



Nanoporous materials as carriers of hydrogen peroxide vapour: A new bio-decontamination technology

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ABSTRACT

High-level bio-decontamination, which involves reducing microorganisms by 99.9999%, is essential in preventing Hospital Acquired Infections and controlling pandemics. This study demonstrates that nanoporous materials, which can retain molecules within their pores, and subsequently release them, can be used in high level bio-decontamination. H₂O₂ vapour is a golden-standard in high level bio-decontamination. This work demonstrates that various nanoporous materials, particularly mesoporous silicas, can be utilized to store and release H₂O₂ in the vapour phase. H₂O₂ concentrations of over 2500 ppm were achieved by desorbing it from the carrier material at low temperatures of 60–80 °C. Generation of H₂O₂-vapour by desorption from nanoporous materials is technically much simpler than vaporization of aqueous H₂O₂ solutions, which use flash vaporization processes occurring at 130–150 °C. This has important technical implications, highlighting the potential of nanoporous materials as carriers for H₂O₂ for high-level bio-decontamination.

1. Introduction

Long before the recent COVID-19 pandemics, there was an increasing awareness that improved cleaning and disinfection of environmental surfaces is needed in healthcare facilities, as previously reviewed in the literature [1–3]. An important example is the hospital-acquired infections (HAI) since various multi resistant pathogens can persist in the healthcare environment for days [4]. Only in American hospitals, the Centre for Disease Control estimates that HAI account for an estimated 1.7 million infections and 99 000 associated deaths per year. Bio-decontamination is relevant in other important sectors as aseptic packaging processes [5] or the maintenance of biological safety cabinets [6].

Traditional cleaning methods (detergent- and disinfectant-based) are notoriously inefficient for decontamination and new approaches have been proposed in recent years [4]. Among these, the more relevant ones are devices that use ultraviolet (UV) light, formaldehyde, chlorine dioxide, peracetic acid, ozone, and hydrogen peroxide (H₂O₂) vapour. All these methodologies have their advantages and disadvantages. For instance, UV light based apparatus allow short bio-decontamination cycles of bio-decontamination, but are expensive and, more importantly, shadow areas are not decontaminated [4]. Formaldehyde and

peracetic acid are effective, but they are also highly toxic, carcinogenic, and leave residues. Chlorine dioxide is effective and non-carcinogenic, but some limitations on venting to the atmosphere may apply [6]. H₂O₂ vapour is highly effective, non-carcinogenic, and does not leave residues, since it decomposes into water and oxygen [2,6].

A drawback in the use of H₂O₂ vapour is due to technological issues related with its vaporization that render the final bio-decontamination equipment expensive and bulky. In fact, present technologies use aqueous solutions, where the H₂O₂ vapour that can be delivered is obviously limited by the Raoult's law and the ratio of H₂O/H₂O₂ vapour is even higher in the vapour than in the solution [7]. To improve peroxide concentration in the vapour phase, and at least ensure that the H₂O/H₂O₂ ratio in the vapour is similar to the original liquid solution, flash vaporization is employed [8]. This has two major consequences: (i) it makes the final equipment expensive, limiting the general use of a bio-decontamination procedure that has proved a high efficacy in reducing 10⁶ times (6-Log reduction or 99.9999% reduction) even the more resistant pathogens, including *Mycobacterium tuberculosis*, *Mycoplasma*, *Acinetobacter*, *C. difficile*, *Bacillus anthracis*, viruses, prions and fungi (ex. *Candida auris*) [3,9]; (ii) the equipment lacks compactness, which precludes the use of the method for applications in small spaces.

Nanoporous materials possess porous with diameter between 1 and

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100 nm [10]. According to IUPAC, nanoporous materials can be classified by pore size, in 3 categories: microporous materials (0–2-nm pores), mesoporous materials (2–50-nm pores), and macroporous materials (>50-nm pores) [10]. These materials have many applications such as adsorbents catalysts and ion exchangers [10–12]. In this work, we present an innovative methodology that utilizes inexpensive nanoporous materials to store and release H_2O_2 vapour for use in bio-decontamination. To the best of our knowledge this methodology has not been previously described in the literature. It will be shown that simple, affordable, and compact devices for bio-decontamination with H_2O_2 can be obtained without any loss of efficiency. These devices can operate in large spaces but are especially adequate for uses in small spaces that range from gloveboxes, bio-safety cabinets, fume hoods, chambers with personal equipment for domestic applications (as masks or cell phones) and terminal sterilization of solid biopharmaceutical molecules.

2. Experimental

2.1. Materials

A screening of various types of nanoporous materials was made, for their capacity to store and release H_2O_2 . A partial list of the tested materials, selected based on their general availability and performance, is shown in Table 1. In Supplementary Information (Section 1) a more complete list of the studied materials, which included clay-based materials and carbon materials, is presented.

To evaluate the specific surface area and porous volumes the usual method of nitrogen adsorption at -196°C was made for selected samples, using an automated apparatus (ASAP 2010 from Micromeritics or NOVA 2200 from Quantachrome). Prior to the adsorption measurements, the samples were outgassed (under vacuum) at 300°C for 2.5 h.

2.2. Loading of the materials with H_2O_2

2.2.1. Loading of H_2O_2 from liquid solution

Porous materials loading was initially made by mixing the powder with a 30 % H_2O_2 solution (Acros Organic, 35 %) in a 1:1 vol ratio for a given time. To improve the separation of the solid material from the liquid H_2O_2 solution, an attempt was made by separating the solid from the liquid, using a dialysis tubing cellulose membrane (Sigma-Aldrich, avg. flat width of 43 mm).

