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Supercritical carbon dioxide-based cleaning and sterilization treatments for the reuse of filtering facepiece respirators FFP2 in the context of COVID-19 pandemic

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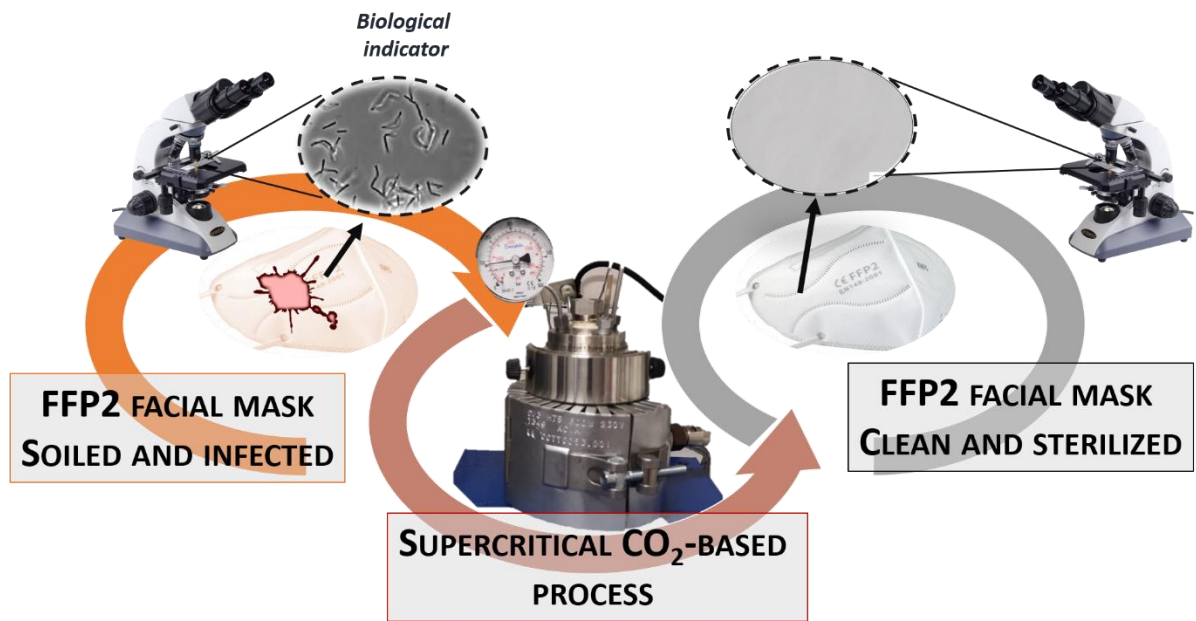
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ABSTRACT

Supercritical Carbon Dioxide treatment of clean and soiled Filtering Face-Piece respirators is shown to meet mandatory requirements for safe reuse of FFP2. Preliminary tests enabled us to select optimal conditions for Supercritical Carbon Dioxide treatment of FFP2, for one hour at 343 K under 7.5 MPa, with a biocide solution. FFP2s from Paul Boyé® were then tested before and after Supercritical Carbon Dioxide treatment with a soiling solution and a biological indicator (*i.e.* spores of *Geobacillus stearothermophilus*) with respect to three objectives: (i) washing of organic deposits, (ii) sterilization (*i.e.* spores inactivation) and (iii) preservation of the filtration performances. The proposed Supercritical Carbon Dioxide treatment fulfils these three criteria, with effective decontamination of spores and promising washing preserving FFP2 requirements for filtration efficiency of aerosol (> 94%) with acceptable pressure drop (< 240Pa). As a simple and low-cost one-step recycling method, Supercritical Carbon Dioxide fits to economical and sustainable development standpoints but also allows one to come back to good practices of face protective equipment use, lowering eventual shortage and tensions for most countries importing these respirators.

Graphical abstract



Keywords: FFP2 face protective equipment; supercritical CO₂; cleaning; sterilization; filtering performances.

1. INTRODUCTION

With the emergence of COVID-19, the need to protect caregivers and to reduce viral transmission in the population led to an explosion of the consumption of single-use Face Protective Equipment (FPE), including surgical masks and Filtering Face-Piece respirators like N95/FFP2. In the first months of the pandemic, severe shortages occurred in several places in the world.^[1] As a result, health authorities had to accept deviations from good practice and standards, which recommend that a FPE should be changed each time the healthcare professional treats another patient, or each time the FPE is touched or withdrawn. Since Covid crisis, N95s or FFP2s are worn by caregivers up to eight hours and surgical masks up to four hours, with related higher risks of transmission of viruses and other microbiological agents for both caregivers and patients. Despite a very significantly increased FPE production, estimated to reach 20 billions in 2020,^[2, 3] it is impossible to come back to the good practice in terms of FPE use, and in several places in the world access to FPE remains a major issue. Besides, the environmental burden associated to FPE production and elimination after use has to be considered.^[4, 5]

For all these reasons, several teams in the world have explored solutions for decontaminating FPEs to allow their reuse. Some of them had already been explored during H1N1 pandemic.^[6] ^{7]} Most efforts concentrated on FFP2/N95, with successful solutions to eliminate SARS-CoV-2, among which hydrogen peroxide,^[8] ethylene oxide,^[9] UV-C,^[10, 11] chlorine dioxide,^[12] dry heat,^[13] moist heat.^[14] Even autoclave was tested, but with very limited success.^[15] These methods proved that the filtration properties of FFP2/N95 and the facial fit remained compliant with standards after several cycles of use/decontamination (number of possible cycles depends on the method, and is estimated between one and ten).

