

Assessment of Fungal Growth on Sodium Polyborate-Treated Cellulose Insulation

José Herrera

Division of Science, Truman State University, Kirksville, Missouri

Cellulose insulation has rapidly gained a large market share among general contractors and homeowners. Recent interest regarding health effects of high concentrations of fungi within indoor environments (building-related illnesses or sick building syndrome) has promoted concern about susceptibility of building materials, including wood products (in general) and cellulose insulation (specifically), to fungal attack. This study reports an assessment of fungal growth on cellulose insulation made from recycled paper and treated with varying concentrations of sodium polyborate within half-scale wall units exposed to variable and high ambient temperatures and relative humidities throughout the summer. Boron-treated and untreated (control) cellulose insulation within the wall units were challenged with a suspension containing high concentrations of spores of five fungal species commonly found in indoor environments. Our results suggest that cellulose insulation treated with sodium polyborate (a) precludes the growth of the five common fungal species; (b) harbors fewer fungal species before and after being challenged with the fungal spore suspension; and (c) is likely having a cytotoxic or sporocidal effect on many, if not all, fungal species. These results suggest that cellulose insulation treated with sodium polyborate, when properly applied and installed, precludes fungal growth for at least 124 days at high temperatures and relative humidities.

Keywords boron, cellulose insulation, fungi, indoor air quality, mold, sick building syndrome

Address correspondence to: José Herrera, Division of Science, Office MG 3034, Truman State University, Kirksville, MO 63501; e-mail: jherrera@truman.edu.

Cellulose insulation made from recycled paper products and treated with boron compounds has been used for several decades and has provided the home construction industry an environmentally friendly alternative to retard fire and to insulate with high R-values and low thermal conductance, while providing high sound proofing at moderate cost.^(1,2)

Recent concerns about building-related illnesses and symptoms, including those mediated by fungi, have led to increased interest regarding the susceptibility of construction materials to fungal growth.⁽³⁾ In some instances, published reports that cellulose insulation materials are particularly susceptible to fungal

attack (e.g., Hyvarinen et al.)⁽⁴⁾ have been generalized by the public and occupational health workers to mean that treated cellulose insulation also is susceptible to attack by fungi. The confusion about what types of cellulose insulation materials are susceptible to fungal attack comes at a time when the public and the construction industry are particularly sensitive to negative information about fungal growth on construction materials (in general) and cellulose insulation (specifically).

I was approached by a consortium of cellulose insulation manufacturers and borate supplier and asked to assess the ability of four different formulations of boron-treated cellulose insulation to prevent or retard fungal growth in a realistic setting using half-scale mock wall units that were prepared based on industry standards and insulated with cellulose insulation impregnated with different concentrations of sodium polyborate (Boron 10; CAS #183290-63-3), one of the most common borates used in the preparation of boron-treated cellulose insulation. To date, and to our knowledge, the efficacy of boron-treated cellulose insulation to retard or prevent fungal growth has rarely been empirically tested (but see Amburgey).⁽⁵⁾

We hypothesized that the cellulose insulation treated with different formulations of sodium polyborate would contain fewer viable fungal spores and less actively growing fungal mycelia than an untreated control assessed in a similar fashion.

MATERIALS AND METHODS

Products to Be Tested

Five cellulose insulation-based insulation formulations were tested: four prepared with different concentrations of sodium polyborate (1) Fiber-lite, Fiberlite Technologies, Inc., Joplin, Mo.; (2) Wallseal, Nu-Wool Inc., Jenison, Mich.; (3) Thermolok, Hamilton Mfg. Inc., Twin Falls, Idaho; and (4) Pest Control Insulation, InCide Technologies, Inc., Phoenix, Ariz.; and a control that had not been chemically treated. During 2002, manufacturing facilities at InCide Technologies randomly selected 5-kg batches of insulation from each of the four manufacturing facilities (plus one untreated control). These batches were shipped to InCide, where the bags were opened to select random subsamples for independent fungal assessment

(Fiberquant Analytical Services, Phoenix, Ariz.). Subsamples of each of the five batches also were shipped to U.S. Borax Corp. (Valencia, Calif.) to determine percentage boron and sodium (by weight).

