30	0	–

Small pieces of the FFP3 masks were put inside a high-pressure autoclave (Thar Process, Pittsburg, PA, USA) and the chamber preheated at 45 °C was pressurized with CO_2 until 200 bar. Optionally, H_2O_2 was incorporated in the autoclave before the pressurization step. Upon system depressurization, FFP3 masks were collected, their dimensions were measured and observed at naked eye, and CCD images taken with a 108 MP camera (Xiaomi Inc., Beijing, China) to evaluate major changes in their size and appearance, such as colour and shape.

2.4.2. Long-term FFP3 masks reprocessing for several cycles

Face masks were subjected to 1 and 10 sterilization cycles at the optimized static conditions (Table 1, condition highlighted in bold) for the evaluation of the integrity of the materials. Entire masks were put inside the autoclave, and 150 ppm of H_2O_2 were incorporated at the bottom of the autoclave by dosing a commercially available solution (30% v/v) at the beginning of each cycle. These tests were carried out at 39 °C and 100 bar during 30 min. Finally, the system was depressurized at a rate of 14 bar/min and the material was subjected to the following cycle or collected for further evaluation.

2.4.3. Evaluation of reprocessed FFP3 masks

Entire face masks subjected to 1 and 10 $scCO_2$ sterilization cycles were analysed to assess whether the exposure to the reprocessing induced relevant changes on the polymeric components of the FFP3 masks. Special attention was paid to the structure of the FFP3 masks filters, which was analysed by scanning electron microscope (FESEM, ULTRA PLUS Zeiss, Oberkochen, Germany) running at a voltage of 5 kV. Prior to their imaging, samples were iridium-sputtered (5–10 nm thickness) depending on the nature of the samples and the required information. The characterization of the filter layers was performed in processed masks (1 and 10 cycles) and in control (unprocessed) masks. All post-sterilization and control samples were visually inspected and scrutinized for any visible sign of degradation, deterioration or changes in texture or colour.

2.4.4. Evaluation of the filtration efficiency

In order to assess the filtration efficacy of the masks, bacterial filtration efficacy, breathability, and splash tests were performed based on EN 14683 standard. All tests were carried out on the finished face mask product and 5 samples per test were used. All face masks were conditioned at 21 ± 5 °C and 85 ± 5% relative humidity for a minimum of 4 h prior to each test.

2.4.4.1. Bacterial filtration efficiency. Staphylococcus aureus (ATCC 6538) were inoculated in 40 mL TSB in an Erlenmeyer flask and incubated with a shaking velocity of 60 rpm at 37 °C for 16 h. The culture was then diluted in peptone water to reach a concentration of 5 × 10^5 CFU/mL. The bacterial challenge was maintained between 1.7 and 3.0 × 10^3 CFU per test.

To perform the test, the extremities of the mask were removed first, and the mask with the test area of minimum 49 cm^2 was clamped between a six-stage cascade impactor and an aerosol chamber. An aerosol of *Staphylococcus aureus* was introduced into the aerosol chamber and drawn through the mask material and the impactor under vacuum. The testing was performed with the inside of the medical face mask in contact with the bacterial challenge. For each sample, the bacterial filtration efficiency percentage B was calculated using the following equation (Eq. (1)):

$$B = \frac{(C - T)}{C} \times 100 \quad (1)$$

where *C* is the mean of the total plate counts for two positive control runs without a sample, and *T* is the total plate count for the face mask sample.

2.4.4.2. Breathability. The differential pressure through a measured surface area of the face mask material at a constant air flow rate of 8 L/min was used to measure the air exchange pressure. From each mask, 5 different test areas of 25 mm diameter were analysed. In order to perform the test, a 4.9 cm^2 area of the face mask was placed between the top and bottom parts of the holder and was clamped using a mechanical clamp with sufficient pressure to avoid air leaks. The differential pressure was then measured using a differential pressure manometer. This procedure was repeated 5 times for each mask. The differential pressure per mask area (ΔP, in Pa/cm^2) was then calculated as follows (Eq. (2)):

$$\Delta P = \frac{(X_{m1} - X_{m2})}{4.9} \quad (2)$$

where X_{m1} is the pressure in Pa at the low-pressure side of the material, X_{m2} is the pressure in Pa at the high-pressure side of the material, and 4.9 is the test area in cm^2.

2.4.4.3. Splash resistance. The resistance of the face mask to penetration of synthetic blood was tested in accordance with ISO 22609:2004. The conditioned face masks were mounted on the device at a distance of 30.5 cm and exposed to a 2 mL splash of synthetic blood on the outer side of the mask at a stream pressure of 16 kPa. The inner side of the mask was then evaluated in terms of the penetration of the synthetic blood within 10 s after performing the test.

2.5. Mechanical tests

The straps of sterilized FFP3 masks were subjected to tensile tests in a TA.XT Plus Texture Analyzer (Stable Micro Systems, Ltd., UK) fitted with a 30 Kg load cell at a crosshead speed of 5 mm/s, according to ASTM standards (D13 Committee, n.d.). In each test, straps of defined dimensions were fixed to the upper and lower clamps with a gap of 25 mm (5 cm specimens) or 75 mm (10 cm specimens), depending on the equipment configuration, and subjected to 5 cyclic loads. In each cycle the strap was stretched 25 mm in length (i.e., 100% strain for 25 mm gap and 33% strain for 75 mm gap). Tests were performed in duplicate at room temperature. The Young's modulus (E) was calculated from the stress–strain plots previous conversion to engineering stress and engineering strain.

2.6. Statistical analysis

All results were expressed as mean ± standard deviation. Statistical analyses of the breathability, the bacterial filtration efficacy results and of the mechanical performance of the straps (1-way ANOVA) were performed using GraphPad Software v9 (GraphPad Software., San Diego, CA, USA) followed by the post hoc Tukey HSD multiple comparison test.