2.2.2. Loading of H_2O_2 from vapour – static mode

The materials, previously weighted and dried in an oven at 100°C , dispersed in the base of small flasks, were then placed in a desiccator saturated with H_2O_2 vapour. This saturation was achieved by the evaporation of a 35% H_2O_2 solution placed at the bottom of the desiccator. The desiccator was covered with aluminium foil to avoid H_2O_2 photodecomposition. For larger amounts of powder, a closed polypropylene box was used. Loading times between 1 and 4 days were

tested. The experiments were made at ambient temperature.

2.2.3. Loading of H_2O_2 from vapour – continuous mode

To prepare large amounts of porous materials, loaded with H_2O_2 , a continuous method was developed. Basically, the approach involved a container only partially filled with liquid H_2O_2 , to allow evaporation of H_2O_2 and its removal by a continuous air flow. The air flow ($1.2\text{ m}^3/\text{h}$) was achieved with a vacuum pump (Diaphragm Vacuum Pump Laboport, 100% oil free, Politetrafluoretileno coated) and made to pass in a glass container (with a volume of 450 cm^3), where the powder to be loaded (50 g) was placed and retained with a welded porous glass filter.

2.3. Determination of the H_2O_2 content

H_2O_2 content after loading in a given solid material was done by H_2O_2 solubilization in water and measuring through an electrochemical assay or spectroscopic assay.

2.3.1. Hydrogen peroxide electrode

For determination of the H_2O_2 content in some samples from the static loading, a hydrogen peroxide electrode (WPI, ISO-HPO-100L) was used. Each solid sample was put in 80 mL of water and the electrode monitored the change of signal corresponding to the release of hydrogen peroxide. H_2O_2 standard solutions were used to build five-point linear calibration curves required for calculations.

2.3.2. Oxygen electrode

For determination of the H_2O_2 content in samples from continuous loading and stability studies a dissolved oxygen electrode (Oxygraph, Hansatech Instruments) was used. The solid material was put in water and the mixture was filtered ($0.45\text{ }\mu\text{m}$ Nylon filters) and diluted (10^{-3}) before measurement. H_2O_2 standard solutions were used to build a five-point linear calibration curves required for calculations. For both, samples and standards, 1 mL was added to the chamber, followed by 20 μL of a 1 mg/L catalase (catalase from bovine liver, $\geq 10\text{ 000 units/mg}$ protein, Sigma Aldrich) solution. The increase in signal after catalase addition, due to the decomposition of H_2O_2 to oxygen and water, was recorded via Oxygraph™ software (Hansatech Instruments) and considered for calculation.

2.3.3. Absorbance

For determination of the H_2O_2 content in some samples from the static loading, the absorbance at 240 nm of the samples was measured. Each solid sample was put in water and the mixture was filtered ($0.45\text{ }\mu\text{m}$ Nylon filters) and diluted (10^{-3}) before measurement. A Jenway 7205 UV-Visible spectrophotometer was used.

2.4. Release of H_2O_2 from the materials

For the release of H_2O_2 from the adsorbent materials, a dedicated equipment was constructed – Fig. S1 in Supplementary Information. Briefly, this equipment (a heating unit) has a heating module with a fan that produces an air flow with a controlled temperature (temperature values between 60 and 80°C were used). The air flow passes through a cartridge, that contains the powder loaded with H_2O_2 , desorbing it, and carrying the H_2O_2 in the vapour state to the surroundings. Efficiency, as discussed below, was measured by the bio-decontamination of a biosafety cabinet and a glove box. The measurement of the H_2O_2 concentration in the vapour phase, as well as the measurement of humidity, was made through a dedicated H_2O_2 probe from VAISALA, model PEROXCAP® Probe HPP272.

2.5. Evaluation of efficacy

2.5.1. Chemical indicators

H_2O_2 vapour chemical indicators (type 4, gke Steri-Record, gke-

Table 1

Materials used for H_2O_2 storage and release tests (a more complete list is given in Supplementary Information).

| Material | Observations |
|--------------------|--------------------------------------|
| Mesoporous Silicas | |
| SBA-15 | Synthesised as described in [13] |
| MCM-41 | Synthesised as described in [14] |
| SG60 | Silica gel from ACROS ORGANICS |
| Zeolites | |
| 3A | Potassium A zeolite from BDH |
| 4A | Sodium A zeolite from Sigma-Aldrich |
| NaX | 13X zeolite from BDH |
| NaY | Y zeolite from Aldrich |
| NH4X | Prepared from 13X by cation exchange |

GmbH, Germany) were used in various situations (ex.: various locations inside a glove box where the bio-decontamination cycles were carried out – see below) allowing for the evaluation of the process of releasing the hydrogen vapour from the adsorbent materials.

2.5.2. Biological indicators

To evaluate the efficacy of microbial sterilization, *Geobacillus stearothermophilus* spore discs containing 10^6 spores inoculated in a glass fibre carrier were purchased from gke-GmbH, Germany. At the end of the bio-decontamination cycle, the biological indicators were aseptically transferred into Tryptic Soy Broth (TSB) with pH-indicator (gke Steri-Record, gke-GmbH, Germany) and incubated at 60 °C, for 7 days. Change of medium colour indicated bacterial growth and failure of the decontamination process.

