Laboratory research on the efficacy of the TDA, Inc. photochemical mold remediation (PMR) technology for contaminated building materials

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Introduction

The goal of the U.S. Environmental Protection Agency (EPA) and TDA, Inc (TDA) Cooperative Research and Development Agreement (CRADA # 901-16) is to evaluate the efficacy of TDA, Inc - "Photochemical Mold Remediation" (TDA-PMR) technology for the remediation of building materials contaminated with molds. The TDA-PMR technology consists of an on-site photochemical generation of active chlorine dioxide (ClO_2) species to deactivate biological organisms in biofilms. Previous EPA research of ClO_2 technologies for fumigation of viable mold contaminated building materials showed the potential of these technologies for remediation of extremely water-damaged buildings.

Through this EPA-TDA CRADA, TDA plans to develop a specific formulation (F) of the photochemical system that is designed for use against fungi and fungal spores. The EPA laboratory evaluations consisted of testing the efficacy of the PMR technology on building materials that support mold growth.

Four mold species frequently isolated from water-damaged buildings were used: *Alternaria alternata*, *Aspergillus versicolor*, *Chaetomium globosum*, and *Stachybotrys chartarum*. These contaminants were individually inoculated onto the surface of six building materials: gypsum wallboard (W), latex-painted gypsum wallboard (L), bare-structural pine wood (PW), concrete (CN), ceiling tile (C) and glass (G). The inoculated building materials were fumigated with the TDA-PMR formulations F1; F2; F3 and F4. Each formulation was tested individually. The reduction in viable spores was determined by subtracting the average log values of colony forming units (CFU) recovered from the treated coupons from the CFU recovered from the positive controls. The target reduction, determined by EPA for previous remediation studies was a 4 log reduction (99.99% inactivation efficiency). F1 and F2 were used for the optimization of the testing conditions (results not shown). Formulations F3 and F4 were the most effective for fumigation of mold-contaminated building materials using an exposure time of 4 hours under a Repti-Sun light @ 9,000 – 10,000 lux. F3 and F4 fumigations showed a ≥ 4-log reduction in CFU for *A.versicolor*, and *S.chartarum* on W, CN and PW. Likewise, a ≥ 4-log reduction in CFU for *A. alternata* was observed on W. A < 4-log reduction in CFU for *A versicolor*, *S.chartarum*, *A.alternata* and *C.globosum* was observed on L and C. Likewise a < 4-log reduction in CFU for *C.globosum* was observed on CN.

Materials and Methods

Mold spores used: *Aspergillus versicolor* (RTI 3843); *Stachybotrys chartarum* (ATCC 201210);*Chaetomium globosum* (ATCC 58948),and *Alternaria alternata* (RTI 3413). Spores were inoculated on each coupon at a concentration of 10^6 – 10^7 spores/coupon. The coupons' preparation and the spiking procedure was performed in accordance with the ASTM guidelines D 6329-98 (2015)

Mold spores were inoculated onto the surface of the six materials: G, L, W, PW, C and CN. Positive control coupons were prepared as the test coupons but were not exposed to the ClO_2. The negative control coupons were not inoculated, but were exposed to ClO_2 with the test coupons. Field blanks were also included. Two types of coupons were prepared – non-vegetative coupons and vegetative coupons. Mold non-vegetative coupons – G, PW, CN - were those where fungal spores were inoculated on the surface of the coupon and fumigated without permitting any growth on the material. Vegetative coupons – W, L, C- were those where the mold spore was inoculated on the surface of the coupon and the coupon was placed in a static chamber for at least 6 weeks to allow active growth to occur on the coupon. Figure 1 and 2 represents the preparation of vegetative wood coupons

Decontamination Tests were performed in a 317 L glove box (Plas Labs, Inc., Lansing, MI). A Repti-Sun light source (9,000 lux) was placed inside the glove box at a distance of 6 cm above the coupons being exposed to PMR

Analyses performed:

Culturability assay**:** inactivation of culturable fungi was quantified on vegetative coupons by comparing the number of colony-forming units (CFU) pre- and post-treatment on the materials coupon. Log change was calculated as follows:

$Log_{10}reduction = Log_{10}CFU_{positive} – Log_{10}CFU_{exposed}$

Where:

