CHARACTERIZATION OF ENVIRONMENTAL CHAMBERS FOR EVALUATING MICROBIAL GROWTH ON BUILDING MATERIALS [1]

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INTRODUCTION

Indoor air biocontamination can be defined as an accumulation of a variety of biological agents and/or their products in one or more source reservoirs, with the potential to become airborne and induce allergic, infectious, or toxic responses in exposed individuals. Biocontaminants may include, but are not limited to, bacteria, fungi, protozoa, microbial toxins, pollens, dust mites, and insect parts.

In addition to potential human health risks, the biocontamination of indoor spaces may have a marked economic impact due to resultant destruction or disfigurement of materials and surfaces. Furthermore, while the microbes responsible for the destruction may or may not themselves be potential aeroallergens, these organisms may provide the initial damage to a building material that allows known aeroallergens to set up a secondary colonization.

It is estimated that at least 20-25% of "problem" buildings have significant microbial contamination (P. Morey [3]). Suspected contributing factors include inadequate building design and maintenance; poor HVAC (Heating, Ventilation, and Air Conditioning) design, maintenance, and operation; and ineffective environmental control related to temperature, RH (relative humidity), carbon dioxide levels, external

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[3] P. Morey, personal communication with K. Foarde.

contaminant sources, and accumulation of condensed moisture on materials and surfaces.

Laboratory studies on the development of optimal growth media, the isolation of pathogens or fastidious organisms, and the transmission of infectious diseases have shown that many factors affect microbial growth. Some of these factors affect the growth of the organism but they can also affect their survival. Conditions that are unfavorable for growth do not necessarily result in death. Many organisms become dormant -- frequently forming spores. Not all microorganisms grow under the same conditions. Often conditions that are favorable for the growth of one organism may preclude another. In the past, little attention has been given in determining exactly which environmental conditions are essential for the growth of organisms implicated in indoor air quality (IAQ) problems.

Pasanen, et al (1991) conducted laboratory studies where organisms were inoculated into growth media on culture plates and placed in chambers with known humidities. These experiments showed that condensation (surface moisture) was essential to the germination of fungal spores and that, if there was sufficient surface moisture, the organisms could germinate even under conditions of low RH. These conditions are found in sub-arctic climates where winter indoor air RH is very low but, because of temperature gradients, condensation results.

In more humid climates, surface moisture is frequently a function of RH. Hydroscopic materials, such as cotton, leather, and cheese, have been found to be more susceptible to mold growth because they absorb more water, making more water available to the mold. In laboratory studies, a minimum moisture content of 10-14% (depending upon the material) allowed fungal growth (Block 1953). The water absorbing properties of the substrate may play an important role in determining the limiting humidity at which mildew will occur.

[4] Experiments have been conducted to investigate the microbial ecology of indoor spaces. A static chamber method has been developed to study the ability of microorganisms to grow on building materials, wherein the RH, temperature, and light in the chambers are controlled. Experiments were designed to simulate some of the microenvironments encountered in buildings. The three microenvironments these initial experiments mimic are: 1) material moisture in equilibrium with indoor air humidity, 2)

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[4] Under the sponsorship of the U.S. Environmental Protection Agency.

catastrophic material-wetting events (i.e., a flood or leaking pipe) with little ventilation, and 3) catastrophic material-wetting situations where there is some ventilation.

This study is part of a more comprehensive project to investigate potential climate control solutions for buildings. The data presented here are some preliminary results from a series of experiments using the static chambers that will define and delineate the effects of some environmental factors on microbial growth, amplification, dissemination, and survival. The ultimate goal of this program is to assist in the development of engineering guidelines for the prevention, mitigation, and control of biocontaminants in indoor air.

MATERIALS AND METHODS

Static Chambers

Static chambers (32 x 39 x 51 cm) were made by modifying acrylic-walled desiccators (Fisher Sci. 08-647-24). Saturated salt solutions were used to maintain specific RHs (ASTM E 104-85) within each chamber. The chambers were placed in a dark, temperature-controlled (21$\pm$3°C) room. The air in the room is HEPA-filtered (High Efficiency Particulate). A 2-in. fan was placed in the chambers during some of the experiments.

