# Organic peroxides—the new generation of highly effective disinfectants for remediation of mouldy buildings

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**ABSTRACT**

Stabilized organic peroxide formulations are the new highly efficient remedies against mould spores in buildings. They are laid out as a fog using a pulse-jet fogger. The effect of one such formulation on spores of *Stachybotrys chartarum* has been studied at the laboratory level. Experiments show that the spores are not only killed, but to a great extent also broken down. This is an important effect because it is very likely that the toxins in the spore wall are also broken down. Thus, the well-known problem with dead *Stachybotrys* spores being as toxic as live ones is eliminated. Our experiments show that 6.5 l of organic peroxide reduces the number of live *S. chartarum* spores by more than 90%. Fogging with organic peroxides is in practical use very cost-effective, one person easily fogs a house in 1–2 h. The fog breaks down naturally; more than 90% is broken down in 24 h, leaving no toxic residues. This also makes stabilized organic peroxides the most environmental friendly on the market for remediation of mouldy buildings.

**OBJECTIVE**

A new suite of organic peroxide formulations has been in the market for some years now, intended for use as disinfectants in mouldy buildings. We have used it for some time in full-scale practical work with very good results. In mouldy buildings we have measured the concentration of fungal spores in air before and after disinfection, and the reduction of spore concentration in air after disinfection has been more than 90% in all cases (unpublished results). In practical use, the peroxides are laid out as a *fog* with a particle size less than 10 µm. In this form they become very reactive (Figure 1).


**Figure 1** Fogging of a room with organic peroxides using a pulse-jet fogger. The peroxide formulation is in its original form a liquid, and in the fogging machine broken down and laid out as a fog, with particle size less than 10 µm. In this form it is very reactive, killing airborne fungal spores with great efficiency. The fog will leak into all these hard-to-reach places, not accessed by ordinary disinfection methods.

Photo: F. Langvad

The peroxide fog is broken down naturally, more than 90% broken down in 24 h.

Even though very good results have been obtained with this method in practical use, the use of organic peroxide has to some extent been disputed. In this investigation we have studied the effect of these remedies in more detail. To do that we have scaled down the method to laboratory level, and used it for laboratory experiments. The results of this study are presented here.

## METHODS

For these experiments two different types of experimental spore chambers were used. The first had a volume of 500 ml and consisted of an autoclavable plastic jar (Nalgene), with a bored out 25 mm hole for the introduction of spore aerosol or organic peroxide fog. This chamber was used for the fluorescence microscopy experiments (Figure 2a). The second chamber was put together using Quick-Fit components, and had a volume of 2 l. This chamber was used to determine reduction of living spores in air after fogging with organic peroxides (Figure 2b).


**Figure 2a** Simple spore chamber (500 ml) used for studying the effect of organic peroxides at the microscope level. Inlet of fog through the bottom hole.

**Figure 2b** Spore chamber (2 l) used to determine the reduction of viable spores after fogging with organic peroxide.

Arrows show inlet of organic peroxide fog.


(1)

Resting spores of *Stachybotrys chartarum* from Malt Extract Agar were suspended in sterile distilled water. Aliquots (0.5 ml) were filtered on 13 mm syringe filters, and the filters were placed in the spore chambers. The organic peroxide (Penetrox, Alron Chemical Co AB, Sweden) was introduced into the chambers as a fog through the bottom hole using an Omron U22 atomizer. The atomizer produces mean drop size of about 5 µm (Figure 3).

**Figure 3** Fogging with organic peroxide (Penetrox) at a laboratory scale. The atomizer produces drop sizes of about 5 µm, making the fog extremely reactive.

Photo: F. Langvad

After 10 min of treatment with Penetrox the filters were mounted in a mixture of 10 µl Calcofluor White (Sigma-Aldrich) + 10 µl Citifluor mounting medium (Agar Scientific), and viewed under the fluorescence microscope using incident UV light with green excitation filter. Included were also untreated filters as controls.

(2) To study the effect of organic peroxides on living spores, spores of *S. chartarum* were introduced into the 2-l spore chamber (Figure 2b) as an *aerosol* from a suspension in sterile distilled water using an Omron U1 atomizer. Various concentrations of Penetrox were used, from 0 to 15 l per 1000 $m^3$ air. After a reaction time of 10 min, Malt Extract Agar plates were introduced into the bottom of the chamber (Figure 2b), and left there for 15 min for spores to settle on the plates. Colonies were counted after proper incubation time at 22°C.