For the determination of D-value, number of colony units remaining after the decontamination was done with the Most Probable Number method [15,16]. The spores were put in reverse osmosis (RO) water and the samples were vortexed and treated in an ultrasonic bath for 30 min, to remove the spores from the carrier and singularize them in the suspension. The resulting solutions were serially diluted in RO water to a level of 10^{-6} . 10 µL of each dilution was inoculated with 190 µL of growth medium in each well of the plate and incubated at 60 °C. The resulting data were interpreted by the means of Most Probable Number tables [17]. Three conditions were evaluated using three peak H₂O₂ concentrations: 379 parts per million (ppm) – 5 g of SG-60 material, glovebox temperature was 24.5–30.0 °C; 468 ppm–5 g of SG-60 material, glovebox temperature was 35 °C throughout the experiment; 2578 ppm–20 g of SG-60 material, glovebox temperature was between 40 and 60 °C throughout the experiment.

2.6. Stability of the stored H₂O₂

To improve the shelf life of the adsorbed H₂O₂, that is to decrease H₂O₂ loss due to desorption and/or decomposition over time, stabilizers were incorporated in SG60. Various types of stabilizers were used such as: sodium phosphate; sulfuric acid; urea, sodium stannate, sodium oxalate, Ethylenediaminetetraacetic acid (EDTA, ThermoFisher, 99.5%), Citric acid (≥99 %, Sigma Aldrich), Diethylenetriaminepentaacetic acid (DTPA, ≥98 %, Sigma Aldrich). The general approach was to physically mix the stabilizer with the adsorbent materials in various proportions, from 1 to 20% (in weight), before H₂O₂ vapour loading. Stability of the final product was evaluated by determining the H₂O₂ retained by the material after a given period in three different storing conditions: ambient temperature, fridge (8 °C) and freezer (−18 °C).

2.7. Evaluation of reusability of silica

To determine the reusability of silica SG60, specifically whether it could be recharged multiple times, after being used, i.e., after the loading and release of H₂O₂, the silica was subjected to a drying and reloading process. Loading in continuous mode was used.

2.8. DNase bio-decontamination and activity determination

The bio-decontamination procedure involved exposing an open vial containing approximately 2000 Kunitz of Deoxyribonuclease (DNase, D4263, Sigma-Aldrich, Deoxyribonuclease I from bovine pancreas) to 100–300 mg of H₂O₂-loaded material in a 50 mL container for seven days at −18.4 °C. Inside the container was also a biological indicator, which was aseptically incubated in TSB media after the 7 days.

To evaluate the effect of the exposure of DNase the H₂O₂-loaded material DNase activity was determined by following absorbance at 260 nm for 5 min in a reaction mix containing 83 mM Sodium Acetate (S8625, Sigma-Aldrich), 4.2 mM Magnesium Sulphate (M1880, Sigma-Aldrich), 0.14% Sodium Chloride (S0817, Sigma-Aldrich), 0.003% (w/v) Deoxyribonucleic Acid (D3664, Sigma-Aldrich) and 250 Kunitz of

DNase.

For heat inactivation, as a negative control, a solution of DNase (500 Kunitz/mL) was subjected to 70 °C for 15 min.

3. Results and discussion

3.1. Loading of the materials with H₂O₂

3.1.1. Loading from liquid peroxide solution

The first attempts of loading the porous materials, were made by mixing the powder with the H₂O₂ solution. However, since the materials were fine powders, the separation from the liquid, by filtration or centrifugation, showed losses of material and losses of H₂O₂ loaded. A second attempt made by separating the solid from the liquid using a dialysis membrane did not solve this difficulty. In fact, because the water could permeate the dialysis membrane, in the end the material was wet. The process of drying the material (for instance, in an oven) implied dramatic losses in the loaded H₂O₂. Additionally, the loading yields from a liquid hydrogen peroxide solution were always very low, about 0.03% in total weight for the best cases. These low loading yields were also reported in the literature by other authors [18], which also employ aqueous H₂O₂ solutions as a source. In this way, the loading from liquid hydrogen peroxide solutions was not pursued further.

3.1.2. Loading of H₂O₂ from vapour – static mode

The amounts of H₂O₂ loaded from vapour phase (static mode for three days) in the mesoporous silicas and zeolite 4A are presented in Table 2, in percent of the total mass. The amounts were measured by the methodology involving the H₂O₂ electrode or through absorbance as described in section 2.3.1. Three repetitions were made for each assay and a typical result is exemplified in Fig. 1. Additional quantitative assays were conducted using the absorbance method as outlined in section 2.3.3.

As registered in Table 2, the material with highest loading is the mesoporous silica SBA-15, closely followed by MCM-41 and SG60. Zeolite 4A demonstrates a notably reduced H₂O₂ loading capacity, which can likely be attributed to its pronounced hygroscopic nature.

Various surface modifications of SBA-15, to enhance the interactions between the material and the H₂O₂ were tried, as summarized in Supplementary Information – Table S2, but did not improve the loading yields. Additionally, as indicated also in Table S2, other types of materials, such as activated carbons or clay-based materials, were also considered but did not present loading yields higher than the samples presented in Table 2.

3.1.3. Loading of H₂O₂ from vapour – continuous mode

Various types of configurations were tested, as schematically described in Fig. S3 – Supplementary Information. Mode E in Fig. S3 was selected, based on a trade-off of conditions such as the process length (the necessary time required to reach at least 15 % of loading yield) and preventing vapour condensation. Loading with continuous mode incorporated higher amounts of H₂O₂ – 18.2 ± 1.5 % – in SG60 after 4 days.