The 5 RH values and the saturated salt solutions used to attain them were:
33% RH - magnesium chloride
54% RH - magnesium nitrate
70% RH - potassium iodide
85% RH - potassium chloride
97% RH - potassium sulfate

Building Materials

All experiments reported in this paper utilized ceiling tile as the test building material. Aged ceiling tile (approximately 10 years old, standard white, textured-face, suspended-ceiling tile) was removed from offices and cut into 3.8 cm squares with a band saw. The pieces of tile were sterilized by autoclaving before inoculation (on the dark side) with microorganisms.

According to manufacturer specifications, the ceiling tile was mineral fiber with a vinyl surface. It contained varying proportions of mineral fiber, glass fiber, gypsum, starch, paper fiber, clay, perlite, silica, styrene acrylic polymer, and phenolic resin.

Moisture Content

The moisture content of the ceiling tile blocks was determined gravimetrically. Ceiling tile blocks were chamber-equilibrated at the test RH for at least 3 days before weighing to obtain the equilibrium moisture weights. Dry weights were obtained after drying for 4 hours at 105°C. The moisture content was computed using the formula:

$$M = [W_b - W_d] / W_d \times 100\%$$

where: M = moisture content, %
W_b = weight of the block, g
W_d = the block weight after drying, g

Test Organism

Literature searches have shown that one of the predominant aeroallergenic indoor air molds isolated from problem buildings is *Penicillium* (Beaumont, et al 1985; Miller, et al 1988). Frequently the species have not been identified. For this initial study, *Penicillium aragonense* was utilized (ATCC # 42228, reported as isolated from air).

Procedure

To study microbial growth at a single RH, chamber-equilibrated ceiling tile blocks were inoculated with approximately 1 X 10^5 colony forming units (CFUs) of *Penicillium aragonense* suspended in 10 µl of water and placed in the static chambers. Duplicate blocks were removed for quantitation on days 3, 8, and 14. Control blocks, inoculated with sterile water and placed in the same chamber, were processed with the inoculated blocks.

The wetted blocks, representing ceiling tiles involved in a flood or other catastrophic event, were evaluated under two conditions: in the first, the air inside the chambers was relatively quiescent (no fan); and in the second, a small fan generated air turbulence and accelerated water evaporation. The wetted ceiling tile (which after

wetting had a moisture content of approximately 40%) were also inoculated with approximately 1 X 10^5 CFUs of *P. aragonense* suspended in 10 µl of water. Again, control blocks were processed with the inoculated blocks on days 3, 7, and 10.

In order to quantify the *P. aragonense* growth, the ceiling tile blocks were removed from the chambers, weighed, and placed in sterile receptacles containing phosphate-buffered saline 0.1% Tween 80. The blocks in buffer were shaken on a wrist-action shaker for 30 min to ensure thorough extraction. Aliquots of block/buffer suspension were diluted and plated on Sabourauds Dextrose Agar. Plates were incubated at room temperature for at least 1 week. Individual colonies (CFUs) were manually counted shortly after visible growth was first observed and again as moderate growth became apparent. Prolonged incubation was necessary to confirm the identification of the mold as *P. aragonense*.

RESULTS AND DISCUSSION

Equilibrium Moisture Determinations

The equilibrium moisture content of blocks that had been chamber-equilibrated for 72 hr is shown in Figure 1. The equilibrium moisture content for the ceiling tiles ranged from a low of 1.6% for blocks placed in the 33% RH chamber, to a high of 5% for blocks equilibrated in the 97% RH chamber.