Assessment by Fiberquant involved filling standard sterile petri dishes with autoclaved (sterilized) subsamples of borate-treated insulation. Subsamples of two formulations (Nu-Wool and InCide) were soaked and mixed separately with 780 μL of sterile distilled water/g of insulation. However, instead of using untreated cellulose insulation as a control, Fiberquant used a sterilized (autoclaved) wood tongue depressor mounted on a piece of clay and enclosed in a standard petri dish containing and just above 3 mL of sterile distilled water. A total of 10 μL of a suspension containing *Alternaria alternata* spores was deposited on the center of all subsamples, including the tongue depressor. Spore suspensions from four additional fungal species (*Aspergillus flavus*, *Aspergillus niger*, *Stachybotrys chartarum*, or *Cladosporium sphaerospermum*) were used separately to challenge four additional replicate sets of 3 plates (for a total of 15 plates). All plates were parafilm and incubated at 30°C for 28 days. After incubation, each plate was removed and observed under a dissecting microscope using 30 \times magnification. Any emerging colonies were measured and the identity of the fungus confirmed using 1000 \times light microscope. The experiment was repeated three times during 2003.

After the subsamples were shipped to Fiberquant and U.S. Borax, the bags were resealed and forwarded to FiberLite. These five formulations were independently spray-applied into five separate half-scale wall units per industry specifications (Figure 1).⁽⁶⁾ The formulation containing the lowest concentration of sodium polyborate was spray-applied first and the one containing the highest concentration was applied last (sequence: control, Nu-Wool, Fiberlite, Hamilton and InCide).

Construction of Wall Units

Construction materials and construction personnel were supplied by Fiberlite Technologies, Inc., and the construc-

tion of the wall units followed, as much as possible, industry standards. A total of five wall units (1.22 m \times 1.22 m) were partially constructed in Joplin, Mo., and shipped to Truman State University (Kirksville, Mo.), completed, and set up on a concrete slab in the northwest corner of the new outdoor Agricultural Science Laboratory (ASL) building at the University Farm (Adair County, Mo.) on May 25, 2004. The portion of the ASL where the experiment took place was roofed and walled on only three sides, thus protecting the wall units from rain and sun but exposing them to ambient temperature and relative humidity throughout the study period.

Each wall unit consisted of 1.3 cm thick oriented strand board (OSB) framed with standard 2" \times 4" pine boards into three 40.6-cm sections (Figure 1). Prior to the addition of the cellulose insulation, a small (1 cm diameter) hole was drilled in the center portion of the middle panel of the OSB of each of the wall units to allow access for a temperature and relative humidity (RH) probe during the study. While not in use, this access hole was covered with a taped cork. Cellulose insulation was spray-applied with tap water in all three panels per manufacturer's protocol⁽⁶⁾ to an average depth of about 7.6 cm at an average density of 48 kg/m³. The leftmost section was sealed in plastic vapor block and left untreated to conduct additional long-term studies.

The sprayed cellulose insulation was allowed to dry and cure in all units for approximately 24 hours before they were sealed with 1.3-cm, industry-grade gypsum boards and secured with four C-clamps placed at the corners of each wall unit. On May 31, 2004, temperature (T) and relative humidity (RH) were measured within all wall units (through the drilled hole) and 1 m above the floor outside the wall units (ambient). The gypsum board covering the rightmost two sections was removed, and eight moisture readings (Delmhorst BD-10 moisture meter, Towaco, N.J.) were taken on the rightmost two panels (four readings on each panel). Then, before the wall units were challenged with a fungal spore suspension, we collected "prechallenge" (pretest) samples of cellulose insulation from each wall unit.

Collection of Samples

A grid (made of 10 cm \times 10 cm quadrants) marked on the margins of the wall units was used to randomly select six quadrants within the two rightmost panels of each unit. Random coordinates consisted of two numbers selected by a computer-based random number generator (*STATISTICA 5.5*; StatSoft, Inc., 1999). The same random coordinates (locations) on each grid were used for sampling all wall units. The coordinates were re-randomized for each of the seven sampling bouts (sampling dates).

During each sampling bout, ambient T and RH (1 m above floor) and T and RH readings within each unit were taken through the drilled hole in the OSB. Then, the clamps and attending gypsum board were removed to measure moisture (eight readings per wall) and collect insulation samples before the gypsum board and clamps were replaced. Assessment of