Table 2
H₂O₂ loadings at ambient temperature (vapour – static method) for the indicated materials.

| Material | Loaded H ₂ O ₂ (%) |
|----------|--|
| SBA-15 | 20.1 ± 0.3 |
| MCM-41 | 14.1 ± 0.4 |
| 4A | 4.7 ± 0.3 |
| SG60 | 14.9 ± 1.3 |

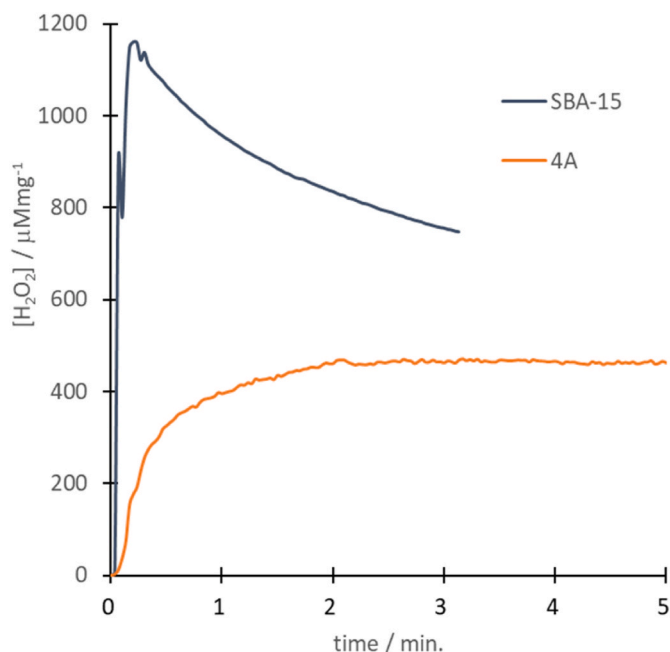


Fig. 1. Detection of the amount of H_2O_2 by the selective electrode (examples for SBA-15 and 4A zeolite).

3.2. Release of H_2O_2 from the materials

As stated above, for the methodology of storing and releasing H_2O_2 with nanoporous materials to be translated into an affordable technology, it would need to operate at a relatively low temperature. Preferably, at a much lower temperature than the ones at which flash-vaporization operates which can achieve 200°C , a temperature that, in fact, can inclusively favour the decomposition of the H_2O_2 molecules [7,8]. Nevertheless, very low releasing temperatures may result in inadequate control of H_2O_2 release, and cause part of the stored vapour to desorb prematurely.

To evaluate the temperature needed to release the H_2O_2 from the materials, thermogravimetric (TG) analyses were made. Fig. 2 a) and b) show the TG, and the respective differential curves (DTG), for a mesoporous material (SBA-15), a microporous material (4A zeolite) and the silica SG60.

As could be expected due to the presence of the extra-framework cations in the zeolite [19], which can promote the interactions with the polar H_2O_2 molecule, the temperature at which most of the H_2O_2 is released, corresponding to the inflexion in the TG signal and the minimum in the DTG curve, is higher for the 4A zeolite than for the SBA-15 material, namely 50°C and 164°C , respectively. While the latter temperature is too high, the former is too low. In fact, even if the minimum in the DTG curve of SBA-15 is at 50°C , it can be seen from the shapes of this curve (Fig. 2 a) that the fall in the mass loss is very steep. To obviate this, the SG60 sample was considered. The minimum in the DTG curve for SG60 is 87°C (Fig. 2 b) and the decrease in the mass loss is less steep than what was found for the SBA-15 material which, potentially, will allow a better control in the release of the H_2O_2 . Additionally, both materials are mesoporous materials, as indicated by their type IV adsorption isotherms of nitrogen at -196°C [10] (Supplementary Information Fig. S4). Even if the mesoporous volume is lower for SG60 ($0.79\text{ cm}^3\text{g}^{-1}$) than for SBA-15 ($1.21\text{ cm}^3\text{g}^{-1}$), both materials present mesopore size distributions with similar maxima of pore sizes (Fig. 3).

To the reasons stated above, and even considering that the capacity of SG60 to store H_2O_2 is lower than the observed for SBA-15, an additional relevant reason favours the selection of the SG60 silica. The bio-decontamination devices obtained based on the storage and release of H_2O_2 in porous materials as described here will require significant