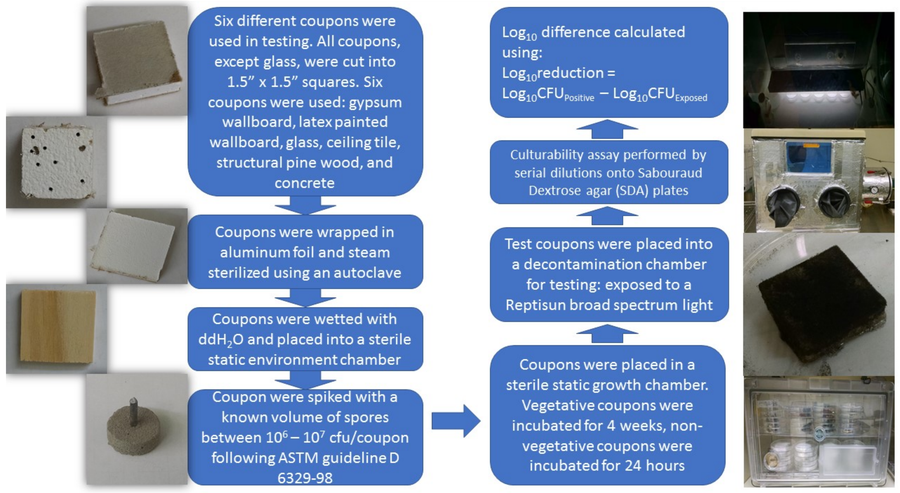
$Log_{10}CFU_C$: mean Log_{10} CFUs of positive controls coupons

$Log_{10}CFU_E$: mean Log_{10} CFUs of exposed coupons

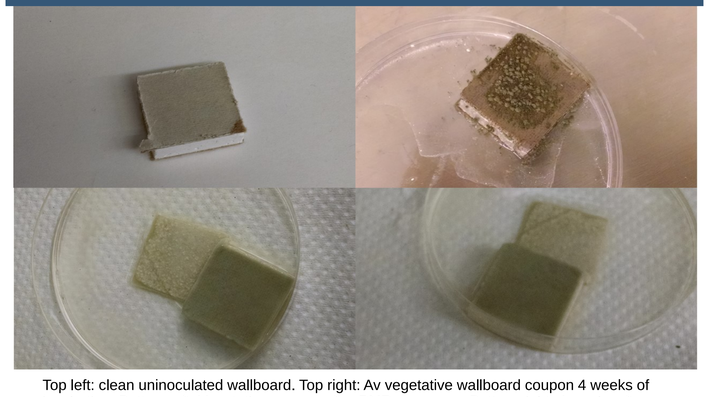
TDA Formulations

Formulation name		
	PMR formulation	
F1	1% sodium chlorite; photo-activator (TDA proprietary); SSDX-12™ (surfactant) and water	
	Part A	Part B-C
F2	Citric and ascorbic acids; water	4% sodium chlorite; photo-activator (TDA proprietary); SSDX-12™ (surfactant product) and water.
F3	Citric acid; SSDX-12	
F4	citric acid and SSDX-12™; polyethylene glycol (PEG) polymer	

Workflow Diagram



Coupon testing cycle



Top left: clean uninoculated wallboard. Top right: Av vegetative wallboard coupon 4 weeks of incubation. Bottom left: Vegetative coupon after PMR exposure. Bottom right: 4 weeks after PMR decontamination test

References

[1] - Betancourt, D. and Serre, S., *Laboratory evaluation of chlorine dioxide fumigation for remediation of building materials contaminated with molds, mycotoxins or allergens*. 2013, US Environmental Protection Agency/ Office of Research and Development/National Homeland Security Research Center, EPA/600/R-13/229.

[2] -*ASTM D 6329-98:Standard guide for developing methodology for evaluating the ability of indoor materials to support microbial growth using static environmental chambers.* 2015: ASTM International (ASTM), West Conshohocken, PA.