Microbial Growth on Moisture Equilibrated Ceiling Tiles

Moisture equilibrated blocks simulate the impact that different room RHs would have on ceiling tile. The test blocks were chamber-equilibrated for 3 days in the 33, 54, 70, 85, or 97% RH static chamber. Figure 2 demonstrates that the *P. aragonense* did not significantly multiply on the blocks placed in any of the chambers except the 97% RH chamber. At 97% RH, the CFUs/block increased from 10^5 (the inoculum) to 10^7. While there was no demonstrable increase in the numbers of *P. aragonense* in the 33, 54, 70, and 85% RH chambers, neither was there a decrease.

Wet Substrate with Little Ventilation (no fan)

Ceiling tile blocks were wetted to contain approximately 40% moisture. The blocks were placed in the 33, 54, 70, 85, or 97% RH static chamber. The moisture content data are presented in Table 1 and show that the tiles lost moisture faster in the chambers with lower RHs. As can be seen in Figure 3, for the wetted blocks (no fan test series) some fungal growth did occur at all the RHs tested, with more growth at higher RHs. At 70% and 85% RH, the fungal counts increased more than two orders of magnitude, while at 97% RH the numbers of organisms reached 10^8 CFUs/ block after only 10 days (an increase of almost three orders of magnitude).

Wet Substrate with Some Ventilation (fan)

As in the previous test series, ceiling tile blocks were wetted to contain approximately 40% moisture and the blocks were placed in the 33, 54, 70, 85, or 97% RH static chamber. Since evaporation was facilitated through the use of a small fan in the chambers, the tiles lost the added moisture more quickly (Table 1) than in the chambers with no fans. Blocks in the three lowest RH chambers had reached less than 3% moisture content within 3 days.

As shown in Figure 4, there was actually a decrease in the numbers of organisms in the 33, 54, and 70% RH chambers. However, at 85% RH, the numbers of fungi increased slowly. At 97% RH, the fungal counts increased almost three orders of magnitude in 10 days.

SUMMARY

The initial data indicate that the static chambers may be successfully employed as part of a laboratory method for the evaluation of microbial growth on building materials. These experiments demonstrate the chambers' usefulness, permitting the examination of ceiling tile under known environmental conditions and measuring the effects on the growth of *P. aragonense*.

Moisture content has been shown to be an important factor for the growth *P. aragonense*. Although these are only preliminary data (single temperature, short duration), a comparison of Figures 1 and 4 indicates that, for this type of ceiling tile, a moisture content of approximately 5% will permit the growth of this organism, while a moisture content of 3% will not. For at least this type of ceiling tile, it may be possible to limit or at least slow the growth of *P. aragonense* if the moisture content can be kept below 3%.

In addition, the data from the wetted tiles (fan vs. no fan test series) allow us to postulate that, if this type of ceiling tile was dried out to below 3% moisture within 3 days, microbial growth might be contained. Hopefully, additional tests using the static chambers will permit the definition of a number of parameters for multiple microorganisms and building materials.

It is important to note that tests at low moisture content indicate that, although the total number of organisms have not increased, a considerable number of viable spores can still be isolated. Should environmental conditions change to be more favorable to the *P. aragonense*, the spores may be capable of growth. Experiments are in progress to confirm this hypothesis.

Additional experiments are underway to confirm the data presented here. Evaluation of the ability of building materials, both new and aged, to support microbial growth under differing environmental conditions is continuing. In addition to a variety of materials used in buildings and various environmental conditions, different organisms will be investigated singly as well as in combination, since that is how they occur in nature.

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Table 1. Percent Moisture of Wetted Ceiling Tiles for No Fan and Fan Test Series

DAY	33% RH	54% RH	70% RH	85% RH	97% RH
No Fan					
0	41	44	43	42	42
3	9.5	19	21	30	41
7	2.1	2.6	4.7	17	35
10	2	2.3	2.6	4.9	29
Fan					
0	39	40	40	44	41
3	2.1	2.5	2.8	12	30
7	2	2.4	2.8	3.6	26
10	1.9	2.2	2.8	3.6	19

Figure 1. Mean Moisture Content of Four Ceiling Tile Blocks Equilibrated for 3 Days at Each Relative Humidity