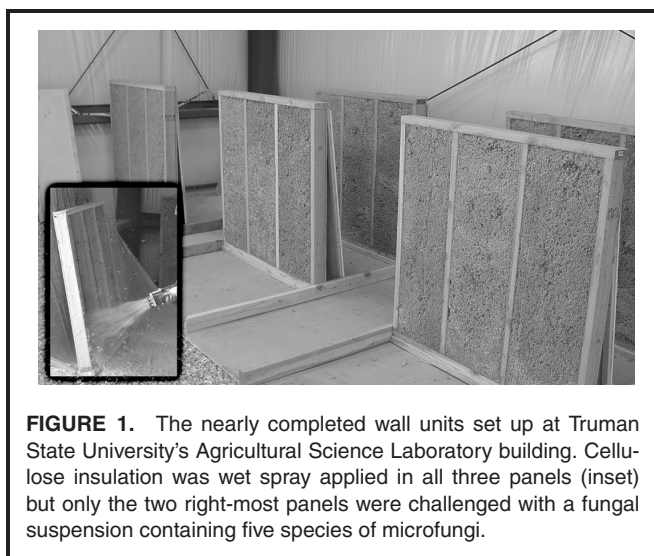


FIGURE 1. The nearly completed wall units set up at Truman State University's Agricultural Science Laboratory building. Cellulose insulation was wet spray applied in all three panels (inset) but only the two right-most panels were challenged with a fungal suspension containing five species of microfungi.

moisture and sampling of the cellulose insulation on each unit generally took less than 5 min.

Collection of insulation samples consisted of taking approximately 0.25 g of insulation material from each of the six randomly selected quadrants with sterile forceps; placing the material into separate, sterile, resealable plastic bags; putting the bags in an iced cooler; transporting them back to Truman State University; and processing the samples within 6 hours.

Fungal Challenge

On May 31, 2004, after the pretest samples were collected, the cellulose insulation in the two rightmost panels of all wall units was challenged with 100 mL of an atomized (aerosol size approximately 10 μ L) spore suspension consisting of about 3000 spores/mL of sterilized distilled water amended with the surfactant, Triton X (approximately 3×10^5 fungal spores in a 1% Triton X solution). The following fungal species and approximate spore concentrations (in spores/mL) were used: *Alternaria alternata* (39 or 1.3% of the total number of spores), *Aspergillus niger* (714 or 23.8%), *Cladosporium cladosporioides* (330 or 11%), *Penicillium chrysogenum* (1737 or 57.9%) and *Stachybotrys chartarum* (72 or 2.4%). All fungal species used in this study were cultured out from the Truman State University mold herbarium.

The identity of the fungal species and the proportion of spores used in this study are modeled after the mean number of airborne mold spores isolated from indoor airspora in apartments from 1998–2002.⁽⁷⁾ Spore stock solutions of individual fungal species were prepared separately and mixed together to make a mixed-species solution that was vortexed and immediately atomized directly onto the surface of the insulation on the two rightmost panels of each wall unit, giving a total spore concentration of 30.27 spores/cm² of cellulose insulation (for comparison, there are usually less than 0.3 spores/cm³ in most indoor environments).⁽⁷⁾

The spore suspension in each wall was allowed to dry for 1 hour before baseline samples were taken (Day 1 of study; sampling protocol described above). Then, each wall unit was resealed with the gypsum board and secured at the margins with the C-clamps. In total, cellulose insulation samples were collected from each of the five wall units on seven occasions: May 31 (pretest), May 31 (Day 1 of study), June 16 (Day 17), June 30 (Day 31), July 30 (Day 61), August 14 (Day 76), and October 2 (Day 124). Sampling was conducted when differences in T and RH between ambient and internal readings within wall units were at their minimum (usually around 11:00 a.m.). In total, we collected 210 samples throughout the study (six samples/formulation/sampling date \times five formulations \times seven sampling dates).

Processing of Samples

Washings

To obtain a more accurate understanding of both the diversity and frequency of fungal species inhabiting the insulation samples, a variation of the washing scheme described by Warcup⁽⁸⁾ was used. This procedure involves washing away ex-

traneous fungal spores and other propagules from the samples and isolating microfungi from cellulose insulation fragments in which those fungi are actively growing.

This process is labor intensive but reduces the possibility of overestimating the number of microfungi that specialize in investing most of their energy in producing many or long-lived spores.⁽⁹⁾ Washing also would increase species richness estimates by increasing the probability of culturing out potentially important nonsporulating or slowly sporulating species actively growing in the insulation. About 0.05 g of each sample was placed separately into a sterile wire mesh cup and washed with a stream of pressurized distilled water and approximately 20 mL of sterile 1% Triton X solution (a detergent designed to wash out extraneous spores) for 5 min. Preliminary experiments revealed that this technique was effective at washing out almost all the extraneous spores while leaving actively growing fungi within cellulose insulation fibers.

Microfungal Assay

After washing the samples, small (approximately 0.5 mm \times 0.5 mm) particles of insulation material were selected using watchman's forceps and imbedded into petri dishes filled with malt extract agar (MEA) containing antibiotics (0.4 g of streptomycin sulfate and 0.2 g of chlortetracycline/L of media) and MEA amended with 8 g of powdered cellulose/L of media (MEA-C; 5 sections/plate). A total of 10 pieces of insulation (5 on each of two plates containing MEA and MEA-C) was assessed per sample. All told, 2100 pieces of insulation were assessed for active fungal growth: 10 pieces/sample/sampling date \times six samples/wall (formulation) \times five walls \times seven sampling dates; constituting a total of 480 plates. Plates containing embedded pieces of insulation material were incubated at 30°C and observed daily for fungal growth for a total of 10 days. I then isolated, identified, and enumerated any microbe arising from the plates using standard, morphological taxonomic techniques.