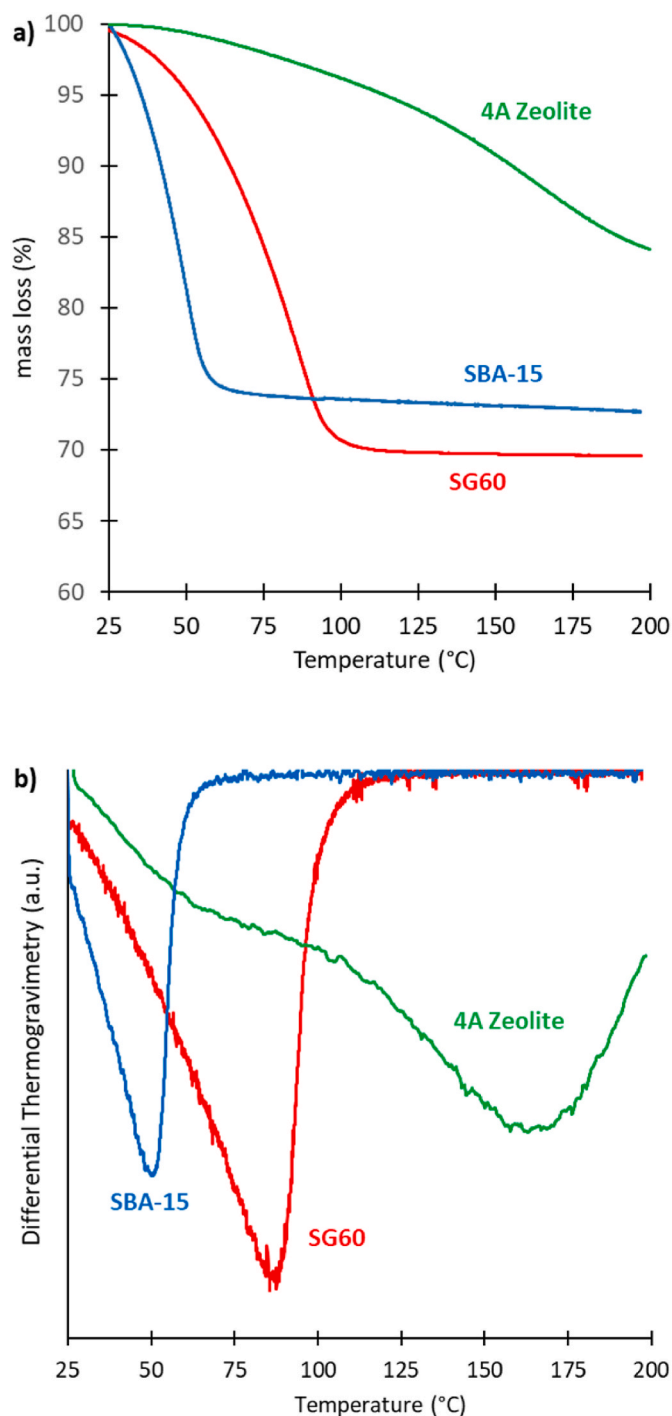


Fig. 2. a) Thermogravimetric and b) Differential Thermogravimetric curves for the indicated materials loaded with H_2O_2 .

quantities of material, for instance for room decontamination. Therefore, it is important that the system is based in an affordable material and, presently, 1 kg of SBA-15 costs nearly 150 times more than the same amount of the SG60 silica studied here. An additional feature of SG60 is that this material offers an additional advantage over zeolites in that it possesses an amorphous nature. This characteristic is less toxic, making amorphous silicas safer to handle than the crystalline structure of zeolites [21].

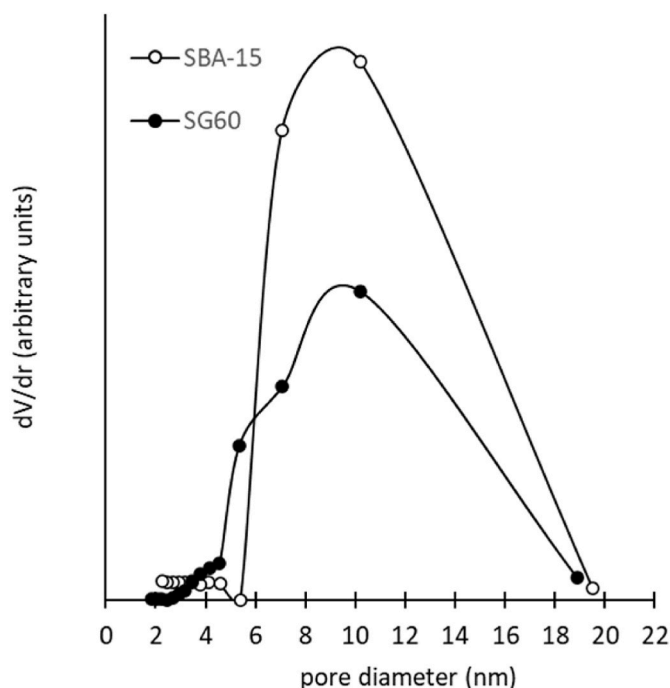


Fig. 3. Mesopore size distributions for the SBA-15 and SG60 samples obtained from the nitrogen adsorption isotherms at -196°C by the Broekhoff-de Boer method [20].

3.3. Stability of the stored H_2O_2 in the SG60 material

The shelf stability of H_2O_2 loaded in SG60 was evaluated since this is an important characteristic for applications in bio-decontamination, namely for product transportation and storage. Three storage conditions were selected (always in the dark): at room temperature, in a fridge (8°C) and in the freezer (-18°C). Illustrative results, obtained from at least 3 replicated experiments, are given in Fig. 4.

It can be noticed from Fig. 4 that storage at room temperature is clearly unappropriated since, even after one week, only 58% of the initial H_2O_2 is present in the material, which decreases for less than 20% of the initial values after one month. Storing the loaded material in the fridge and, particularly, in the freezer improves the stability.

The decomposition of H_2O_2 is a complex process that can proceed by a number of complex reaction pathways [22,23]. To reduce the H_2O_2 decomposition, mostly for aqueous H_2O_2 solutions, a number of stabilizers had been proposed in the literature between organic and inorganic

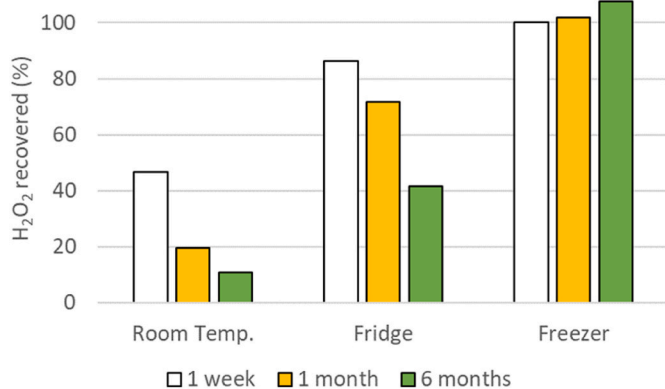


Fig. 4. Percentage of H_2O_2 recovered from loaded SG60 material after one week, one month, or six months stored at room temperature, in the fridge (8°C) and in the freezer (-18°C).