3. Results and discussion

3.1. Definition of the operating window for the supercritical CO_2 sterilization of FFP3 masks

The main operating parameters mastering the efficacy of the $scCO_2$ sterilization process are temperature, pressure, treatment time, presence of additives and depressurization rates (Ribeiro et al., 2019; Perrut, 2012). Thermal treatment can deactivate a wide variety of microorganisms, including resistance forms. A classical steam sterilization procedure requires a minimum operating temperature of 121 °C, according to ISO standards (ISO 17665-1:2006(en), n.d.). The temperature effect on the $scCO_2$ sterilization efficacy relies on an increase of the solubility of CO_2 in the liquid cell medium, as well as the fluidity of the cell membrane. Both features enhance the acidification process and therefore the inactivation ability of $scCO_2$. In comparison to steam/dry heat sterilization, only mild operating temperatures are required, but temperatures below 40 °C usually start compromising the efficacy of the process (Zhang et al., 2006). Pressure is also essential for the efficacy of the process as higher pressures correlate with higher amounts of CO_2 dissolved in the intra- and extracellular media. However, this influence is easily observable at mild pressures (80–150 bar), and an increase in pressure above 200 bar does not significantly alter the sterilization efficacy (Perrut, 2012). Treatment time is also a critical parameter as it sets the contact time of bacterial cells with CO_2. The addition of other additives, such as hydrogen peroxide, peracetic acid, or even water, boosts the bactericidal activity of the $scCO_2$ and they are often used in low contents (in ppm) as chemical additives to achieve the sterilization of the most resistant forms, such as bacterial spores (Bernhardt et al., 2015; Leggett et al., 2015). Finally, the sterilization efficacy of the process can be favoured by tuning the depressurization rate, inducing a mechanical burst of cells (Fraser, 1951; Foster et al., 1962).

A trade-off solution between the processing parameters and the material integrity was firstly set for the development of an efficient sterilization for the reprocessing of contaminated FFP3 masks. Since the main component of the mask is polypropylene (PP), the thermal stability of the polymer must be considered under a $scCO_2$ atmosphere. Due to the plasticizing effect of compressed CO_2, PP may experiment swelling depending on the processing temperature and pressure (Hasan et al., 2010; Gong et al., 2017).

FFP3 face masks were subjected to 45 °C and 200 bar of CO_2 during a 2-hour period to confirm the resistance of the materials to a CO_2 atmosphere saturated with H_2O_2. After the sterilization process, the collected materials showed no relevant changes in the textiles of the mask layers (Fig. S1). Similar results were obtained for masks processed under $scCO_2$ atmosphere in absence of additives. Based on these encouraging results, the sterilization procedures were designed in a more conservative manner under milder operating conditions. For instance, the working parameters were maintained below these thresholds of temperature (<45 °C), pressure (<200 bar), contact time (<120 min) and additive incorporation (<1200 ppm).

3.2. Sterilization of *B. pumilus* spores and contaminated FFP3 masks

The resistance of *B. pumilus* towards $scCO_2$ sterilization was previously reported and the incorporation of additives is usually required to achieve the inactivation of resistance forms (Zhang et al., 2006; Shieh et al., 2009; Santos-Rosales et al., 2019, 2022). For instance, the spores of this microorganism were recently proposed as the biological indicator to control $scCO_2$-based sterilization protocols (Santos-Rosales et al., 2021).

Temperature was fixed at 39 °C since protocols achieving high sterilization levels of *B. pumilus* dry spores under this working temperature were previously reported (Santos-Rosales et al., 2021, 2022). Regarding the processing pressure, an upper limit of 100 bar was defined to avoid deformations in the PP mask filters. A controlled depressurization during ca. 7 min was performed to prevent morphological changes in the masks. Namely, $scCO_2$ treated PP experimented was reported to transition from colourless material towards a slight yellow colouration due to a quick depressurization (Shieh et al., 1996). This aspect could be particularly detrimental for the multiple reprocessing of FFP3 masks.

At the former pre-set processing parameters, an optimization of the efficacy of the sterilization was performed in terms of additive content and overall contact time (Table 1). Commercial suspensions of *B. pumilus* spores were employed, which are known to be more vulnerable to $scCO_2$ compared to dry forms (Kamihira et al., 1987; Nakamura et al., 1994; Kumagai et al., 1997; Dillow et al., 1999). However, the use of H_2O_2 as additive was required to achieve complete inactivation of the initial bioburden (10^6 spores). Regarding the purpose of the developed method, the presence of residual H_2O_2 in the sterilized FFP3 mask can induce an irritation effect on the skin during usage. Moreover, inhalation of highly concentrated solutions of H_2O_2 can induce severe irritation and inflammation of the respiratory tract (Watt et al., 2004; Urban et al., 2019). Therefore, only low contents of H_2O_2 (less than 600 ppm) were incorporated during $scCO_2$ treatment, avoiding direct contact of the liquid with the samples.

The inactivation of *B. pumilus* spores was firstly evaluated through a specific staining method. Accordingly, Live/Dead staining was shown to work not only with eubacteria (Boulos et al., 1999) but also with archaea (Leuko

et al., 2004), eukaryotic cells (Zhang and Fang, 2004) or bacterial endospores (Laflamme et al., 2004). The selected stain only allows the differentiation between intact and damaged cytoplasmic membranes. However, it is often used to assess active and dead cells (Berney et al., 2007) since membrane-compromised microorganisms can be considered as dead (Nebe-von-Caron et al., 2000). Regarding the dyes, the green fluorescing (SYTO9) can enter all cells, therefore both control (untreated) and sterilized samples would be stained. In contrast, spores with damaged membranes get also stained with propidium iodine. In our case, untreated spores (Fig. 2A, B) only had green fluorescence and sterilized spores (Fig. 2C, D) displayed the green fluorescence paired with an intense red colouration. In addition, treated spores were also cultured in TSB to confirm the absence of microbial growth (cf. Section 2.3.1).