Microscopic Assessment

To determine if there were any nonculturable microbial growth on the insulation samples, we assessed a total of 420 pieces of cellulose insulation microscopically (two pieces/sample/sampling date \times six samples \times five walls (formulations) \times seven sampling dates). Individual slides containing a drop of lactophenol acid fuchsin, covered with a coverslip were assessed for the presence of microbial cells or mycelia using an epifluorescence, phase-contrast microscope at 100–1000 \times magnification. Presence of bacteria and fungi were confirmed with a high-resolution digital imaging system (CIAS; CID Imaging, Inc., Camas, Wash.).

Statistical Analyses

Overall fungal growth rates for each of the five formulations were arc-sine transformed and compared with each other using an analysis of variance (ANOVA) with formulation as the independent variable, transformed growth rate as the dependent variable, and sampling date as the covariate (Ho: growth rate

for boron-treated samples = growth rate for control samples). When necessary, a Tukey's Honest Significance Difference (HSD) multiple comparison test was used to determine differences among the formulations.⁽¹⁰⁾

RESULTS

Elemental analyses of the cellulose insulation samples revealed that the highest concentration of boron existed in the Hamilton samples (2.45%), followed by Nu-Wool (2.35%), Fiberlite (2.18%), InCide (1.69%), and the untreated control (<0.01). Sodium levels also were highest in Hamilton (2.34%) but were followed by Fiberlite (2.11%), InCide (1.14%), Nu-Wool (0.17%), and control (0.05%). Neither percentage of boron nor sodium in the formulations appeared to be obviously related to fungal growth rates on the cellulose insulation.

Independent assessment of fungal growth on the cellulose insulation or wooden tongue depressors by Fiberquant indicated that none of the 30 boron-treated subsamples (five fungal species × two formulations × three replicate runs) exhibited fungal colonies. Conversely, all but 5 of the 15 (five fungal species × three replicate runs) tongue depressors (control) showed growth of fungal colonies with which they were challenged. Only those tongue depressors challenged with *Alternaria alternata* spores did not exhibit growth on the wood on any of the three replicate runs.

Cellulose insulation within the control wall unit (untreated) was yellowish after the 17th day of the study (June 16) and remained discolored through the rest of the study. No other obvious visual differences existed among the five different formulations.

Cellulose insulation particles embedded on MEA and MEA-C plates during the first three sampling bouts did not exhibit any obvious differences with respect to number of fungi or composition of the fungal community. Consequently, to simplify the comparisons and to increase our sampling size, we opted to pool data obtained from MEA and MEAC plates.

Moisture Measurements

Moisture readings and RH within the walls significantly decreased over time (univariate regression analysis, $F = 214.4$, $df = 278$, $p < 0.0001$; $F = 153.6$, $df = 33$, $p < 0.0001$, respectively; Figure 2).

Fungal Measurements

A total of 1959 cellulose insulation particles were assessed for fungal growth. Of those, 258 (or 13.2%) harbored fungi; most of these (197 or 76%) were isolated from untreated (control) cellulose insulation. Nu-Wool had 21/258 (8.1%), Fiberlite had 20/258 (7.8%); Hamilton had 11/258 (4.3%), and InCide had 9/258 (3.5%). Almost all the boron-treated samples harboring fungi, however, were observed during the first sample less than 12 hours after the insulation had been challenged with fungal spores (Day 1; Figure 3).

Although the fungal challenge increased the percentage growth rate of both control and boron-treated insulation, it was