substances [24–27]. Since adsorbed H_2O_2 is a new field of studies, there were not, to our knowledge, specific stabilizers reported in the literature for this type of system. As so, a trial-and-error study was made with the more common stabilizers used in liquid phase. The general approach, as mentioned in the Experimental Section, was to mix the stabilizer in various proportions with the SG60 sample, using incipient wetness, and evaluating the H_2O_2 retained by the material after a given period of time, in different storing conditions. Since stability in the freezer (-18°C) was achieved for the material even without stabilizers, for a period of six months, the study focused on the improvement of stability at room temperature and at the fridge temperature (8°C).

As mentioned, a large number of stabilizers were tested, and the obtained results are given in the Supplementary Information Section 5. In Fig. 5A, we present the results for the systems that showed the more favourable improvement in the stabilization of H_2O_2 loaded in the SG60 material, for storage at room temperature. These systems were based in the addition of EDTA, DTPA or citric acid. It can be seen from this figure that, although some loss of H_2O_2 is always evident, for a two-month storage period EDTA or citric acid (2% weight) present similar results. However, when the results for storage in the fridge (8°C) are also considered – Fig. 5B, citric acid, at 1 or 2 % performs better. The H_2O_2 content of SG60 material mixed with EDTA or citric acid, after storing at 3 months at room temperature, is still sufficient to make the efficient bio-decontamination of small spaces such as biosafety cabinets – as discussed in the following section.

3.4. Bio-decontamination efficacy of H_2O_2 loaded in SG60

Next, we characterized the bio-decontamination efficacy of H_2O_2 desorbed from the SG60 material. The release of H_2O_2 from the porous material to the surroundings to be bio-decontaminated was made using a

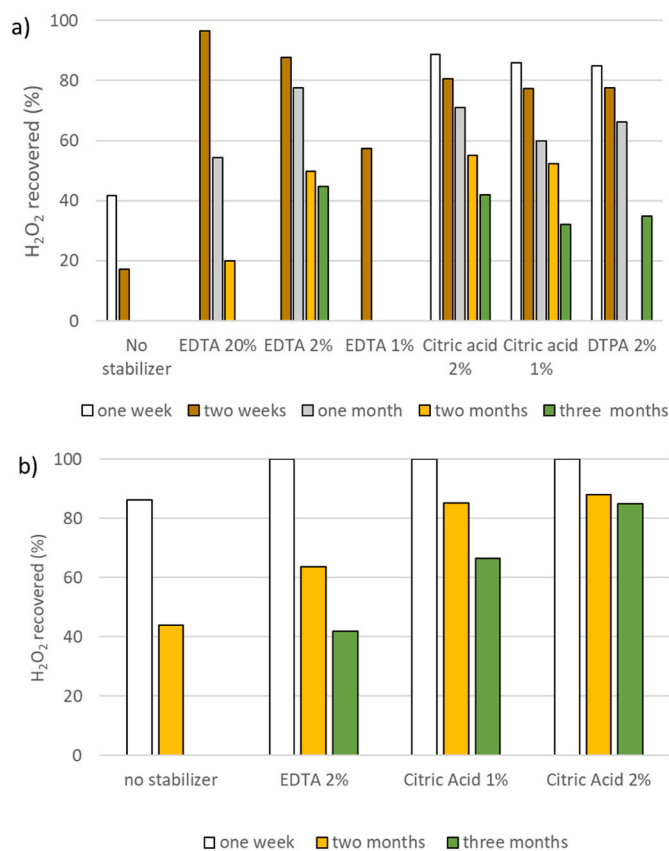


Fig. 5. Percentage of H_2O_2 recovered from SG60 material mix with EDTA, DTPA or Citric Acid, in the indicated weight proportion. Samples were stored at (a) room temperature or (b) in the fridge (8°C).

dedicated unit to heat the powder with hot air flow and desorb the H_2O_2 – Supplementary Information, Section 1. A given amount of powder is enclosed in a cartridge in which the top and bottom are two layers of High Efficiency Particulate Absorbing (HEPA) filter. These layers of HEPA filter retain the powder particles but allow a stream of hot air (between 60 and 80 °C) to pass and, therefore, enable the release of H_2O_2 in the vapour phase through the top HEPA filter and into the closed environment to be bio-decontaminated.

We started by performing a qualitative assay in a glovebox (total volume of 0.25 m³, see Fig. S6) by using a standard procedure that uses chemical and biological (*Geobacillus stearothermophilus* spore discs) indicators [28], as described in the experimental section. Fig. 6 schematically indicates the places in the glovebox where the indicators were located (positions 1 to 10).