To achieve the one-step treatment of numerous FFP2/N95, collective solutions, such as hydrogen peroxide, ethylene oxide, autoclave, have to be bactericide to avoid cross-contamination between masks of different users (which may occur if masks are not isolated from each other by microporous envelopes). Solutions which are only virucide, such as UV-C, chlorine dioxide, dry or moist heat, can therefore only be used to treat the masks of one user at a time, to avoid bacterial cross-contamination.

A common limit of these recycling methods is that FFP2/N95 cannot be wetted and hence washed. Indeed, efficient filtration at reduced pressure drop of FFP2s rely on the electrical properties, lost by contact with liquids, as shown with Isopropyl alcohol spraying or dipping.^{[16-}

^{18]} This is a major concern, since no one would like to use a dirty FPE. Besides, a first washing step is usually required to guarantee the efficiency of any sterilization or decontamination process and even imposed by World Health Organization.^[19] Washing indeed eliminates organic deposits, known to be able to protect microorganisms from decontamination agents.^{[20,}

^{21]}

It is therefore mandatory that the proposed decontamination method is efficient, even if the FFP2 has been soiled by normal use (e.g. with salivary or nasal secretions or face powder). The previously described decontamination methods were tested with real organic deposits (saliva or proteins), however these soiling protocols were not compliant with European standards.^[22-24]

Therefore, in order to reuse cleaned masks with microbiological safety, the ideal solution would be to find a cleaning method of organic deposits preserving filtration performances of FPEs.

Supercritical fluid technology has been recognized as a promising alternative for sterilization treatment as it can proceed under moderate temperatures and pressures^[25]. At supercritical state carbon dioxide (above 304 K, 7.38 MPa) is nontoxic and non-flammable being effective against targeted microorganisms by physical and chemical means^[26, 27]. Sterilization by supercritical carbon dioxide (SSCD) has shown real practical success mainly when applied to vegetative

forms, while the task to inactivate resistant bacterial spores has yet not been productive. In some cases, water or chemical additives have been used to achieve sterilization^[28, 29]. Microbiological latent forms known as spores are highly resistant to such sterilization treatment and the inactivation mechanism is poorly understood^[30, 31]. High temperature or high-pressure conditions can kill the spores, even the most high-heat resistant ones^[32, 33], however for our application on face protective equipment, both high temperature and humid treatment are deleterious for the mask integrity.

Only one reference has been found mentioning SSCD potential interest for FPE decontamination^[34], although no cleaning and no filtration efficiencies were reported despite the good SSCD-based decontamination process on both endospores and SARS-CoV-2^[34]. We aimed to explore SCCD for FPE decontamination as a one-step process to simultaneously clean and sterilize FPEs, preserving filtration performances beyond the normative limits. More specifically, we demonstrated that SCCD can be used as a cleaning and sterilizing method keeping the filtration performances of a FFP2 at the level of the normative requirements to mark an FFP2 as reusable after SCCD cleaning.

2. METHOD

After an optimization phase, described in SF1, performed with several types of FFP2 half masks, we used FFP2s provided by Paul Boyé, France, (ref. MPB2.1 136/20 M4), compliant with European standard EN 149 :2001+A1:2009^[35]. These FFP2s with a “duckbill” shape are made of two flat parts in polypropylene fibres, with no exhalation valve, with a nose piece for adjustment and two welded elastic braids.

2.1. Supercritical carbon dioxide-based cleaning and sterilization protocol

A scheme of the experimental set-up using supercritical CO₂ developed for the cleaning and the sterilization of masks is given in Figure 1. Briefly, the high-pressure reactor used in this study consists of a stainless steel batch autoclave manufactured by Top Industrie with a