An optimized sterilization procedure was developed requiring only 30 min of exposure to $scCO_2$ environment and using 150 ppm of H_2O_2 (Table 1). Neither decreasing additive content further nor using shorter contact times were able to reduce the bacterial endospore load. Since the sterilization procedure was controlled with endospores, which are the more resistant forms, the inactivation of other microorganisms is implicit (Zhang et al., 2006). Thus, results in this section verify the development of a powerful tool under mild processing conditions not only for the inactivation of endospores but also vegetative bacteria, viruses, and even fungi in contaminated materials.

Sterilization of dry porous materials, including masks, can be challenging due to poor accessibility regions and absence of water. Therefore, all the tested parameters for sterilizing spore suspensions were assessed in the more complex and realistic scenario of contaminated FFP3 masks. Due to the deep penetration capacity of $scCO_2$, the experimental settings developed for the inactivation of liquid spore suspensions rendered identical results in contaminated FFP3 masks.

3.3. Evaluation of reprocessed FFP3 masks

Entire FFP3 face masks subjected to 1 and 10 sterilization cycles and using the optimized conditions (100 bar, 39 °C, 150 ppm of H_2O_2, 30 min, depressurization at 14 bar/min) were observed under a scanning electron microscope (SEM) to confirm that the porosity, surface and diameter of the filters were not compromised when subjected to several sterilization cycles (Fig. 3). The fibrous structure of the filtering cloths was unaltered after the $scCO_2$ processing with no differences in comparison to the control samples, regardless of the number of sterilization cycles. Additional images with a variety of magnifications can be found as Supplementary material (Figs. S2, S3, S4, and S5). These results are in line with previous reports where masks subjected to 5 cycles of $scCO_2$ sterilization did not cause any visible damage on their surface properties (Bennet et al., 2021).

3.3.1. Bacterial filtration efficacy (BFE) and breathability

To investigate whether 1 and 10 sterilization cycles affected the integrity of the masks, the bacterial filtration efficacy (BFE) testing was performed according to UNE-EN 14683 standard concerning surgical mask performance (Standards, n.d.). Among the different categories of face masks, surgical masks are the only masks specifically designed and regulated to prevent bioaerosol dissemination from the wearer into the environment. Masks specified under the abovementioned standard are classified into two types according to their bacterial filtration efficiency (BFE) level: type I masks have a BFE ≥ 95%; type II masks have a BFE ≥ 98%. In this

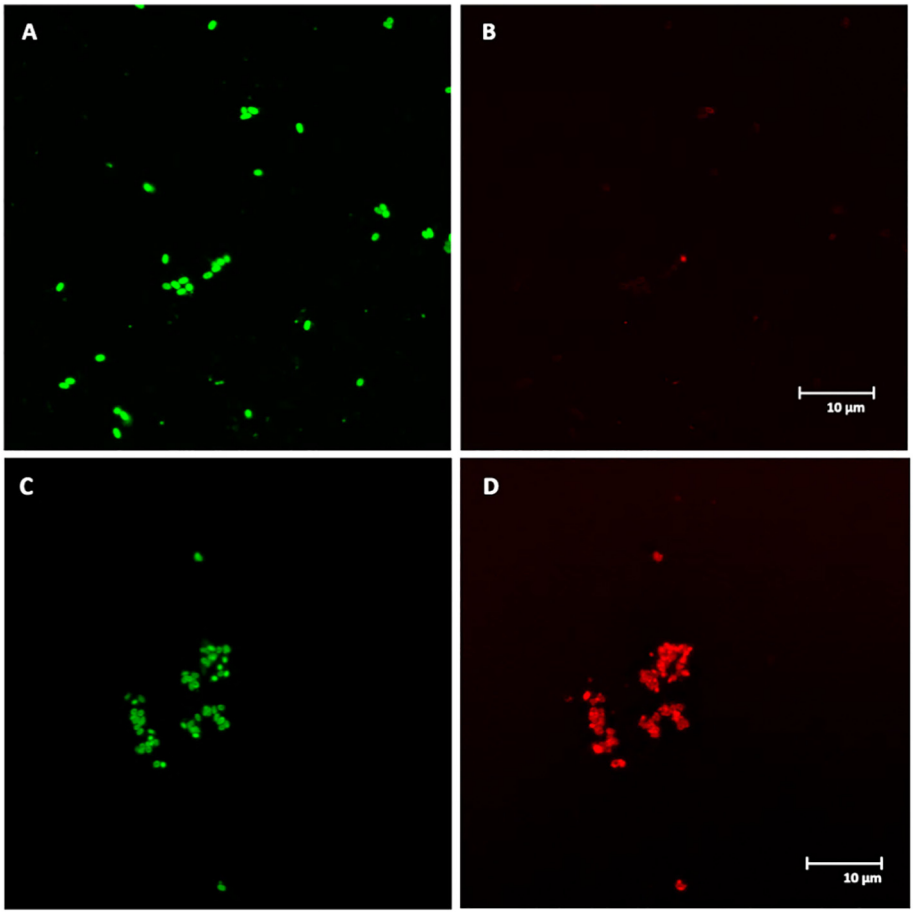

Fig. 2. Representative results of the membrane integrity assay of *B. pumilus* spores after $scCO_2$ sterilization (100 bar, 39 °C, 30 min, 600 ppm H_2O_2), stained with Live/Dead BacLight. Untreated spores (A, green filter; B, red filter) compared to sterilized samples (C, green filter; D, red filter). Images were not merged since green signal overlapped red signal. Complete spore inactivation was achieved under the abovementioned processing parameters.