Results and Discussion

The efficacy of chlorine dioxide fumigation for the remediation of building materials contaminated with molds has been previously reported. (1) The objective of our study was to ascertain the biocidal efficacy of TDA – PMR chlorine dioxide technology against four mold species - *Alternaria alternata*, *Aspergillus versicolor*, *Chaetomium globosum*, and *Stachybotrys chartarum* – which are frequently isolated from water-damaged buildings. A series of experiments was performed using mold-inoculated coupons of the following building materials: glass slides, gypsum wallboard, latex-painted wallboard, unpainted pine wood, ceiling tile and concrete) as the test coupons. All these represent construction materials allowing us to evaluate as realistically as possible in a controlled laboratory setting the efficacy of PMR as a decontamination approach in the built environment. Glass was chosen as a control material that would give the best-case inactivation results. The building materials have the potential to impede the inactivation of biological contaminants by a remediation technology, whereas glass is a smooth hard surface and is thus less likely to interfere**.**

The inoculated building materials were fumigated with the TDA-PMR formulations F1; F2; F3 and F4. Each formulation was tested individually. The PMR efficacy testing was divided in two phases – Phase 1 and Phase 2. Phase 1 consisted of 6 decontamination tests using F1 and glass coupons inoculated either with *S. chartarum* or *A. versicolor* to optimize testing parameters (results not included). With these tests it was determined the following:

the optimal F1 contact time under the Reptisun light was 3 hrs.

the required light intensity (Reptisun lamp) was 9,000 lux

the coupons surface needed to be re-wetted with 500 ul of the formulation (F1) every hour

Sodium thiosulfate (2%) was stoichiometrically added to the coupon surface after 3 hours of exposure to neutralize the F1 reaction. Neutralization tests were necessary to achieve the exact exposure time in each run and to prevent biocidal or bacteriostatic effects of the formulation during extraction, processing and culturing of the mold spores following the exposure period.

Phase 2 consisted of testing F2, F3 and F4 on inoculated gypsum wallboard, latex-painted wallboard, unpainted pine wood, ceiling tile and concrete coupons using the optimized conditions determined in Phase 1.

Table 1: Log_{10} Reduction (± combined standard error of the mean) of CFUs on all materials

	Wood Mean ± SE		Concrete Mean ± SE		Wallboard Mean ± SE		Latex Painted Wallboard Mean ± SE		Ceiling Tile Mean ± SE	
Formulation	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4
Aspergillus versicolor RTI 3843	4.05±0.07	4.05±0.07	4.39±0.12	4.41±0.19	4.39±0.70	5.09±0.07	< 4.00	< 4.00	< 4.00	< 4.00
Stachybotrys chatarum ATCC 201210	4.28±0.09	4.32±0.08	3.56±0.14*	3.56±0.14*	5.04±0.05	5.04±0.05	< 4.00	< 4.00	< 4.00	< 4.00
Chaetomium globosum ATCC 58948	--	--	< 4.00	< 4.00	--	--	< 4.00	< 4.00	< 4.00	< 4.00
Alternaria alternata RTI 3413	4.77±0.01	4.77±0.01	4.64±0.03	4.64±0.03	4.92±0.18	4.27±0.39	< 4.00	< 4.00	< 4.00	< 4.00

* The minimum detection limit for the test organism varied based on the dilution and amount plated for the coupon. When 0 (zero) counts were detected on all the plates from 1 coupon, 0.5 was used as the number of CFUs detected and used to calculate of the number of CFUs on a coupon. The Log_{10} mean on the Sc unexposed concrete coupons was 6.26. All Sc exposed coupons were 0 counts.

Because adverse health effects differ by organism and susceptibility of the exposure population, no standard acceptable level of contamination exists, nor does any required level of efficacy for decontaminating building materials in the field. Therefore, a key issue in evaluating the efficacy of any biocide, including PMR, is to determine the acceptable number of CFU remaining after treatment. The target reduction, determined by EPA, was a 4 log_{10} reduction (99.99% inactivation efficiency). Therefore, the challenge level was based on being able to quantify a 4 log_{10} reduction in CFU.

Table 1 shows the PMR decontamination tests using F3 and F4. Due to the variable CFU results obtained with F2, TDA Inc. in agreement with the EPA laboratory decided to discontinue testing this formulation. A 4 log_{10} reduction in CFU was seen on gypsum wallboard, concrete and unpainted pine wood for *Alternaria alternata, Aspergillus versicolor*, and *Stachybotrys chartarum*. The target 4 log_{10} reduction in CFU was not attained for the organisms tested in latex painted wallboard and ceiling tile.

Additional testing (data not shown) was done to determine log_{10} reduction 8 weeks after testing. A 4 log_{10} reduction in CFU was maintained on gypsum wallboard, concrete and unpainted pine wood for *Alternaria alternata, Aspergillus versicolor*, and *Stachybotrys chartarum*. It shows that no further regrowth of the biocontaminants could be detected (within the tests' detection limits) after 8 weeks of the decontamination challenge.