maximal internal volume of 300 mL. A ceramic band heater is used to heat the system. The pressure in the autoclave is reached with a high-pressure pump (metering pump manufactured by MiltonRoy). All experiments were performed in batch mode for a duration of 1h. One or two masks are introduced in the autoclave with 500 μL of a solution 50:50 in vol. of absolute ethanol (min 99.9%, reference ET00051000, Scharlau) and hydrogen peroxide (H_2O_2 , 30% w/w in H_2O , reference H1009, Sigma-Aldrich). Here, the ultimate goal is to use the minimum of water for the experiment (i.e. in order to preserve filtration performances). Thus, 500 μL of solution in 300 ml of CO_2 is the minimum efficient volume to perform the sterilization process. The preheater and the reactor are heated to 353 K and 343 K, respectively. Once both temperatures are reached, 7.5 MPa of CO_2 (300 mL of CO_2 4.8, Messer) is added inside the autoclave thanks to a HP pump (CO_2 injection, pressurization way on Fig. 1c). The autoclave is brought to the targeted conditions: 7.5 MPa and 343 K, during 1h. After the sterilization treatment, the back pressure regulator allows a fast depressurization at 7.0 MPa/min with two short stages at 4.0 and 2.0 MPa in order to increase the depressurization absolute slope value (depressurization way on Fig. 1c). After depressurization down to atmospheric pressure the autoclave is opened. Then, dried, uncontaminated and clean FFP2 masks are collected for characterization. CO_2 never crosses the equilibrium curve, which prevents apparition of capillary forces; this is a crucial point to preserve the filtration capacity of masks.

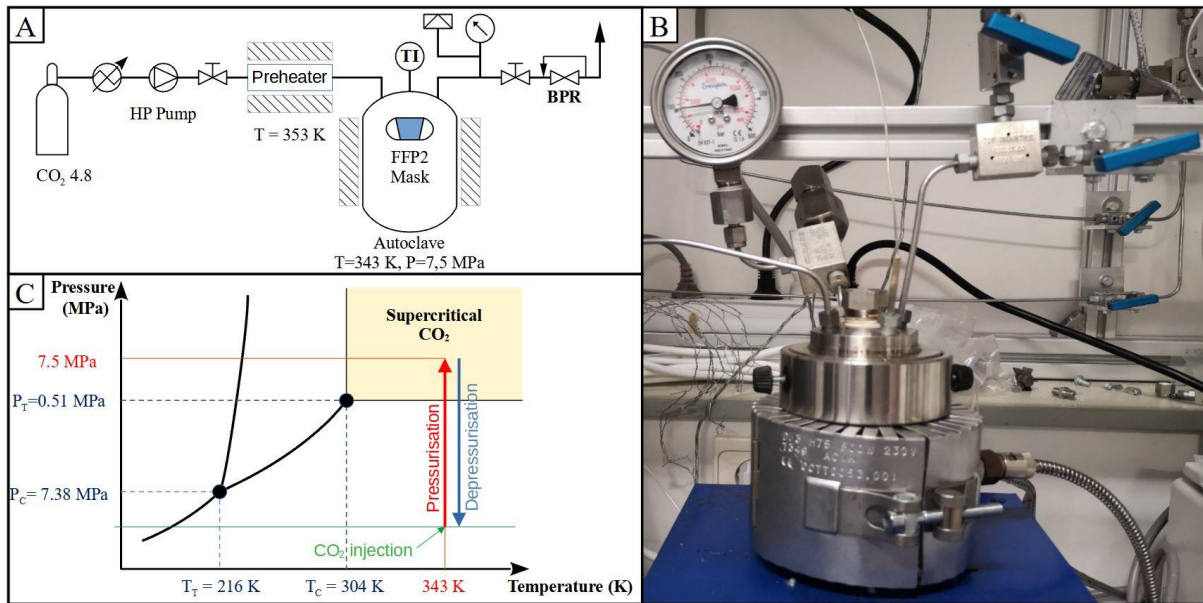


Figure 1: (A) Scheme of the experimental set-up for mask cleaning and sterilization, (B) Picture of the experimental set-up, (C) CO₂ phase diagram and thermodynamic pathway during the sterilization and cleaning of masks.

In order to determine the efficient sterilizing condition, different CO₂ pressures and temperatures, as well as the addition of different reagents (total volume 1 mL), were first tested on steel spore discs (Excelsior). We used *Geobacillus stearothermophilus* spores (Cell Line 7953, 10⁶ spores/disc) as a biological indicator and a surrogate for coronavirus as this strain is known to be very resistant to sterilization processes [29]. The steel spore discs were removed from the Tyvek packaging before the sterilization test and placed in the high-pressure autoclave under sterile conditions (Fig. 1). After exposure to different conditions (Table 1), the spore discs were aseptically transferred in 10 mL of TSB (Tryptone Soya Broth, Sigma-Aldrich). For each test, one spore disc that was not exposed to the sterilizing process was also used as a positive control. Cultured spore discs were incubated at 333 K for 48h. The growth medium turbidity allowed us to detect fast spores' germination. Development of vegetative cells was directly estimated by optical microscopy using a Thoma counting chamber. This counting method is a quick and reliable method to evaluate the sterilization efficiency of a process using spore discs

before the CFU counting method. The sterilization estimation is given in percentage of efficiency to decrease the development of spores' germination, when compared to the positive control (i.e. sample not treated and with germinated cells). A sterilization efficiency of 100% prevents the spores' germination and indicates a $6\log_{10}$ reduction. The different tested conditions and the treatment results are described in the table 1. All the experiments were performed in duplicate.

Table 1: Summary of the tested parameters to determine the efficient sterilization protocol.