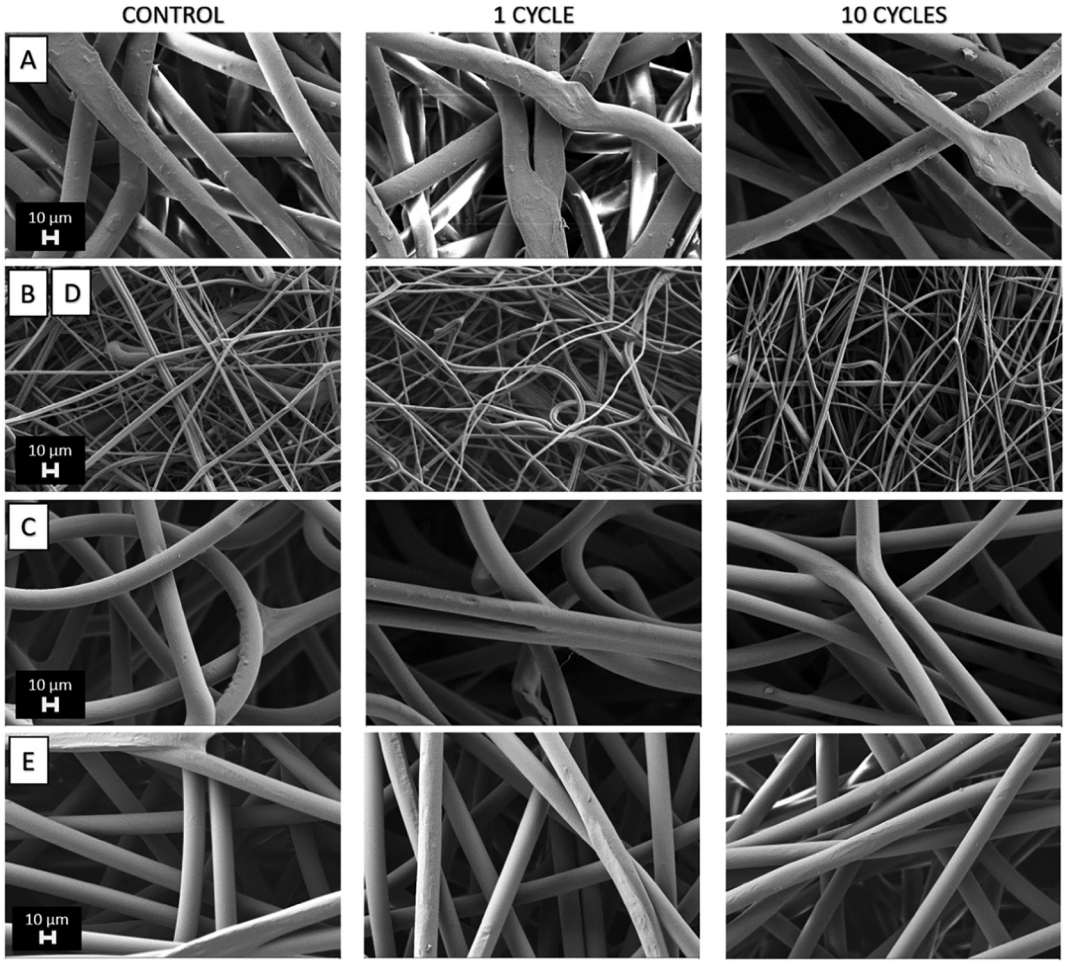

Fig. 3. SEM images of the different filters, (A) spunbond PP fabric filter (50 g/m^2), (B, D) cotton fabric filters (40 g/m^2), (C) melt-blown PP filter (25 g/m^2), I spunbond PP fabric filter (30 g/m^2) of the tested FFP3 masks. Morphological differences between control and sterilized filters (1 or 10 cycles) were not observed.

section, results showed that the $scCO_2$ sterilization treatment induced significant reduction in the BFE values (Fig. 4). Mask subjected to 1 and 10 cycles presented the same performance during the filtration test, displaying an identical 1.5% reduction in BFE value. Nevertheless, sterilized masks presented an overall BFE value above 98%, accomplishing the target values for type II masks, even though filtering masks (FFP3) are not considered medical devices. In a context of urgency, results in this section place great value on $scCO_2$ sterilization for the reprocessing of FFP3 mask while ensuring a suitable filtering performance according to UNE-EN 14683.

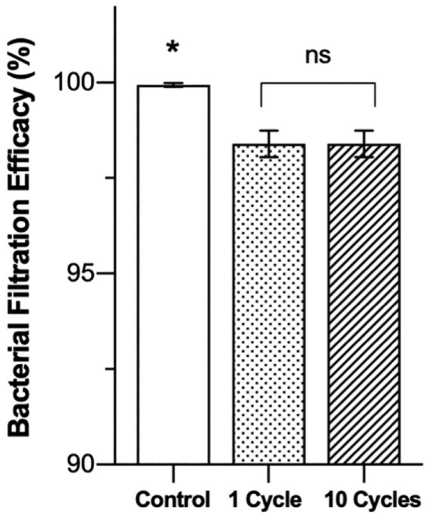

Fig. 4. Bacterial Filtration Efficacy of FFP3 masks before (control) and after (1 and 10) sterilization cycles. Asterisk denotes significant differences (1-way ANOVA; $p < 0.05$). The $scCO_2$ sterilization induced significant modifications in BFE results of the masks, although values were in the range set in the UNE-EN standards.

Resistance of a material towards the air flow can be measured by the pressure differential across the analysed area. From these values, a reliable parameter indicating the ease of breathing trough can be obtained (Kwong et al., 2021). Breathability is an essential criteria regarding costumer comfort during use and, if inadequate, might lead to an inappropriate PPE use (Pei et al., 2020). In this work, the breathability of FFP3 masks sterilized by $scCO_2$ was analysed and compared to untreated masks (Fig. 5). The sterilization treatment had a significant impact on the mask breathability properties. Namely, a downward trend was identified in the differential pressures as the sterilization cycles were performed. Masks subjected to one sterilization cycle experimented a decrease of 4% in the pressure drop values compared to the control masks. However, this reduction was not consistent with further reprocessing cycles and masks subjected to 10 sterilization cycles only suffered decreases close to 7%. Despite the $scCO_2$ treatment enhanced the FFP3 breathability, the filtration efficacy of the masks was not compromised to the point of avoiding use in a hospital setting.