**RESULTS**

Resting, live fungal spores will appear dark against a green background when stained and viewed in the fluorescence microscope as explained under Methods. Injured and dead spores will vary from light green to yellow. Figure 4a shows resting spores of *S. chartarum* without any special treatment as seen under the fluorescence microscope. The majority of spores appear dark, indicating they do not have an active chitin synthesis, and hence the fluorochrome is not taken up. Some dead spore is seen as light green, due to the fluorochrome leaking into the cells.

Figure 4b shows spores of *S. chartarum* after treatment with Penetrox. Very few spores remain intact. Dead spores appear brighter than the background, due to leakage of the fluorochrome into the cells (D). Spores that are broken down will eventually fade into the same colour as the background (Bro). When completely broken down they can only faintly be seen.

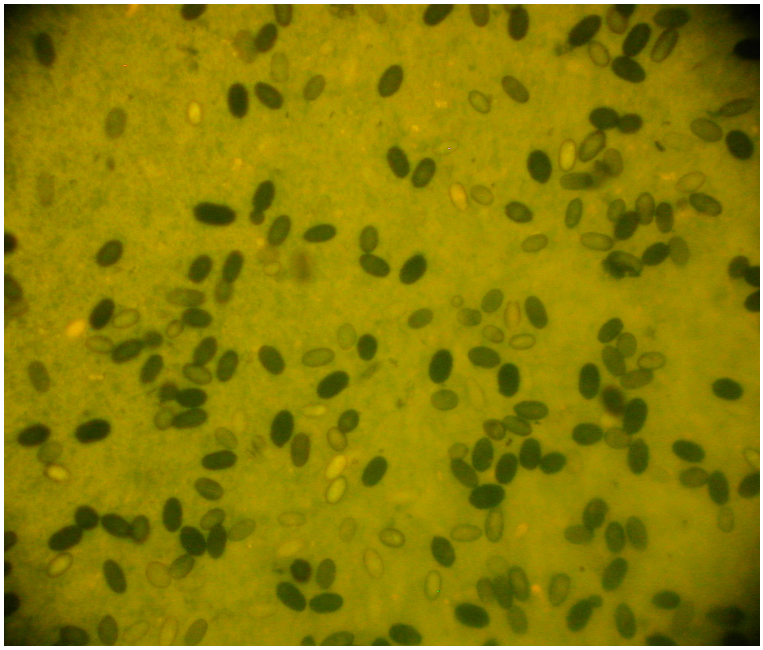

**Figure 4a** Spores of *Stachybotrys chartarum* as seen under the fluorescence microscope. The majority of spores appear dark, indicating that they are resting but live. Resting spores do not have an active chitin synthesis, and hence the fluorechrome is not taken up.

Photo: F. Langvad

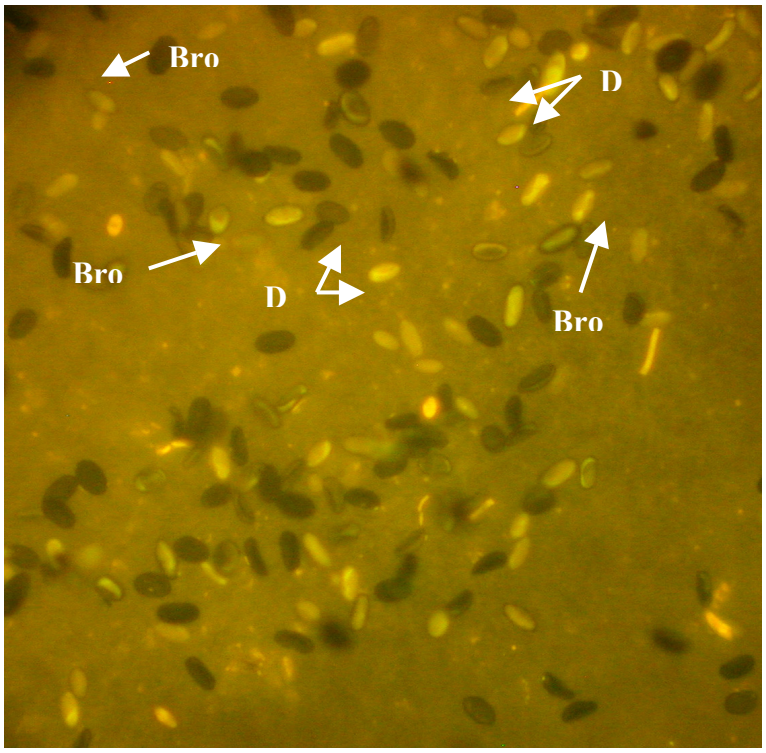


**Figure 4b** Spores of *S.chartarum* treated with Penetrox. Dead spores appear brighter than the background, due to the leakage of the fluorochrome into the cells (D). Spores which are broken down will eventually fade into the same colour as the background (Bro). When completely broken down they can only faintly be seen.
Some live cells can still be seen.