CO₂ pressure (MPa)	Reactor temperature (K)	Adding reagent	Treatment duration (hours)	Sterilization efficiency (%)
30.0	368	No	2	27
30.0	368	Yes (1/3 ethanol, 1/3 acetone, 1/3 acetic acid (v/v))	2	29
18.0	363	No	2	4
16.0	363	No	2	20
15.0	368	No	2	24
15.0	358	No	2	21
10.0	343	No	2	14
10.0	343	Yes (25% ethanol and 75% H ₂ O ₂ (v/v))	2	100
10.0	343	Yes (50% ethanol and 50% H ₂ O ₂ (v/v))	2	100
10.0	343	Yes (75% ethanol and 25% H ₂ O ₂ (v/v))	2	100
10.0	343	Yes (90% ethanol and 10% H ₂ O ₂ (v/v))	2	100
10.0	343	Yes (90% ethanol and 1% H ₂ O ₂ (v/v))	2	100
10.0	343	Yes (100% ethanol)	2	24
7.5	343	Yes (50% ethanol and 50% H ₂ O ₂ (v/v))	1	100

The optimal conditions to perform efficient sterilization (*i.e.* 100% efficiency to inhibit spores development) require the use of a co-solvent (mixture of ethanol and H₂O₂). The selected conditions to perform cleaning and sterilization experiments on FFP2s are a CO₂ pressure and temperature of 7.5 MPa and 343K, with the addition of a mixture of ethanol and hydrogen peroxide (50/50, v/v). This allows to decrease both the pressure and temperature conditions as well as the treatment duration in order to clean and preserve the filtration capabilities of FFP2s.

2.2. Soiling protocol

According to the recommendations of standards, ^[22-24] we soiled 18 new FFP2s with a solution of 3g/L of bovin serumalbumin (BSA) and 3mL/L of sheep erythrocytes, to respect the dirty soiling conditions required for demonstrating efficiency of disinfection. Using a pipette, 10 µl of both BSA and sheep erythrocytes were deposited in duplicate within a defined area on the FFP2 (*i.e.* about 1 cm², see Fig. 2B).

The residual erythrocytes and the residual proteins have been determined by image analysis. In order to characterize residual erythrocytes, pictures of both treated and untreated FFP2 were taken, and these images were treated with ImageJ (java.version: 1.8.0_112, source: ImageJ (nih.gov)) to plot mean-intensities. The residual proteins were determined by a Bicinchoninic acid Assay (Pierce™ BCA Protein assay kit, ThermoScientific). 100µl of BCA reagent were deposited on each dried BSA droplet (duplicate deposits). After 15 min of reaction (*i.e.* BCA reagent reacts with the proteins and produces a purple-color dependent on the protein concentration) at Room Temperature (RT), a picture was taken and this image was again treated with ImageJ to plot mean-intensities. Each picture was first treated by extracting the red component, then normalized to both the maximum and minimum pixel intensities (*i.e.* non treated background and black ink) for a greyscale analysis. Finally, the mean intensity was considered on defined area (1.5 cm²). For each soiling material deposit (*i.e.* 3g/L of BSA and 3mL/L of sheep erythrocytes), the residual values were evaluated by determining the ratio of

the residual soiling material intensity after treatment (I) to the intensity on the untreated unit (I₀) (Fig. 2).

2.3. Sterilization protocol

Since spores are considered the standard to meet the target of Sterility Assurance Level (SAL, the probability of a non-sterile item in a population) of $6 \log_{10}$ reduction, the microorganism *Geobacillus stearothermophilus* (G.s) known to be highly resistant to sterilization processes was chosen in this study. [29] Different conditions (CO₂ temperature and pressure as well as the use of co-solvents) were tested on spores before validation of the efficient sterilization protocol on *G. stearothermophilus*. This set of results is presented in Table 1 (see part 2.1). Once the sterilization protocol was approved, the tests were performed on FFP2s.

For the subculture method, five droplets with serial dilutions of spores (*i.e.* 10^6 to 10^2 spores) in the soiling solution were deposited on one FFP2. The deposit was done in triplicate (Fig. 3). After drying at room temperature (about 60 min), this FFP2 was treated with SCCD conditions. The same serial dilutions were performed for one untreated FFP2 and used as a reference. After treatment, 1 cm² pieces around each spot where spores had been deposited were cut in a sterile way (15 pieces for each FFP2). Both treated and untreated pieces were placed in TSB (Tryptone Soya Broth, Sigma-Aldrich), vortexed for two minutes and incubated at 333 K for 48h. Turbidity in the medium indicated growth of vegetative cells. For each dilution, growth was estimated by direct cell counting with a Thoma chamber (0.1 mm depth). Grown cultures were transferred on agar plates (0.1 mL) to determine the CFUs for each dilution.

