INVESTIGATION OF EFFECTIVENESS OF MOLD DISINFECTANTS AND CHEMICALS ON TOTAL CELL NUMBERS OF MOLD ON BUILDING MATERIALS

Judith Mueller and Urban Palmgren

ABSTRACT

In this investigation mold disinfectants H20 (30%), H202 (5%) with hydroxy-acids (fruit acids) and isopropanol (70%) were tested on *Aspergillus versicor* growth on wall paper pieces. The control treatment was H20. The aim of the study was to examine if the chemicals have an effect on the total cell count. The disinfectants had four incubation times: 0h, 2h, 24h and one week. The results have shown that the amount of reduction of the total cell count is not sufficient to replace the remediation of infected material with disinfection. In addition, the biochemical activity and the CFU have shown a habit of recovering after only one week, without nutrients and fluids available. This emphasizes the ineffectiveness of disinfectants to remove the biomass. Furthermore, the long term effect of mold disinfectants has not been investigated. In regard to preventive health protection, indoor mold contamination should be removed and replaced rather than treated by disinfectants.

KEYWORDS: mold, total cell numbers, disinfection, mold disinfectants

INTRODUCTION

Disinfection is the inactivation of microorganisms and inhabition of the ability of reproduction and division. The disinfectant varies in the effect and action. The destruction of the cell does not always occur but even airborne cell components can have an allergic and toxic potential (WHO, 2009). Disinfection of mold and bacteria on building material is a common alternative to a costly remediation. Guidelines in the USA and Europe recommend the removal of microbiologic contaminated material. Depending on the parts and the size of the contamination the remediation is costly and complex. Disinfection seems to be an easy and fast alternative. Tests shows, that chemicals could minimize the presence of colony forming units of microorganisms. However, within these studies, the biomass was not taken into consideration. The aim of our investigation was to examine if the total cell numbers (biomass) of mold on samples was affected by mold disinfectants. Furthermore the metabolism activity of the cells and the CFU were investigated. As a result we tested the hypothesis "mold disinfectants and chemicals do have an effect on total cell numbers of mold on building materials".

MATERIALS AND METHODS

In our assay, we tested the effectiveness of mold disinfectants as well as other chemical substances for the purpose of mold disinfection. We want to prove that

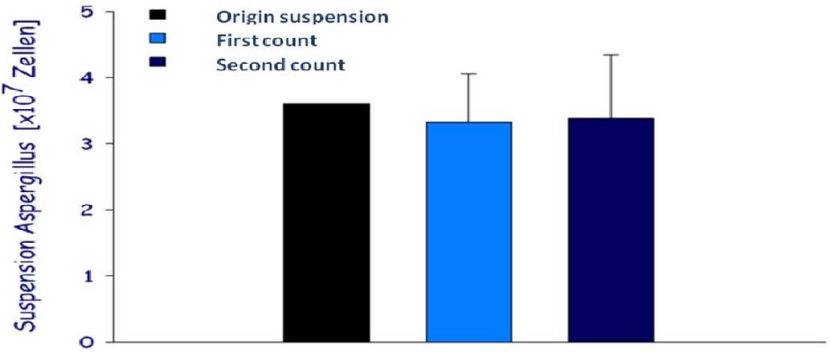
disinfection cannot replace decontamination of microbiological contaminated material while the chemical substances fail to destroy the biomass. The disinfection only affects the CFU; therefore, without testing the total cell count, the measured success of chemicals as mold disinfectants may be based on false positive results. Our research tests the effects on CFU as well as the total cell number and biological activity, thereby giving us a more accurate result of the effectiveness chemicals has towards mold.

The studies were based on mold contamination of wall paper. A pure culture of *Aspergillus versicolor* was transferred in 10 ml of sterile buffered solution and worked as the mold suspension. The mold suspension was spread on 2x2 cm wall paper and incubated on DG18 Agar for one week. The colonies of the suspension and the wallpaper were analyzed in preexperiments to ensure that samples were homogenous. The pieces of wallpaper were treated with three different substances: H_2O_2 (30 %), H_2O_2 (5%) with fruit acid and Isoporpanol. For every treatment and incubation time, three replicates have been done. Further, the samples with these substances are tested in different incubation times: immediately, after 2 hours, after 24 hours and after one week in regard to the reduction of the biomass and the reactivation of the biological activity and the effect on the CFU. The contaminated samples were washed with 10 ml of sterile buffered solution to achieve a sample solution. The control group was treated with pure water and analyzed in the same way. The total number of microorganism/ cm^2 was investigated by the Camnea method (Palmgren et al., 1986) dyed with acredin orange and counted with an epifluorescence microscope. The biochemical or metabolism activity of the mold was investigated with the dye "fluorescein diacetate" (Palmgren, 1984) and counted with an epifluorescence microscope. Colony formed units (CFU) were assessed on three standard media (Samson, 2010) TGE for bacteria, DG18 and Malt agar for mold, incubated at 25 °C counted as well as differentiated after seven days.