Photo:F.Langvad.

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Figure 5 shows the reduction of viable *S. chartarum* spores as a function of concentration in air of Penetrox.

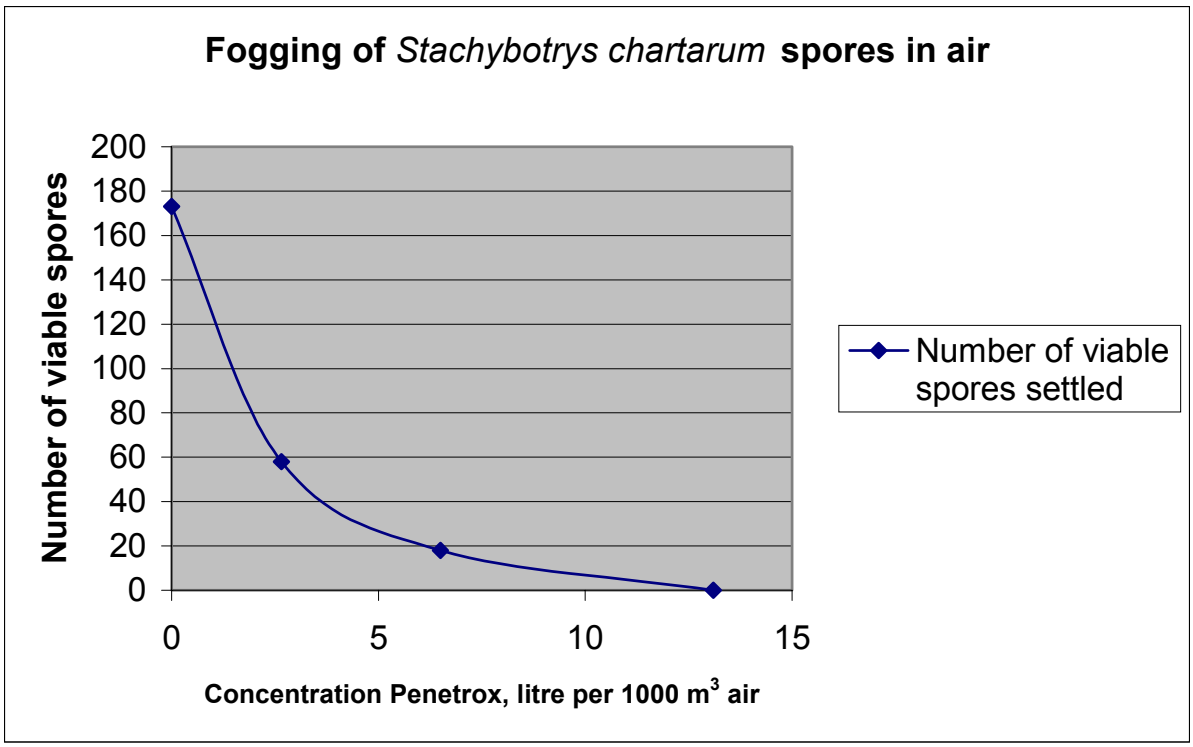


**Figure 5** Reduction of the number of viable spores in an air space as a function of concentration of Penetrox. A concentration of 6.5 l per 1000 $m^3$ air reduces the number of viable spores with 90%.

## CONCLUSION

The results show that organic peroxides are very efficient in killing spores of *S. chartarum* in air when laid out as a fog. Not only are the spores killed, but they are also to a great extent destroyed. Therefore, it is very possible that toxins, like Satratoxin H in *S. chartarum* spores, are broken down. This is very important, since dead spores of this fungus are as dangerous as living ones. This makes these remedies almost ideal for use as disinfectants in mouldy buildings. One person easily fogs a house in 1–2 h. Furniture and other belongings may be safely left in the house. The fog breaks down naturally, less than 10% being left after 24 h, and leaving no toxic residues. Three days after fogging the family may safely move back again. This makes these compounds the most environmental friendly in the market for remediation of buildings.

The new remedies make the use of chlorine (hypochlorite) obsolete.