For the filtration method, triplicates of 10^5 spores in soiling solution were deposited on one FFP2 to provide a worst-case scenario. The six cut pieces (*i.e.* both treated and untreated FFP2s) were incubated for ten minutes at room temperature in 5 mL of saline buffer (NaCl 2M). Serial dilutions (up to 10^2) of each piece were filtrated (0.22 μm) and the filters were deposited on agar plates to determine the CFUs for each sample. For both subculture and filtration methods,

the sterilization effect of the SCCD was evaluated by a reduction of the number of living cells (N) after treatment, with respect to the number of living cells initially added to the untreated unit (N_0). All experiments were carried out under sterile conditions and performed at least in duplicate.

2.4. Compliance of FFP2s filtration properties before and after SCCD protocol

We tested FFP2's filtration performances according to norms to EN149:2001+A1:2009(F) and EN13274-7:2019(F),^[35, 36] respectively for mask and flat filters cut in FFP2 (32 mm diameter), as detailed in SF1. These filters are challenged with upstream concentrations of 5 to 25 mg/m³ of Paraffin droplets suspended in air as liquid test aerosols with a median number diameter of 400 nm (Q1= 290, Q3= 549), for a size distribution with a Geometric Standard Deviation of 1.65, requested for the polydisperse test aerosol. These tests, compliant with norms, were performed on flat filters cut in FFP2 (32 mm diameter), at 15.8 cm/s face velocity related to the reference peak inhalation flowrate of 95 Liters per minute (Lpm) on 100 cm² filtering media. Aerosol concentrations were measured with optical detection by light diffusion required for particulate respirator certification.

Filtration performance is expressed as the total mass filtration efficiency (mass fraction of particles collected in the filter). To select optimal operating conditions of the SCCD protocol in terms of temperature and pressure, preliminary tests were first conducted by filtration at 9.6 cm/s of solid NaCl aerosol measured by emission spectroscopy (see SF1). The influence of SCCD protocol on mass-based filtration efficiency is highlighted with non-dimensional results by dividing the efficiency after each SCCD treatment by the reference value for new filters. Number-based filtration efficiency has also been characterized versus electrical mobility diameter of particle, as detailed in SF1.

The selected SCCD protocol was then tested by measuring filtration efficiencies of filters cut in FFP2 at 15.8 cm/s and resistance (pressure drop) to corresponding velocities for inhalation

flows of 95 and 30 Lpm as well as to an exhalation flow of 160 Lpm on 100 cm² filtering media, as prescribed by the norms. We chose to perform the most stringent filtration test prescribed with norm EN149, with paraffin aerosol. At least three different filters per lot of FFP2s were tested before and after one or two SCCD treatments, on clean new FFP2s or on FFP2s soiled with spores, following the soiling protocol presented above.

The initial efficiency, the resistance to inhaled and exhaled air and their evolution upon exposition to the Paraffin aerosol were measured three times 30 seconds, successively upstream, downstream and upstream the filter. Short term and time-averaged mass concentrations, with related mass-based filtration efficiencies, were then calculated with standard deviations.

To check if such FFP2 could be reused after treatment with respect to European norms, ^[22-24] the loading and the storage tests have also been performed on flat filters cut in FFP2 treated by SCCD, then loaded with 4 mg of Paraffin aerosol, before the final measurements, after 24h storage in ambient conditions (as detailed in SF2).

2.5. Data analysis

All experiments of the soiling and sterilization protocols were carried out at least in duplicate. The presented data are the means of at least two replicated separated experiments. Significant differences were determined by Student's t test (p-value <0.05).

For the filtration properties characterization, a spreadsheet (Microsoft Excel[®]) was used to calculate the mass concentrations from measured size distributions and the total mass efficiency defined above. Average efficiencies among the different filters tested from different locations on one FFP2 and from different FFP2s treated in the same conditions from the same set of masks, are given with standard deviation and p-values to highlight significant discrepancies from the 94 % minimal filtration efficiency for FFP2 certification, determined by Student's t test, with p- values at least below 0.05, specified in Figure 4 and Table SF2-4.

3. RESULTS

3.1 SCCD cleaning effect on FFP2s

To evaluate the cleaning effect of this treatment we compared residual values of erythrocytes and BSA deposits (3% and 0.3%, respectively) on both treated and untreated FFP2. SCCD treatment visually removed most of the blood and proteins on FFP2 (Fig. 2). Visual quantification confirmed great removal efficiency for both soiling material deposits (*i.e.* erythrocytes and BSA) with only $1.09 \pm 0.01\%$ of residual erythrocytes and $6.5 \pm 0.4\%$ of residual proteins on the FFP2 after SCCD treatment (Figure 2).