RESULTS AND DISCUSSION

In the preparation of the mold suspension the amount of *Aspergillus versicolor* was counted with an epifluorescence microscope. The suspension was cultivated on wall paper and washed out after a week to compare the concentration of the mold between the origin suspension and the contamination of the wall paper samples. The results are stated in figure 1. The pre-tests have shown that the chosen method were successful to prepare homogenous samples.

Figure1: Total cell numbers of *Aspergillus* suspension and Pre-tests



The prepared samples were treated with the chemicals and the total cell numbers were counted after an incubation time of 0, 2, 24 hours and one week.

Figure 2: total cell count after different incubation times of disinfectants.

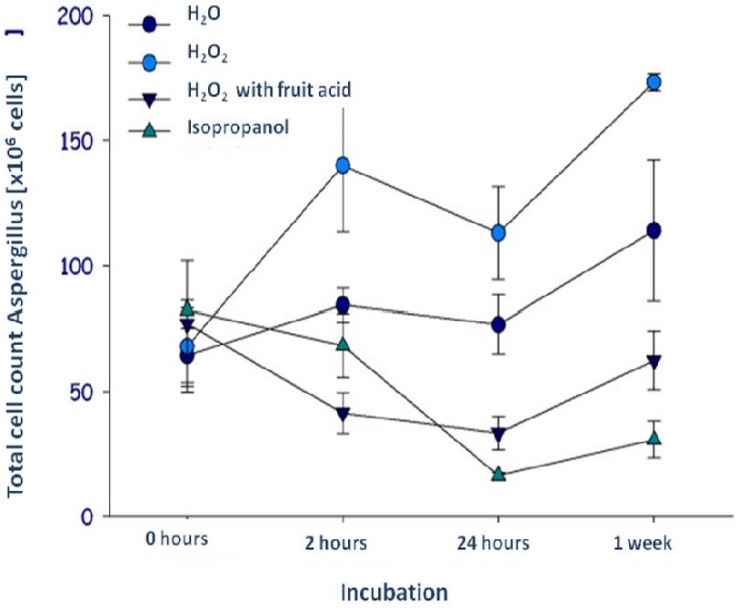
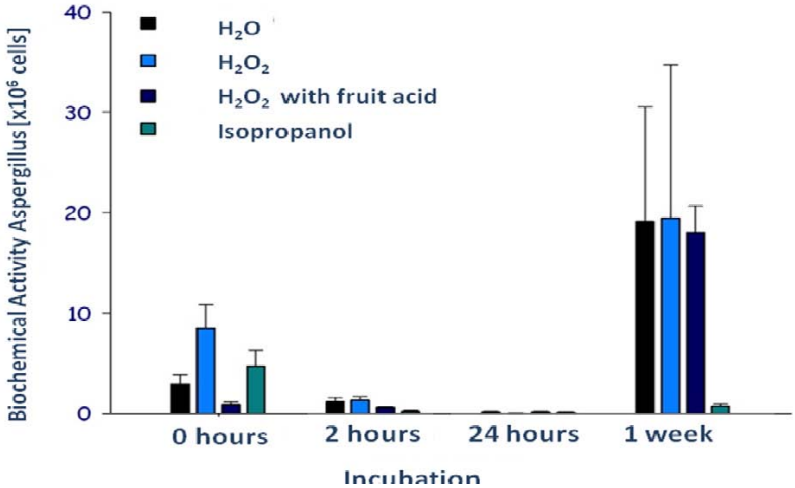


Figure 2 shows the results of the different disinfectants. Water was used as the control group and has shown only a slightly difference between the four cell counts. Hydrogen peroxide has shown an increase of microorganisms, hydrogen peroxide with fruit acid a slightly decrease and isopropanol has shown the highest reduction. The *Aspergillus* suspension started with about $3.6x10^7$ microorganism per ml. The highest reduction was detected after the incubation time of 24 hours to a cell number of $1.4x10^7$. With all disinfectants, a recovery and an increase of total cell numbers could be seen after one week of incubation.

All samples and replications were also investigated for metabolism or biochemical activity with the dye "fluorescein diacetate" and were counted after the same incubation times. The decrease of activity was significant. After 2 hours almost no activity could detected.