3.3.2. Splash resistance

The evaluation of synthetic blood penetration on medical face masks is required from a regulatory perspective to ensure the suitable performance of PPEs (Standards, n.d.). Accordingly, the splash test was performed

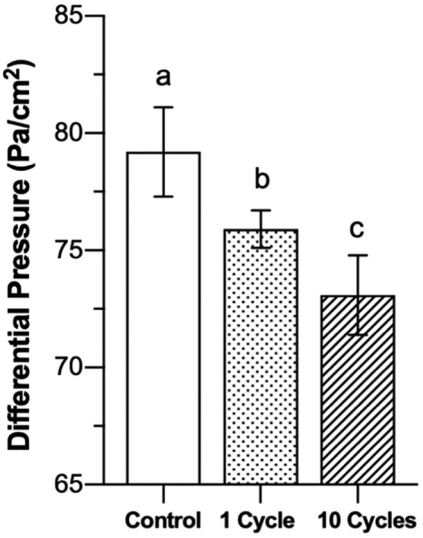

Fig. 5. Breathability values of FFP3 masks before (control) and after several (1 and 10) sterilization cycles. Equal letter denotes statistically homogeneous groups (1-way ANOVA; $p < 0.05$). The $scCO_2$ sterilization induced a significant increase in the breathability values of the treated masks but did not have practical relevance.

following ISO 22609:2004 and the results were determined in a qualitatively manner by visual inspections. $ScCO_2$ sterilization did not induce any modifications on the performance of the FFP3 masks in this regard (Fig. 6). Footprint of the splash blood can be easily observed in the outer part of the masks (A, C, E), but no trace of synthetic blood was seen on the inner sides of the masks (B, D, F) resulted intact regardless despite being subjected to multiple sterilization cycles. All the tested materials passed the splash test for a set stream blood pressure of 120 mmHg, which corresponds to the pressure threshold defined by ISO standards.

3.3.3. Mechanical performance of the elastic straps of the FFP3 mask

The respirator integrity following disinfection is crucial to demonstrate the preservation of the functional properties of the masks. The strap behaviour of the sterilized FFP3 mask was investigated through tensile tests following ASTM standards (D13 Committee, n.d.). Modifications in this regard could compromise not only the patient comfort levels but also the appropriate fit once used. In this context, the implementation of the $scCO_2$ sterilization for the recycling of FFP3 masks could be jeopardized if the mechanical properties of the elastic straps are remarkably affected.

In a recent study, peracetic acid was used to decontaminate N95 masks and the strap elasticity was not affected after 5 decontamination cycles (John et al., 2021). However, steam sterilization was reported to be incompatible for the reprocessing of N95 masks since critical losses of strap elasticity were observed after a single sterilization cycle (Grinshpun et al., 2020).

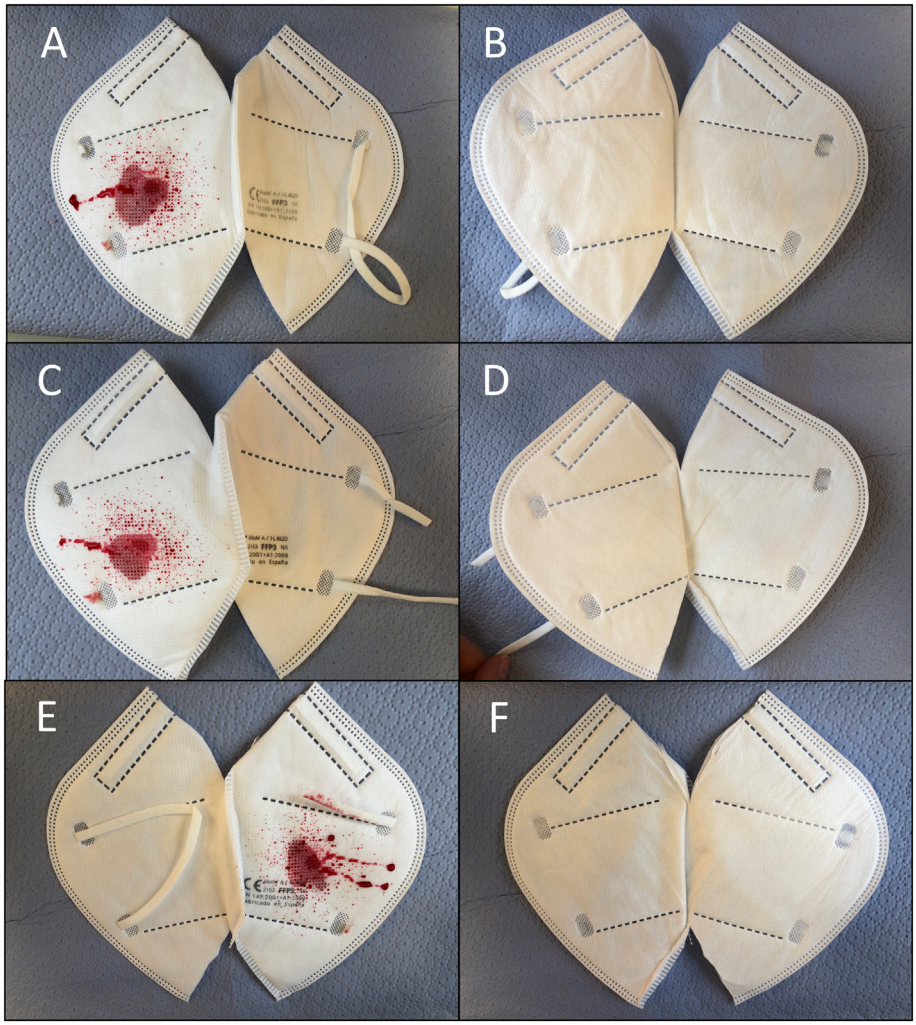

Fig. 6. Results of blood splash penetration tests on (A, B) controls, (C, D) masks subjected to 1 sterilization cycle and (E, F) masks subjected to 10 sterilization cycles. (A, C, E) The blood splash can be identified in the outer part of the masks, while (B, D, F) the inner parts are clean.