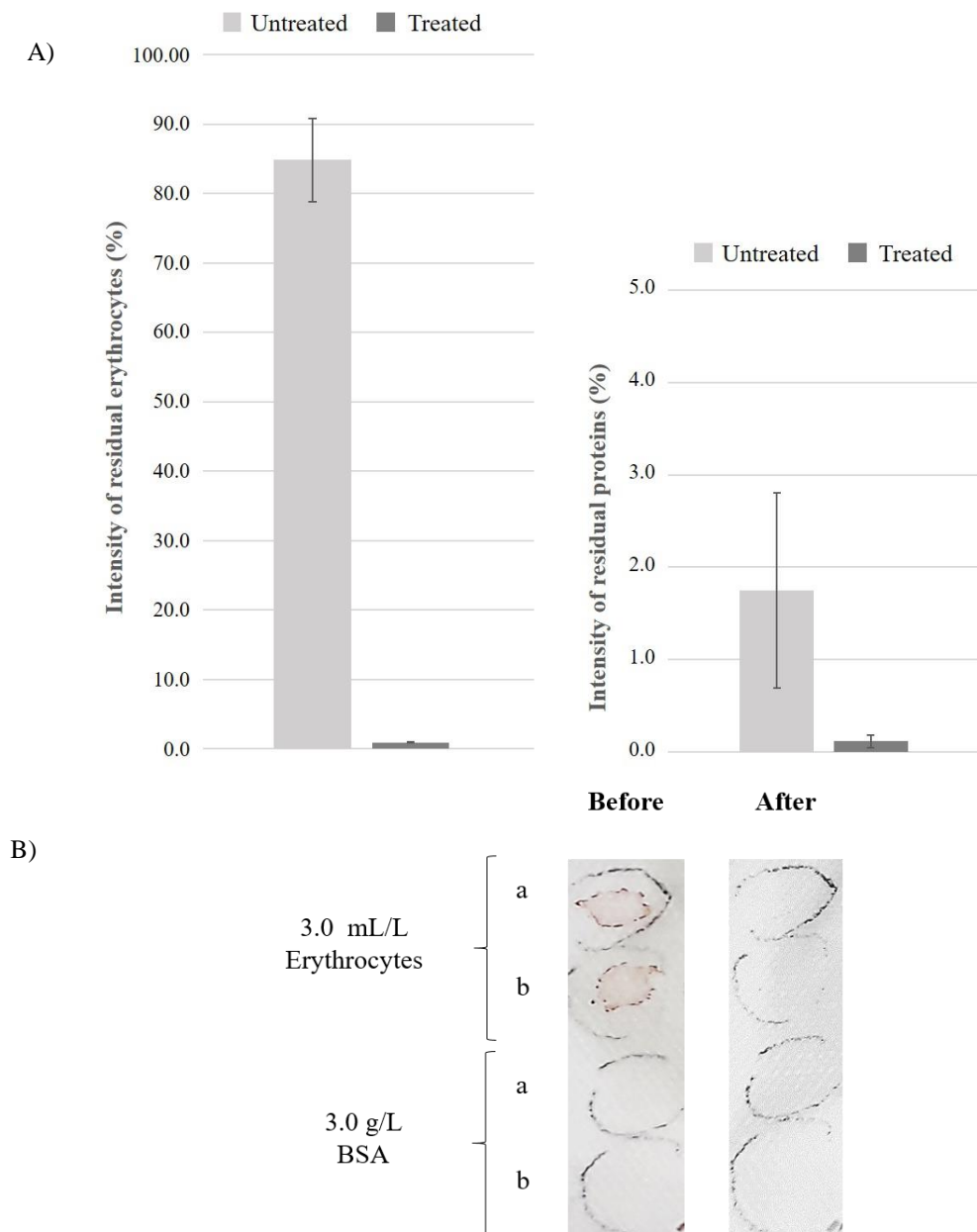


Figure 2: (A) Percentage of residual soiling material, residual erythrocytes (left) and residual proteins (right) after our SCCD treatment (343 K and 7.5 MPa for 1h) determined by the ratio of intensity (image analysis) of both residual erythrocytes and proteins with and without treatment (I/I_0). Significant differences were determined by Student's t test (p -value <0.05), (B) Pictures of FFP2 before and after treatment with 3.0 g/L of BSA and 3.0 mL/L of erythrocytes in duplicate deposits, for image analysis.

3.2. SCCD sterilization effect on FFP2s

We used both subculture and filtration methods to evaluate the biocide effect on *G. stearothermophilus* spores (the commercial solutions we used were calibrated at 10^6 spores for the subculture method, and at 10^5 spores for the filtration method). For both methods, the SCCD treatment (*i.e.* 343 K and 7.5 MPa for 1h) demonstrated a great efficiency. SCCD treatment totally inactivated spores' population ($> 6 \log_{10}$ of the initial 10^6 and 10^5 spores' deposits, *i.e.*, subculture and filtration counting methods, respectively (Table 2 and Figure 3).

Table 2: Inactivation tests of *G. stearothermophilus* by SCCD (343 K and 7.5 MPa for 1h) according to both counting methods (*i.e.* subculture and filtration) after 48h of growth at 333 K on TSB agar media. NT, untreated sample; Tr. treated sample.

Counting methods	Tests	Log CFUs		Sterilization efficiency (%)
		NT	Tr.	
Subculture	1	5.98	0	100
(10^6 spores deposit)	2	6.10	0	100

	3	5.53	0	100
Filtration	1	5.42	0	100
(10 ⁵ spores deposit)	2	5.21	0	100
	3	4.58	0.17	96.3



Figure 3: Pictures of FFP2 with serial dilution spores deposit (in the soiling solution composed of a mixture of erythrocytes and proteins) in triplicate, before (top) and after (bottom) SCCD treatment (343 K and 7.5 MPa for 1h).