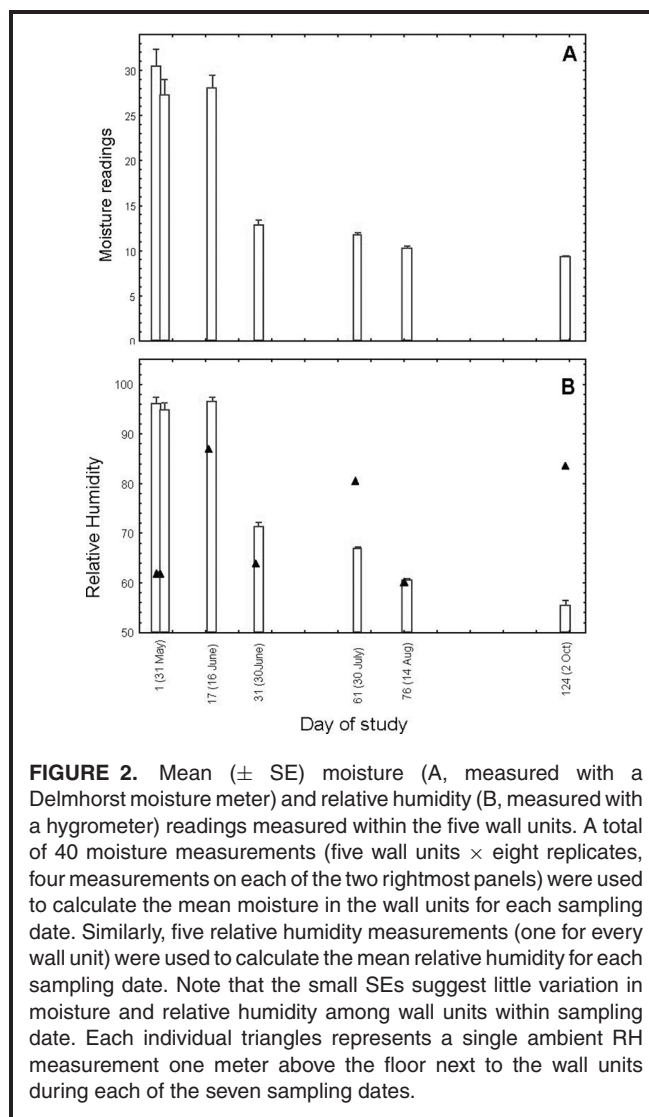


FIGURE 2. Mean (\pm SE) moisture (A, measured with a Delmhorst moisture meter) and relative humidity (B, measured with a hygrometer) readings measured within the five wall units. A total of 40 moisture measurements (five wall units \times eight replicates, four measurements on each of the two rightmost panels) were used to calculate the mean moisture in the wall units for each sampling date. Similarly, five relative humidity measurements (one for every wall unit) were used to calculate the mean relative humidity for each sampling date. Note that the small SEs suggest little variation in moisture and relative humidity among wall units within sampling date. Each individual triangles represents a single ambient RH measurement one meter above the floor next to the wall units during each of the seven sampling dates.

apparent that the untreated samples (control) had significantly higher growth rates during the pretest (Day 0) than the boron-treated samples (Figure 3). Furthermore, control samples were more likely to harbor more than one fungal species. Of the 258 cellulose insulation particles that were exhibiting fungal growth, 36 of them had more than one fungal species. All 36 of these particles originated from control wall units.

In addition, although we sprayed the insulation in our units with only five fungal species, we recovered a total of 23 species of microfungi, almost all of them from the control wall (21 of 23 species found on untreated cellulose insulation). Boron-treated samples contained an average of only 4.0 species ($N = 4$; $SE = 0.71$), but of those four species, an average of 2.5 species (or 63%) were one of the five original (core) species sprayed on the cellulose insulation. Conversely, only 4 of the 21 species found in control particles (19%) were core species. The remaining 17 species accounted for 44% (98/224) of the instances of fungal growth on control particles. The appearance of most of these "non-core" species growing on the particles occurred

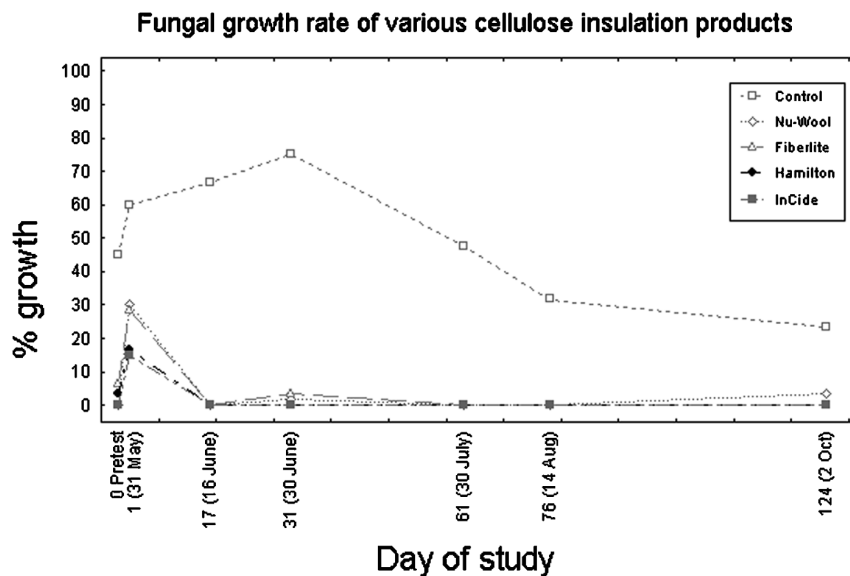


FIGURE 3. Percentage fungal growth on the five formulations of cellulose insulation tested throughout 2004; four of the products were treated with sodium polyborate and one was left untreated (control).