Table 2
Young's modulus values of FFP3 mask straps after being subjected to $scCO_2$ sterilization compared to untreated straps. Straps were subjected to 5 consecutive deformation cycles (33% deformation/cycle).

FFP3 masks	Tensile cycle	Young's modulus (MPa)
Control	1st	0.198 ± 0.001
	5th	0.221 ± 0.005
1 Cycle	1st	0.202 ± 0.010
	5th	0.227 ± 0.002
10 Cycles	1st	0.244 ± 0.001
	5th	0.247 ± 0.001

To simulate a realistic scenario, specimens of 10 cm were cut and subjected to cyclic tensile tests applying a 33% deformation per cycle. Under this experimental setup, the straps presented a perfectly elastic behaviour when subjected to stress, and the deformation-recovery profiles obtained after the 5 cyclic charges were symmetrical for all the tested specimens, including those sterilized with $scCO_2$ (Fig. S6). The straps maintained their initial dimensions after the consecutive deformation cycles. Overall, the $scCO_2$ did not induce any modification on the mechanical response of the straps, and the values of the Young's modulus of the material were statistically identical (1-way ANOVA, $p < 0.05$), even after 10 reprocessing cycles (Table 2).

In a more exhaustive approach, specimens of 5 cm were subjected to cyclic tensile tests and applying a 100% deformation per cycle. Similar results were found regarding the mechanical behaviour of the straps under this experimental setup (Fig. S7). Namely, the $scCO_2$ sterilization induced certain increase on the Young's modulus values compared to control straps. This effect was constant and dependent on the sterilization cycles (Table 3), although was not statistically significant (1-way ANOVA, $p < 0.05$). Overall, the developed sterilization method based on $scCO_2$ preserved the mechanical properties of the processed straps.

4. Conclusions

In this work, the successful sterilization of FFP3 masks while preserving their filtration properties was achieved using $scCO_2$ technology. High sterilization levels (logR-6) were achieved for spores of *B. pumilus*, a microorganism particularly resistant towards the $scCO_2$ sterilization. At 39 °C and 100 bar, only 30 min were required to achieve complete inactivation of the initial bioburden at the realistic scenario of contaminated FFP3 masks. This sterilization procedure represents an efficient, fast, safe and sustainable method for the multiple reprocessing (at least for 10 cycles) of face masks, in the absence of an alternative procedure. Although $scCO_2$ sterilization had an impact on the filtering and breathability properties of the FFP3 masks, the general performance of the PPEs was not remarkably compromised, even for those subjected to 10 sterilization cycles. Overall, the developed sterilization conditions underline the $scCO_2$ sterilization as a promising tool for the decontamination of FFP3 face masks and to allow their reuse in a context of urgency, such as the COVID-19 pandemic with a crucial impact on restricting the pathogen spread. The extension of this technology to other medical and technical equipment than FFP3 masks is currently under evaluation. PPEs from several suppliers will be also reprocessed with the developed $scCO_2$ method to assess their universal performance after sterilization. In addition, scale-up tests are also being performed to validate the applicability of the technology to a pilot scale. The low logistic complexity of the $scCO_2$ sterilization encourages its implementation in hospital settings, fostering the incorporation of this technology to the healthcare arsenal.

Table 3
Tensile mechanical properties of FFP3 mask straps after being subjected to $scCO_2$ sterilization compared to untreated straps. Straps were subjected to 5 consecutive deformation cycles (100% deformation/cycle).

FFP3 masks	Tensile cycle	Young's modulus (MPa)
Control	1st	0.258 ± 0.011
	5th	0.333 ± 0.030
1 Cycle	1st	0.257 ± 0.001
	5th	0.326 ± 0.007
10 Cycles	1st	0.265 ± 0.010
	5th	0.308 ± 0.018

CRediT authorship contribution statement

Víctor Santos-Rosales: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Clara López-Iglesias:** Conceptualization, Investigation, Methodology, Writing – original draft. **Ana Sampedro-Viana:** Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Carmen Alvarez-Lorenzo:** Methodology, Writing – original draft, Writing – review & editing. **Samaneh Ghazanfari:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Beatriz Magariños:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Carlos A. García-González:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154089.

References

Aslanidou, D., Karapanagiotis, I., Panayiotou, C., 2016. Tuneable textile cleaning and disinfection process based on supercritical CO2 and Pickering emulsions. J. Supercrit. Fluids 118, 128–139.
Bennet, D., et al., 2021. Evaluation of supercritical CO2 sterilization efficacy for sanitizing personal protective equipment from the coronavirus SARS-CoV-2. Sci. Total Environ. 780, 146519.
Berney, M., Hammes, F., Bosshard, F., Weilenmann, H.-U., Egli, T., 2007. Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight kit in combination with flow cytometry. Appl. Environ. Microbiol. 73, 3283–3290.
Bernhardt, A., et al., 2015. Improved sterilization of sensitive biomaterials with supercritical carbon dioxide at low temperature. PLoS ONE 10, e0129205.
Boulos, L., Prévost, M., Barbeau, B., Coallier, J., Desjardins, R., 1999. LIVE/DEAD BacLight: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. J. Microbiol. Methods 37, 77–86.
Cadnum, J.L., et al., 2020. Effectiveness of ultraviolet-C light and a high-level disinfection cabinet for decontamination of N95 respirators. Pathog. Immun. 5, 52–67.
Calvo, L., Casas, J., 2018. Sterilization of biological weapons in technical clothing and sensitive material by high-pressure CO2 and water. Ind. Eng. Chem. Res. 57, 4680–4687.
Cinquemani, C., Boyle, C., Bach, E., Schollmeyer, E., 2007. Inactivation of microbes using compressed carbon dioxide—an environmentally sound disinfection process for medical fabrics. J. Supercrit. Fluids 42, 392–397.
Commissioner, 2021. O. of the. Investigating Decontamination and Reuse of Respirators in Public Health Emergencies. FDA.
D13 Committee. Test method for breaking strength and elongation of textile fabrics (grab test). http://www.astm.org/cgi-bin/resolver.cgi?D5034-21.