All the chemical indicators proved bio-decontamination, by changing their colour from purple to rose during the bio-decontamination cycle. After 7 days, the culture medium remained purple in all tubes containing the biological indicators exposed to the bio-decontamination cycle indicating a 6-Log reduction (that is, a 99.9999% reduction) in the *Geobacillus stearothermophilus* spore concentration. Only the non-exposed biological indicator (control tube) was yellow after 24 h, indicating microbial growth. To obtain a more quantitative characterization of the bio-decontamination efficacy of H_2O_2 loaded in SG60, D-values were determined. The D-value is the time required to achieve a log reduction, that is, to kill 90% of the present microorganisms. Three sets of experiments with different H_2O_2 vapour profiles were carried out (Fig. 7). Biological indicators containing 1 million spores were exposed to H_2O_2 vapour and the number of surviving spores was measured by the Most Probable Number Method, as described in the experimental section. After an initial 5-min lag phase, in which little sporicidal activity was detected, a rapid phase of spore killing ensued (Fig. 7b). This lag phase was probably caused by the time needed to build up H_2O_2 levels in the vapour phase. In the fast phase, D-values of 1–2 can be calculated depending on the precise conditions, which compares very well with H_2O_2 generated from liquid solutions [29]. Thus, after being released into the vapour phase, the H_2O_2 loaded in SG60 has a very high bio-decontamination efficacy.

3.5. Transportation

Transportation safety is an important issue when dealing with concentrated solutions of H_2O_2 . As liquid H_2O_2 is classified as an Oxidizing Substance (Division 5.1, Oxidizing Liquid) [30], belonging to the Packing group II, the H_2O_2 loaded SG60 was tested.

Relevant samples were subjected to Division 5.1 Oxidizer Analysis in accordance with the United Nations Transport of Dangerous Goods-Manual of Tests and Criteria, seventh revised edition (2019) [31]. The tests were performed by an external ISO 2001:2015 Certified Laboratory, which concluded that the material does not appear to be a Division

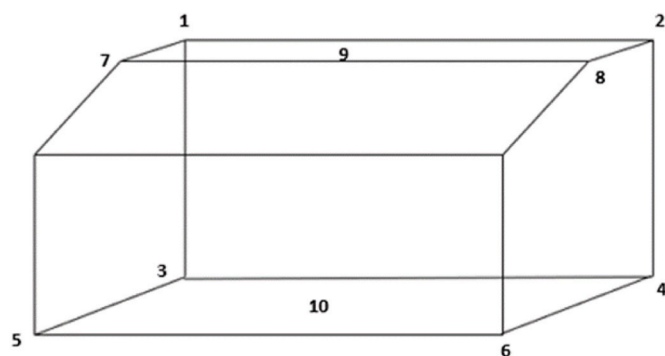


Fig. 6. Locations of the chemical and biological (*Geobacillus stearothermophilus* spore discs) indicators in the glovebox.

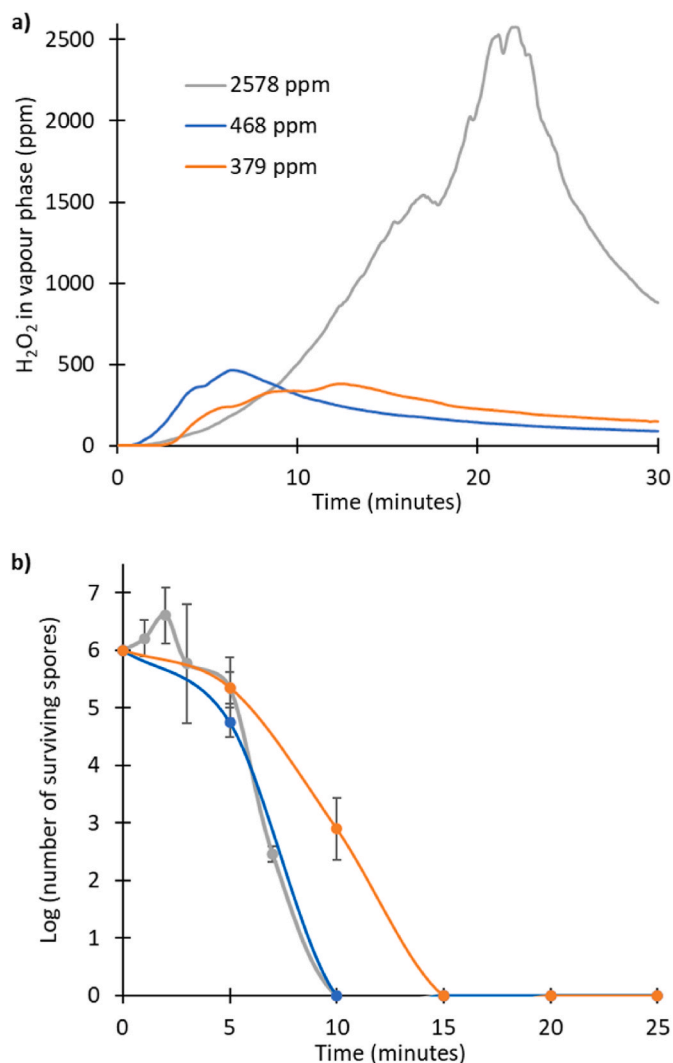


Fig. 7. Determination of D-values: a) profile of H_2O_2 vapour; b) profile of \log_{10} of surviving *Geobacillus stearothermophilus* spores. The lines correspond to three conditions labelled according to the peak H_2O_2 concentration reached: 379 ppm, 468 ppm and 2578 ppm.

5.1 Solid Oxidizer, as defined by the United Nations and The United States Department of Transportation criteria. Additional information on the methodology and test results can be found in Supplementary Information (Section 7).

3.6. Reusability of SG60

Today, circular economy is key issue for economic and environmental sustainability. Thus, SG60 material utilized in bio-decontamination cycles was subjected to hydrogen peroxide recharging to assess its viability for reuse. Table 3 provides data on H_2O_2 levels in both new SG60 and reused SG60, silica that has been recycled after the loading process. Each row in the table corresponds to a separate batch.