after Day 15 of the study (88.8% or 87/98). Only one of these non-core species (*Fusarium semitectum* complex) was isolated from one cellulose insulation particle on Day 0, and three non-core species (*Epicoccum nigrum*, *Chaetomium globosum*, and *Geotrichum* sp.) isolated from a total of 10 particles on Day 1.

Because there were so few instances of fungal growth on the boron-treated cellulose insulation, we opted to pool all dates of the study and test differences in growth rates among the formulations with a more conservative one-way ANOVA. The ANOVA and a subsequent post hoc Tukey's HSD test

revealed that untreated cellulose insulation (control) samples were much more likely to harbor fungi (ANOVA $F = 33.80$, $df = 4$, $p < 0.0001$), and that all boron-treated groups differed from the control group ($p = 0.00013$) but not from each other ($p > 0.90$).

Although there were too few instances of fungal growth on which to examine the dynamics of the growth process on boron-treated samples, the control samples show a strong relationship between growth rate, moisture content, and RH (Figure 4). That is, as relative humidity and moisture content decreased, growth rate also decreases.

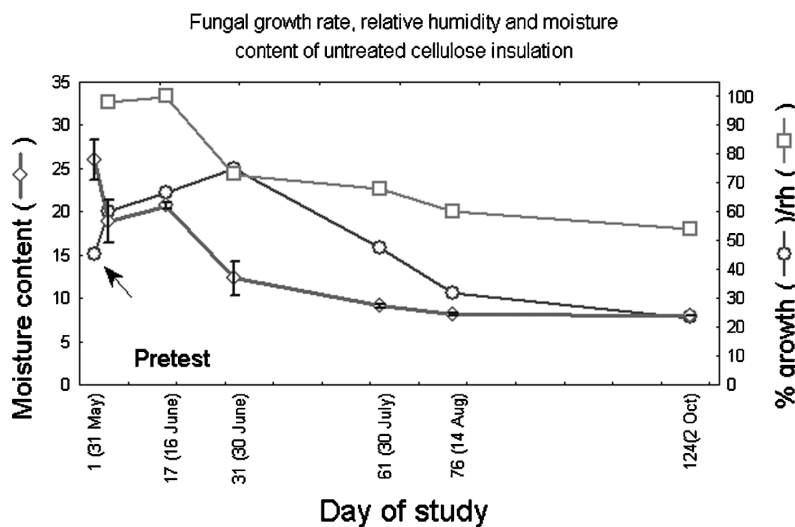


FIGURE 4. Comparison of mean (\pm SE) moisture content (diamonds; measured with a Delmhost moisture meter; $n =$ eight measurements per sampling date), relative humidity (squares; measured with a hygrometer; one measurement per sampling date) and percentage fungal growth (circles) measured on untreated (control) cellulose insulation during 2004.

Microscopic Assessment

The microscopic work suggested that a large number of microbes exist as commensals on all cellulose insulation formulations studied. Almost all (141/210 or 67%) cellulose insulation samples contained viable microbes. Although we were unable to determine conclusively the identity of the viable cells, post hoc analyses with two general types of standard media (tryptic soy and nutrient agar) suggested that these organisms are primarily Gram positive bacteria or inactive microbial propagules that normally inhabit many types of insulation, including fiberglass.^(11,12) Many of these cells in our study appear to be unculturable on standard media, since only a small number of particles yielded colonies within 7 days on standard media (tryptic soy and nutrient agar). The presence of actively growing mycelia discernable under the microscope was rare: only 5 of the 420 samples assessed (1.2%) were observed to contain actively growing mycelia. All of these observations of mycelia were made from control samples, spread sporadically throughout the sampling dates (2 on Day 17; 1 each on Day 0, 31, and 61 of the study).

DISCUSSION AND CONCLUSIONS

Our results clearly indicate that cellulose insulation made from recycled paper and treated with sodium polyborate (CAS #183290-63-3) is unlikely to exhibit fungal growth even when challenged with artificially high concentrations of viable fungal spores. In addition, although the growth rate was statistically higher in the untreated wall unit and we observed some yellowing of the cellulose insulation, we saw no additional obvious visual evidence of fungal growth on the untreated cellulose insulation through Day 124 (last day of study) of sampling. These observations were bolstered by a concurrent (and unpublished) experiment in the laboratory that placed small samples of untreated and boron-treated cellulose insulation in partitioned petri dishes with one section of the dish filled with distilled water (at 100% RH). These samples did not show any obvious visual evidence of fungal growth even after 150 days.