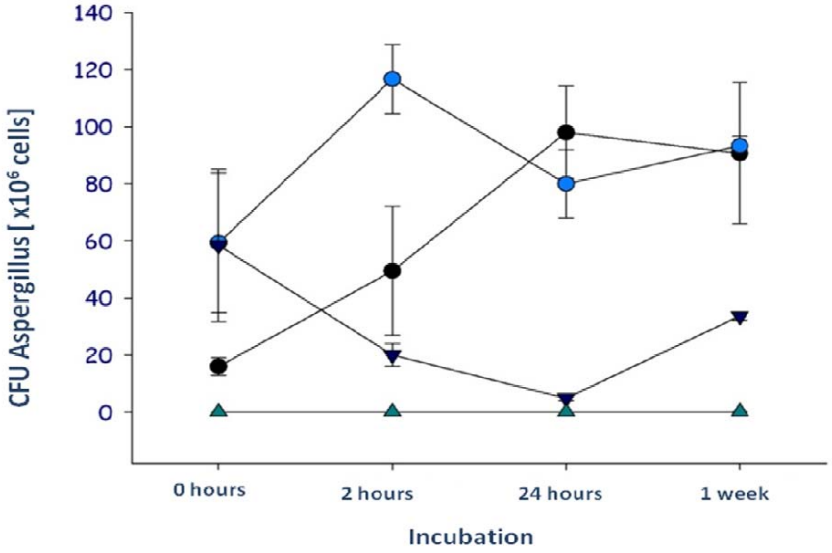
Furthermore, the water treated samples hardly show biochemical activity. Surprisingly after one week incubation all samples could recover and show metabolism activity. Only the samples treated with isopropanol alcohol had a slightly lesser recovery.

Figure 3a: biochemical activity after different incubation times of disinfectants



After counting the total cell number and the biochemical activity all samples were incubated for one week to investigate the CFU. The control showed a rapid increase until 24h incubation and a slight decrease after one week. Hydrogen peroxide could not reduce the CFU, however hydrogen peroxide with fruit acid has shown a reduction but also an increase after one week incubation. The alcohol only had an extreme effect on CFU, where even after one week incubation, the CFU were underneath the limit of detection.

Figure 3b: CFU after different incubation times of disinfectants.



In the German mold remediation guidlines (Tischer, Chen, Heinrich, 2011) the removal of contaminated material is endorsed in regard to preventive health protection. Further, dead and dried microorganisms can produce allergic and toxin components. Due to this publication mold disinfectants produces praise their products as not only to kill but also to destroy the biomass. After disinfection, the components are still in the porous indoor material and emit these components to the indoor air. If the products can lead to a lyses of the microorganisms, the allergen potential is still present on the surface of the cells and can also emit to the indoor air. These investigations and the results show the reduction of total cell number of *Aspergillus versicolor* occur, but only in a marginal percentage. The number of 10^7 per ml could not be reduced to tolerable microbial contamination. The camnea method dye intact spores and mycelia and a prediction about cell components cannot be given after this investigation. Within the biochemical activity a reduction could be

seen but also a recovery after one week. This leads to the assumption that the treatment did shock rather than kill the microorganisms. However, the recovering was visible and in the further experiments with a longer period of recovery should be recovery investigated. Normally one expects a recovery if the availability of water is still present. In our investigation we have stored the samples dry and no nutrients or fluids were available. Hence we can confirm that the trend of recovering take place without the aid of nutrients of fluids.

On this stage of the study we conclude that the tested chemicals could not reduce the biomass of contaminated building materials and could not substitute the remediation.

Disinfections cannot replace the decontamination of microorganism in building material and hence our null hypothesis is rejected.

In further studying an increase of replication is necessary. Furthermore, at the present time the same investigation is carried out with real samples and the results will be published in the near future.

REFERENCES:

- Palmgren U, Ström G, Blomquist G and Malmberg P. Collection of airborne microorganisms on Nuclepore filters, estimation and analysis, CAMNEA-Method. *J. Appl. Bact.* 1986:61, p. 401-406.
- Palmgren U. Microbial and biochemical changes occurring during deterioration of Hay and Preservative effect of urea, *Swedish J. agric Res.* 1984:14 p.127-133
- Samson RA et al. Food and indoor fungi, CBS-KNAW Fungal Biodiversity Centre Utrecht, Netherlands, 2010.
- Tischer, Chen and Heinrich. Association between Domestic Mould and Mould Components, and Asthma and Allergy in Children: a systematic Rewiew., 2011 in Press.
- WHO, Dampness and Mould. Guidelines for Indoor Air Quality, 2009.