de Man, P., et al., 2020. Sterilization of disposable face masks by means of standardized dry and steam sterilization processes; an alternative in the fight against mask shortages due to COVID-19. J. Hosp. Infect. 105 (2), 356–357.
De-la-Torre, G.E., et al., 2022. Binational survey of personal protective equipment (PPE) pollution driven by the COVID-19 pandemic in coastal environments: abundance, distribution, and analytical characterization. J. Hazard. Mater. 426, 128070.
Dillow, A.K., Dehghani, F., Hrkach, J.S., Foster, N.R., Langer, R., 1999. Bacterial inactivation by using near- and supercritical carbon dioxide. Proc. Natl. Acad. Sci. 96, 10344–10348.
Farke, J., 2020. N95 Filtering Facepiece Respirator Ultraviolet Germicidal Irradiation (UVGI) Process for Decontamination and Reuse. p. 19.
Foster, J.W., Cowan, R.M., Maag, T.A., 1962. Rupture of bacteria by explosive decompression. J. Bacteriol. 83, 330–334.
Fraser, D., 1951. Bursting bacteria by release of gas pressure. Nature 167, 33–34.
Garcia-Gonzalez, L., et al., 2007. High pressure carbon dioxide inactivation of microorganisms in foods: the past, the present and the future. Int. J. Food Microbiol. 117, 1–28.
García-González, C.A., Concheiro, A., Alvarez-Lorenzo, C., 2015. Processing of materials for regenerative medicine using supercritical fluid technology. Bioconjug. Chem. 26, 1159–1171.
Gong, K., Panuganti, S.R., Chapman, W.G., 2017. Study of solubility and swelling ratio in polymer-CO2 systems using the PC-SAFT equation of state. J. Appl. Polym. Sci. 134, app.44804.
Grinshpun, S.A., Yermakov, M., Khodoun, M., 2020. Autoclave sterilization and ethanol treatment of re-used surgical masks and N95 respirators during COVID-19: impact on their performance and integrity. J. Hosp. Infect. 105, 608–614.
Hartanto, B.W., Mayasari, D.S., 2021. Environmentally friendly non-medical mask: an attempt to reduce the environmental impact from used masks during COVID 19 pandemic. Sci. Total Environ. 760, 144143.
Hasan, M.M., Li, Y.G., Li, G., Park, C.B., Chen, P., 2010. Determination of solubilities of CO2 in linear and branched polypropylene using a magnetic suspension balance and a PVT apparatus. J. Chem. Eng. Data 55, 4885–4895.
Heinzerling, A., et al., 2020. Transmission of COVID-19 to health care personnel during exposures to a hospitalized patient - Solano County, California, february 2020. MMWR Morb. Mortal. Wkly Rep. 69, 472–476.
Ho-mu, L., Zhiying, Y., Li, F.C., 1993. Inactivation of leuconostoc dextranicum with carbon dioxide under pressure. Chem. Eng. J. 52, B29–B34.
Hutkins, R.W., Nannen, N.L., 1993. pH homeostasis in lactic acid Bacteria1. J. Dairy Sci. 76, 2354–2365.
Ishikawa, H., Shimoda, M., Kawano, T., Osajima, Y., 1995. Inactivation of enzymes in an aqueous solution by micro-bubbles of supercritical carbon dioxide. Biosci. Biotechnol. Biochem. 59, 628–631.
ISO 17665-1:2006(en). Sterilization of health care products — moist heat — part 1: requirements for the development, validation and routine control of a sterilization process for medical devices. https://www.iso.org/obp/ui/#iso:std:iso:17665:-1:ed-1:v1:en.
John, A.R., et al., 2021. Scalable in-hospital decontamination of N95 filtering face-piece respirator with a peracetic acid room disinfection system. Infect. Control Hosp. Epidemiol. 42, 678–687.
Kamihira, M., Taniguchi, M., Kobayashi, T., 1987. Sterilization of microorganisms with supercritical carbon dioxide. Agric. Biol. Chem. 51, 407–412.
Kumagai, H., Hata, C., Nakamura, K., 1997. CO2 sorption by microbial cells and sterilization by high-pressure CO2. Biosci. Biotechnol. Biochem. 61, 931–935.
Kutralam-Muniasamy, G., Pérez-Guevara, F., Shruti, V.C., 2022. A critical synthesis of current peer-reviewed literature on the environmental and human health impacts of COVID-19 PPE litter: new findings and next steps. J. Hazard. Mater. 422, 126945.
Kwong, L.H., et al., 2021. Review of the breathability and filtration efficiency of common household materials for face masks. ACS Nano 15, 5904–5924.
Laflamme, C., Lavigne, S., Ho, J., Duchaine, C., 2004. Assessment of bacterial endospore viability with fluorescent dyes. J. Appl. Microbiol. 96, 684–692.
Leggett, M.J., et al., 2015. Resistance to and killing by the sporicidal microbicide peracetic acid. J. Antimicrob. Ther. 70, 773–779.
Leuko, S., Legat, A., Fendrihan, S., Stan-Lotter, H., 2004. Evaluation of the LIVE/DEAD BacLight kit for detection of extremophilic archaea and visualization of microorganisms in environmental hypersaline samples. Appl. Environ. Microbiol. 70, 6884–6886.
Liao, L., 2020. Can N95 respirators be reused after disinfection? How many times? ACS Nano 14 (5), 6348–6356.
Lin, H.-M., Yang, Z., Chen, L.-F., 1992. Inactivation of Saccharomyces cerevisiae by supercritical and subcritical carbon dioxide. Biotechnol. Prog. 8, 458–461.