Although there were few apparent visual differences among the cellulose insulation samples sprayed into the wall units, several microbiological differences existed at the start and continued throughout our study:

- Our assessment revealed that untreated cellulose insulation contained about 9–10 times more viable fungi than the boron-treated samples prior to being challenged with our fungal spore suspension (Figure 3). It is likely that the fungal inhibiting properties of the treated cellulose insulation were functioning even before our study began.
- Control samples contained a disproportionate amount of “non-core” fungal species. These species occurred rarely during Days 0 and 1 of the study and were isolated more frequently toward the end of the study. This suggests that (a) non-core species of microfungi likely were introduced

and grew after the start of our study (Day 1), and (b) because almost all fungi isolated during the pretest (Day 0) were members of the core group of five fungal species sprayed on Day 1, we propose that the five core species used in this study accurately model the fungal community that can grow on cellulose insulation.

- Control samples contained more fungal species per sample. That is, cellulose insulation particles were observed to harbor multiple species of fungi on 36 occasions; all were collected from control samples.
- Although, the percentage of fungal growth jumped in both control and treated samples after being challenged with our spore suspension, this increase was transient in the treated samples and remained high (and even increased for the first 30 days) in the control samples.

These differences suggest that although there were no apparent differences obvious to the naked eye with respect to fungal growth between the control and treated cellulose insulation, sodium polyborate appears to have a cytotoxic effect on fungal mycelia and/or has an inhibiting or sporocidal effect on asexual fungal spores (conidia).

Assessment of cellulose insulation samples also showed that as the summer progressed, both moisture and RH readings within all wall units decreased (Figure 2), and this decrease correlated well with a concomitant decrease in the percentage of untreated cellulose insulation particles exhibiting fungal growth (Figure 4) but not ambient RH measurements (Figure 2b). This suggests that water availability has a strong influence on the likelihood that spores and/or mycelia will remain viable and actively grow on the cellulose insulation. Since we did not see any significant fungal growth in the treated cellulose insulation after Day 1 of the study, we can only draw conclusions about untreated cellulose insulation.

Data from direct observations of the treated and untreated cellulose insulation on slides imply that most fungal growth observed on our slides was likely caused by viable and slow growing or dormant fungal spores. This is not surprising since cellulose insulation provides a recalcitrant substrate that is consumed only by a subset of specialized microbial species. Many fungal species observed on our plate samples are likely feeding (and slowly growing) on small amounts of organics trapped within the cellulose insulation (see Ref. 12 for a similar finding with fiberglass). Furthermore, we suspect that since the control cellulose insulation was not treated with sodium polyborate, these fungal propagules were able to remain viable until water activity dropped below some critical value. Had the water activity (indirectly measured by RH and moisture content) remained high, we suspect we would have observed high growth rates (as measured by the plate samples) in our controls. The fact that growth rates decreased in control samples, and the lack of obvious visual fungal growth on the control wall units, confirms that cellulose insulation is a difficult substrate to breakdown and consequently requires high water activities and specialized fungal species adapted to break down and grow on this substrate.

Nevertheless, although our study was not designed to address this, we admit that had the water activities remained high for a longer time, we may have seen more actively growing mycelia on our slides or visible signs of fungal growth on our samples. Moreover, our study would have benefited from: (a) additional and contemporaneous laboratory studies that addressed the degree to which microfungal populations grow on boron-treated or untreated (but sterilized) cellulose insulation in a standardized laboratory growth chamber environment; (b) the addition of more replicate wall units, which would have increased the likelihood to observe within-treatment variation; (c) more frequent or more accurate readings of water activity within the cellulose insulation, which may have given a more accurate or better insight about the fungal community dynamics and the threshold values for fungal growth on the different cellulose insulation formulations; and (d) although most fungi associated with indoor environments grow well in MEA at 30°C,⁽¹³⁾ it is possible that our estimates of fungal diversity may have changed had we grown our samples at different temperatures or in different media.

Because it is likely that sodium polyborate does inhibit the growth or kills fungal cells, the exact biochemical mechanism remains unknown. Based on this study and other post hoc studies carried out in our laboratory, it appears that sodium polyborate may prevent the growth of mycelia, the actively growing and cellulose insulation-decomposing portion of the fungal life cycle. More studies on the nature of the effect of boron compounds on fungal growth should yield additional information on the mechanism(s) that protect boron-treated cellulose insulation (in particular) and wood products (in general).⁽¹⁴⁾

Finally, although small differences in fungal growth rates existed among the four formulations containing sodium polyborate, the post hoc Tukey's test suggested that these differences were not significantly different, and that differences in boron concentrations present in the four treatment formulations may not statistically change the likelihood of fungal growth.