3.3. FFP2s filtration performances before and after exposition to the SCCD protocol:

The selected conditions of the SCCD protocol are met under 7.5 MPa CO₂ at 343K for 60 minutes, with 0.5 mL of a biocide 50/50 solution of Ethanol and Hydrogen Peroxide 30%.

Figure 4, depicts the mean mass efficiencies of different filters from the same lot of FFP2, before and after treatment. The corresponding pressure drops and mass efficiencies are gathered for all filters in Table SF2-2, with standard deviation of measurements for each filter, as well as for the mean values among different filters per set of masks, presented here for new and treated filters, versus the treatment and the number of SCCD treatments on clean or soiled FFP2.

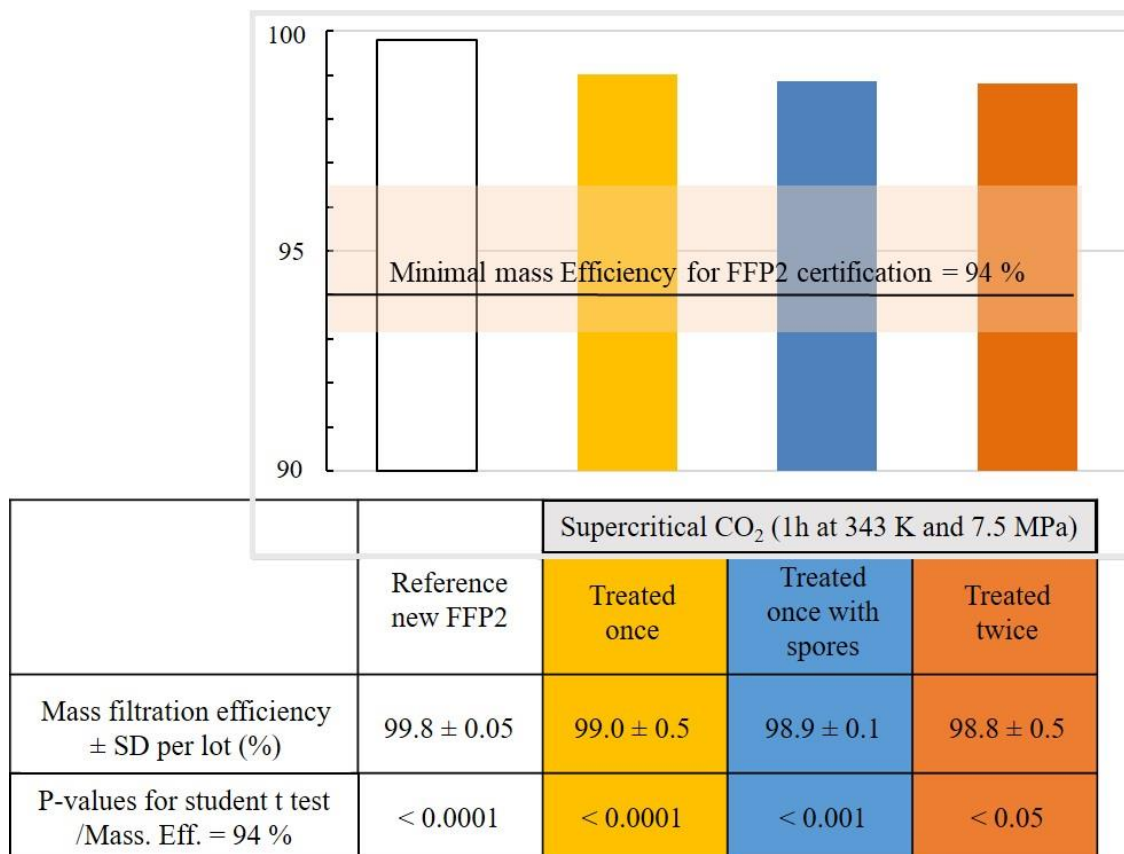


Figure 4: Mean mass efficiencies per lot of new and treated Filters cut in FFP2 (Paul Boyé®) after SCCD treatment, with all p-values for significant discrepancies from 94 % critical efficiency ($p < 0.001$).

The reproducibility of calculated pressure drops and efficiencies for different filters from the same FFP2, as well as from different ones from the same lot, supports that new FFP2 as well as treated one are homogeneous, with larger uncertainties on lower efficiencies (see Table SF2-3).

One or two cycles of the proposed SCCD treatment do not alter the filtration performances beyond the normative limits for FFP2: mean mass filtration efficiencies keep higher than 98.9% (SD= 0.05%), above the minimal 94% (see SF2 for SD and uncertainties), and 150 to 175 Pa pressure drops are measured at 15.8 cm/s corresponding to 95 Lpm on 100 cm² filtering media (uncertainty < 9%, with SD from 3 to 15 Pa), below the required maximal 240 Pa (see Table SF1-2). Finally, after the loading and the 24 hours storage, Paul Boyé's FFP2s initially treated by SCCD protocol kept a filtration efficiency above 98.2%, compliant with the normative requirements for certification of FFP2, which could thus be reused after SCCD treatment (see Figure SF2-4).