Lindsley, W.G., et al., 2015. Effects of ultraviolet germicidal irradiation (UVGI) on N95 respirator filtration performance and structural integrity. J. Occup. Environ. Hyg. 12, 509–517.
Lore, M.B., Heimbuch, B.K., Brown, T.L., Wander, J.D., Hinrichs, S.H., 2012. Effectiveness of three decontamination treatments against influenza virus applied to filtering facepiece respirators. Ann. Occup. Hyg. 56, 92–101.
Matthew, P., Steven, M.H., Kevin, M.S., Lisa, J.W., 2013. Supercritical Fluid Processing of Materials for Regenerative Medicine. Recent Patents on Regenerative Medicine. 3, pp. 237–248.
Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K., Hakoda, M., 1994. Disruption of microbial cells by the flash discharge of high-pressure carbon dioxide. Biosci. Biotechnol. Biochem. 58, 1297–1301.
Nebe-von-Caron, G., Stephens, P.J., Hewitt, C.J., Powell, J.R., Badley, R.A., 2000. Analysis of bacterial function by multi-colour fluorescence flow cytometry and single cell sorting. J. Microbiol. Methods 42, 97–114.
Pei, C., Ou, Q., Kim, S.C., Chen, S.-C., Pui, D.Y.H., 2020. Alternative face masks made of common materials for general public: fractional filtration efficiency and breathability perspective. Aerosol Air Qual. Res. 20, 2581–2591.
Peng, Y., Wu, P., Schartup, A.T., Zhang, Y., 2021. Plastic waste release caused by COVID-19 and its fate in the global ocean. Proc. Natl. Acad. Sci. U. S. A. 118, e2111530118.
Perrut, M., 2012. Sterilization and virus inactivation by supercritical fluids (a review). J. Supercrit. Fluids 66, 359–371.
Ribeiro, N., et al., 2019. A new era for sterilization based on supercritical CO2 technology. J. Biomed. Mater. Res. B Appl. Biomater. 108.
RIVM. Reuse of FFP2 masks. https://www.rivm.nl/en/documenten/reuse-of-ffp2-masks.
Rowan, N.J., Laffey, J.G., 2020. Challenges and solutions for addressing critical shortage of supply chain for personal and protective equipment (PPE) arising from coronavirus disease (COVID19) pandemic – case study from the Republic of Ireland. Sci. Total Environ. 725, 138532.
Rowan, N.J., Laffey, J.G., 2021. Unlocking the surge in demand for personal and protective equipment (PPE) and improvised face coverings arising from coronavirus disease (COVID-19) pandemic – implications for efficacy, re-use and sustainable waste management. Sci. Total Environ. 752, 142259.
Rubio-Romero, J.C., Torrecilla-García, J.A., Calero-Castro, S., Pardo-Ferreira, M.del C., 2020. Disposable masks: Disinfection and sterilization for reuse, and non-certified manufacturing, in the face of shortages during the COVID-19 pandemic. Saf. Sci. 129, 104830.
Saliu, F., et al., 2021. The release process of microfibers: from surgical face masks into the marine environment. Environ. Adv. 4, 100042.
Santos-Rosales, V., et al., 2019. Sterile and dual-porous aerogels scaffolds obtained through a multistep supercritical CO2-based approach. Molecules 24, 871.
Santos-Rosales, V., et al., 2021. Supercritical CO2 technology for one-pot foaming and sterilization of polymeric scaffolds for bone regeneration. Int. J. Pharm. 605, 120801.
Santos-Rosales, V., Magariños, B., Alvarez-Lorenzo, C., García-González, C.A., 2022. Combined sterilization and fabrication of drug-loaded scaffolds using supercritical CO2 technology. Int. J. Pharm. 612, 121362.
Shieh, Y.-T., et al., 1996. Interaction of supercritical carbon dioxide with polymers. I. Crystalline polymers. J. Appl. Polym. Sci. 59, 695–705.
Shieh, E., Paszczynski, A., Wai, C.M., Lang, Q., Crawford, R.L., 2009. Sterilization of Bacillus pumilus spores using supercritical fluid carbon dioxide containing various modifier solutions. J. Microbiol. Methods 76, 247–252.
Spilimbergo, S., Bertucco, A., 2003. Non-thermal bacterial inactivation with dense CO2. Biotechnol. Bioeng. 84, 627–638.
Standards, E. EN 14683 + AC. https://www.en-standard.eu. https://www.en-standard.eu/csn-en-14683-ac-medical-face-masks-requirements-and-test-methods/.
Torrente, M., et al., 2021. To burn-out or not to burn-out: a cross-sectional study in healthcare professionals in Spain during COVID-19 pandemic. BMJ Open 11, e044945.
Urban, M.V., Rath, T., Radtke, C., 2019. Hydrogen peroxide (H2O2): a review of its use in surgery. Wien Med Wochenschr 169, 222–225.
Viscusi, D.J., et al., 2011. Impact of three biological decontamination methods on filtering facepiece respirator fit, odor, comfort, and donning ease. J. Occup. Environ. Hyg. 8, 426–436.
Watt, B.E., Proudfoot, A.T., Vale, J.A., 2004. Hydrogen peroxide poisoning. Toxicol. Rev. 23, 51–57.
Wilson, A.J., Nayak, S., 2019. Disinfection, sterilization and disposables. Anaesthesia Intens. Care Med. 20, 603–608.
Zhang, T., Fang, H.H.P., 2004. Quantification of Saccharomyces cerevisiae viability using BacLight. Biotechnol. Lett. 26, 989–992.
Zhang, J., et al., 2006. Sterilization using high-pressure carbon dioxide. J. Supercrit. Fluids 38, 354–372.