In conclusion, our results suggest that at least over the span of more than 124 days and at high ambient relative humidities and temperatures (in the Midwest), cellulose insulation treated with sodium polyborate restricts the growth of five common species of indoor molds. Based on the diversity of different fungal species introduced on control samples after the study started, it is also likely to inhibit growth of most (if not all) species of mold. Studies have confirmed this finding on wood or wood products using sodium polyborate and other boron compounds.^(14,15)

ACKNOWLEDGMENTS

Undergraduates Melvin Omodon, Niba Nchuto, Christina Gray, and Lindsay Ricketts of Truman State University

ably assisted in the processing of some samples. Thanks are due to Bill Kuntz and the Truman State University farm for allowing us to conduct our summer-long study in the Agricultural Science Laboratory building. Jimmy Story from Missouri Enterprise introduced the opportunity to conduct research on cellulose insulation.

Portions of this study were financially supported by a consortium of cellulose insulation manufacturers headed by In-Cide Technologies, Inc.

REFERENCES

1. **U.S. Army Corps of Engineers, Construction Engineering Research Lab:** *Thermal Comfort Strategies: A Report on Cellulose Insulation* by B.M. Deal, R.J. Nemeth, M. Adams, and L.P. DeBaillie (CERL-TR- 97/22). Champaign, Ill.: Construction Engineering Research Lab, 1996.
2. **Siddiqui, S.A.:** *A Handbook on Cellulose Insulation*. Malabar, Florida: Robert E. Kreiger Publishing Co., Inc., 1989.
3. **Burge, P.S.:** Sick building syndrome. *Occup. Environ. Med.* 61:185-190 (2004).
4. **Hyvarinen, A., T. Meklin, A. Vepsäläinen, and A. Nevalainen:** Fungi and actinobacteria in moisture-damaged building materials: Concentrations and diversity. *Int. Biodeterior. Biodegrad.* 49:27-37 (2002).
5. **Amburgey, T.L.:** The need for co-biocides when treating with borates. In *Proceedings of the First International Conference on Diffusible Preservatives*. Madison, Wisconsin: Forest Products Research Society, 1990. pp. 51-52.
6. **Cellulose Insulation Manufacturers Association (CIMA):** *Standard Practice for the Installation of Sprayed Cellulosic Wall Cavity Insulation* (Technical Bulletin #3). Ypsilanti, Michigan: CIMA, 1996. [Online] Available at http://www.cellulose.org/pdf/cellulose_bulletins/tech_bulletin1.pdf (Accessed February 20, 2005).
7. **Herbarth, O., U. Schlink, A. Muller, and M. Richter:** Spatiotemporal distribution of airborne mold spores in apartments. *Mycol. Res.* 107:1361-1371 (2003).
8. **Warcup, J.H.:** Isolation of fungi from hyphae present in soil. *Nature* 175:953-954 (1955).
9. **Parkinson, D., and D.C. Coleman:** Microbial communities, activity and biomass. *Agric. Ecosyst. Environ.* 34:3-33 (1991).
10. **Zar, J.H.:** *Biostatistical Analysis*. Englewood Cliffs, New Jersey: Prentice Hall, 1984.
11. **Van Loo, J.M., C.A. Robbins, L. Swenson, and B.J. Kelman:** Growth of mold on fiberglass insulation building materials—A review of the literature. *J. Occup. Environ. Hyg.* 1:349-354 (2004).
12. **Ezeonu, I.M., D. Price, S.A. Crow, and D.G. Ahearn:** Effects of extracts of fiberglass insulations on the growth of *Aspergillus fumigatus* and *A. versicolor*. *Mycopathologia* 132:65-69 (1995).
13. **Samson, R.A., E.S. Hoekstra, and J.C. Frisvad:** *Introduction to Food and Airborne Fungi*, Seventh Edition. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures, 2004.
14. **Morrell, J.J., C.M. Sexton, and A.F. Preston:** Effect of moisture content of Douglas fir heartwood on longitudinal diffusion of boron from fused borate rods. *Forest Prod. J.* 40:37-40 (1990).
15. **Laks, P.I., C.G. Park, and D.L. Richter:** Anti-sapstain efficacy of borates against *Aureobasidium pullulans*. *Forest Prod. J.* 43:33-34 (1993).