The evidence suggests that SG60 effectively retains its loading capacity after utilization, thereby enabling a sustained and repeated recycling process. Thus, the material can be employed multiple times without a significant decline in its capacity to retain H_2O_2 , offering practical advantages in terms of resource utilization and cost-effectiveness.

Table 3H₂O₂ content in new and reused SG60 material after loading (continuous mode).

| | %H ₂ O ₂ Content | |
|--------------------|--|---------------|
| | New silica | Reused silica |
| | 18.2% | 15.9% |
| | 20.1% | 16.6% |
| | 15.4% | 17.5% |
| | 19.0% | 15.8% |
| | 20.0% | 21.9% |
| | 17.3% | 15.6% |
| | 18.8% | 17.1% |
| | 15.7% | 16.6% |
| | 18.5% | 17.5% |
| | 18.6% | 15.8% |
| Average | 18.2% | 17.0% |
| Standard Deviation | 1.5% | 1.8% |

3.7. Bio-decontamination of biological molecules

Biopharmaceuticals are an emerging class of drugs in which microbial decontamination poses a problem. It is usually assumed that bio-decontamination protocols inactivate these products. Our proof-of-concept with the enzyme deoxyribonuclease (DNase) experiment shows that the bio-decontamination of these products is possible without loss of activity.

DNase is a type of nuclease enzyme that catalyses the hydrolytic cleavage of phosphodiester linkages in the DNA molecule. In Fig. 8, the activity of DNase is followed as variation in absorbance at 260 nm ($\Delta\text{Abs } 260 \text{ nm}$) along time for the test samples and controls. As expected, for the heat inactivated protein sample, DNA cleavage did not occur and thus no significant variation in absorbance was verified. For the enzyme samples exposed to 100 mg of H₂O₂ loaded material, a variation in absorbance was verified, indicating that the enzyme maintained its activity. Log 6 bio-decontamination was achieved in 2 out of the 3 samples, shown by the negative biological indicators after incubation. For the enzyme samples exposed to 300 mg of H₂O₂, while H₂O₂ inactivated the biological indicators, a loss of DNase activity was observed in all samples.

Although the mass of H₂O₂-loaded material should be optimized to ensure the 6-log the bio-decontamination without compromising the enzyme integrity, these experiments show the potential for the low temperature bio-decontamination.

4. Conclusions

In this work we showed for the first time that H₂O₂ can be stored upon adsorption from vapour phase in a nanoporous material and later, after gentle heating at temperatures between 60 and 80 °C, rapidly released in the gas phase in concentrations that enable efficient bio-decontamination. Various types of nanoporous materials can be used for this end, from zeolites to mesoporous silicas, including relatively cheap materials such as Silica Gel 60 (SG60). The stability of the H₂O₂ loaded in SG60 is modest at room temperature but can be preserved in the freezer (at −18°) for over six months. For samples where the SG60 is mixed with EDTA or citric acid in concentrations between 1 and 2%, the stability of the adsorbed H₂O₂ is clearly improved. We proved that the silica SG60 can be reused after loading and release of H₂O₂, maintaining its capacity of H₂O₂ retention. In addition, we also showed that at sub-zero temperatures there is a slow release of H₂O₂ vapour from the porous material that can be used to decontaminate biological products without loss of activity. In conclusion, the utilization of nanoporous materials for the adsorption and subsequent desorption of H₂O₂ represents an innovative and simple method for an efficient use of H₂O₂ in its vapour phase, enhancing current bio-decontamination procedures and opening the field to new possible applications, such as the bio-decontamination of biopharmaceuticals.

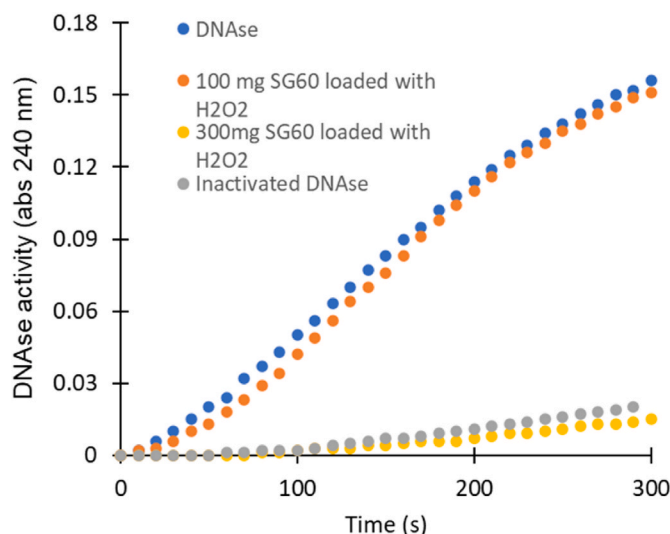


Fig. 8. Bio-decontamination of DNase containing vials without loss of enzymatic activity. Tubes (50 mL) containing DNase were exposed to 100 mg and 300 mg of SG60 loaded with H₂O₂ for 1 week in a freezer (−18 °C). Both amounts were sufficient to achieve a 6-log decontamination. Samples with untreated DNase (DNase) and heat Inactivated DNase (70 °C for 15 min) are also shown.

CRediT authorship contribution statement

Fadhil Musa: Writing – review & editing, Validation, Project administration, Investigation, Funding acquisition, Formal analysis. **Raquel Nogueira:** Methodology, Investigation. **Margarida Beiral:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Fernando Antunes:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **João Pires:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JP, FA, and FM are founders of Delox Lda., a company that was created to develop applications in bio-decontamination, using H₂O₂ desorbed from nanoporous materials. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.micromeso.2024.113056>.

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