4. DISCUSSION

Our first tests evaluated the combination of temperature, CO₂ pressure and the use of a reagent to help inactivating the spores of *Geobacillus stearothermophilus*. Results are shown in table 1, and for all the “dry” conditions tested (temperature and SSCD pressure without reagent) the reduction of spore growth was very low (less than 30% of efficiency) indicating high resistance of these spores to high temperature and SSCD treatment. Higher temperature and pressure treatments are more efficient to inactivate the spores, as reported in the literature [32] but our application on facial mask requires mild temperature to maintain facial mask integrity (i.e. filtration efficiency). To do so, we added small amounts of reagents (different solvents) using mild-temperature and SSCD pressure tests. For our application, synergetic effects of SSCD, mild-temperature and a low volume mixture of reagents (ethanol and H₂O₂) allowed an effective sterilization. H₂O₂ is known to be a strong oxidant and the combined effect of SSCD and H₂O₂ is reported to be highly effective to kill spores [30, 34]. Addition of ethanol enhances also the inactivation of bacterial spores (as reported in [37]). As a result, the use of mild temperature and pressure conditions of SSCD along with ethanol and H₂O₂, indicates an efficient one-step process in inactivating the *G. stearothermophilus* spores on facial masks.

The solvent properties of SCCD, used along with a mechanical action, enable the efficient cleaning of soiled textile.^[38] The combination of solvents and both mild temperature and pressure conditions was promising to clean the soiled facial masks without affecting the filtration properties of FFP2s beyond the normative limits, i.e. with efficiencies above 94% and pressure drops below 240 Pa. Colorimetric measurements on the facial masks verify the efficient cleaning of such combination.

The method requires no very sophisticated machines, so that the industrialisation of this solution should be easy; we have already started to work on that. Supercritical CO₂-based cleaning and sterilizing machines can be implemented in various sizes, capable to serve as well the needs of a medical ward as the needs of a complete hospital or even more. This versatility will be a major logistic advantage. The process will minimize human intervention (since all Face Protective Equipments (FPEs) of a ward or of a hospital can be put all at once in a single machine).

5. CONCLUSION

To our knowledge, the proposed SCCD protocol is the first one able to sterilize FFP2s with a promising cleaning without altering filtration performances beyond the normative limits expected for FFP2 certification (efficiency > 94% and pressure drop < 240 Pa). The soiled Paul Boyé[®] FFP2s treated once or twice with SCCD successfully passed the loading test and the conservation test required for certification as reusable. This patented SCCD treatment^[39] would thus deserve to be tested by certification organisms as a potential cleaning and decontaminating method preserving filtration performances of these FFP2s for at least two cycles of use / decontamination. Since filtration performance goes from 99.8 for a new FFP2 to 98.9 % for a twice treated Paul Boyé[®] FFP2, it can be expected that SCCD could be applied up to five to 10 cycles before reaching the minimal 94% efficiency.

G. stearothermophilus is known to be highly resistant to sterilization processes. In our study, the spore inactivation during high pressure CO₂ and mild temperature reached high efficacy

with the use of a strong oxidant (i.e. hydrogen peroxide), while spores treated with pure CO₂ were not killed. The spore inactivation mechanism is not yet understood, but it seems to be caused with perforation of the spore outer layers, especially when the process is run in the presence of a strong oxidant additive in CO₂ [30]. Indeed, these experimental conditions are strong enough to weaken the spore layers thus preventing the spores' germination. So far, few studies focus on SCCD effect on virus inactivation [34]. When considering coronavirus, which are a type of enveloped viruses like Covid-19 (i.e. the genetic material is packed within an outer layer of lipids and proteins) in comparison with non-enveloped viruses which have a hard outer shell called capsid, our experimental conditions (i.e. supercritical CO₂ and strong oxidant) seem ideal to solubilize membrane lipids causing membrane disruption, therefore, coronavirus inactivation. This study, using supercritical CO₂ for microorganisms' inactivation, deserve to be extended to coronavirus in order to validate Covid-19 inactivation as microparticles on filtering face-Piece respirators.

The availability of a simple solution to recycle FPEs will have a major impact in the fight against Covid-19 or other infectious diseases conveyed aerosols or microparticles. It will significantly reduce the tension on the availability of such equipment, thus facilitating their access to all countries and reducing the risk of local shortage putting caregivers at risk. Safe reuse of FPEs would enable to restore to good practice, to protect from infectious risks. Finally, a simple and low-cost one-step recycling method is interesting not only from an economical viewpoint, but also for the impact on environment, since 20 billions of FPEs were used in 2020, corresponding to tens of thousands of tons of PolyPropylene.

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