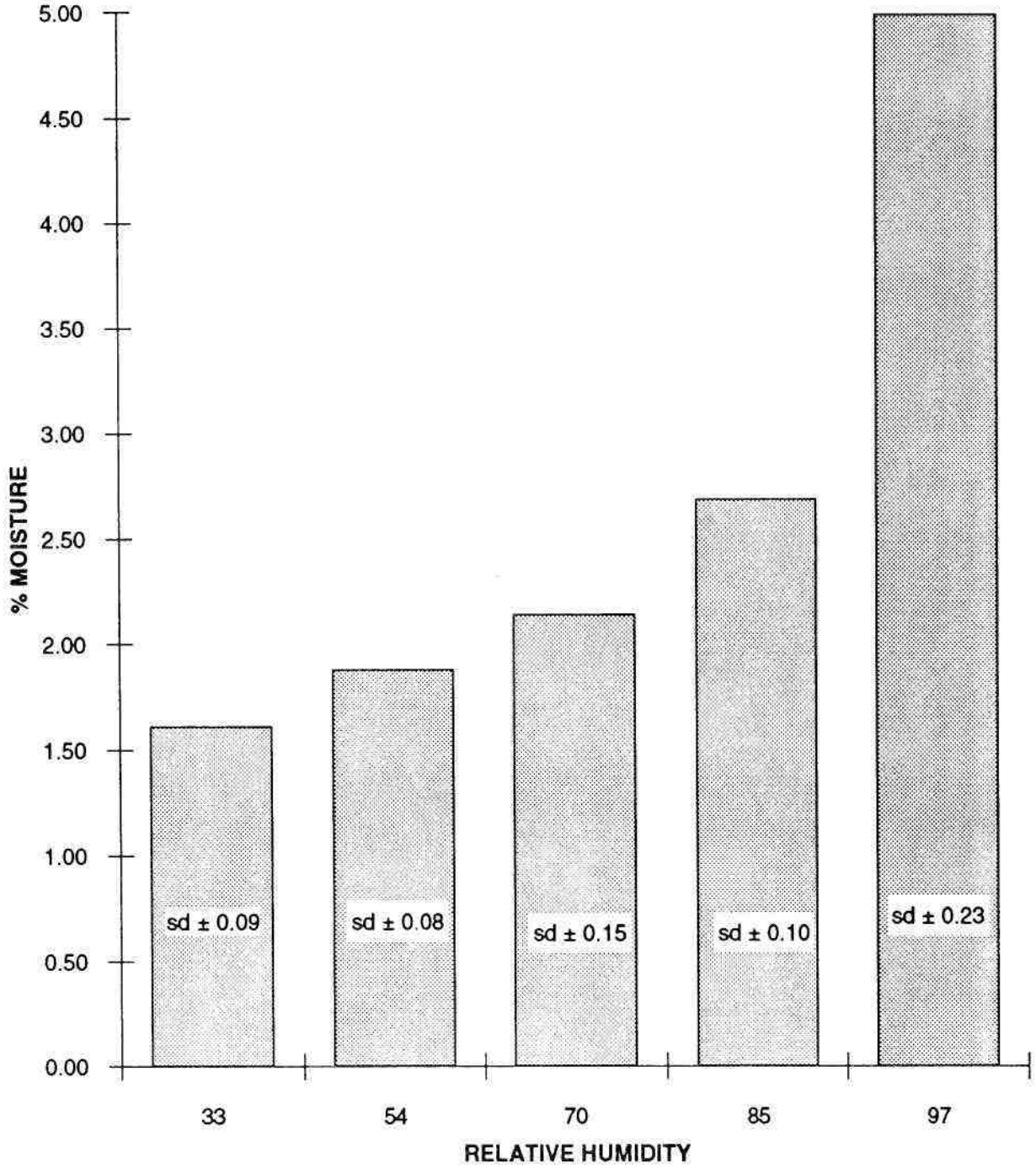

Figure 2. Penicillium Growth on Chamber Equilibrated (3 days) Ceiling Tile Pieces

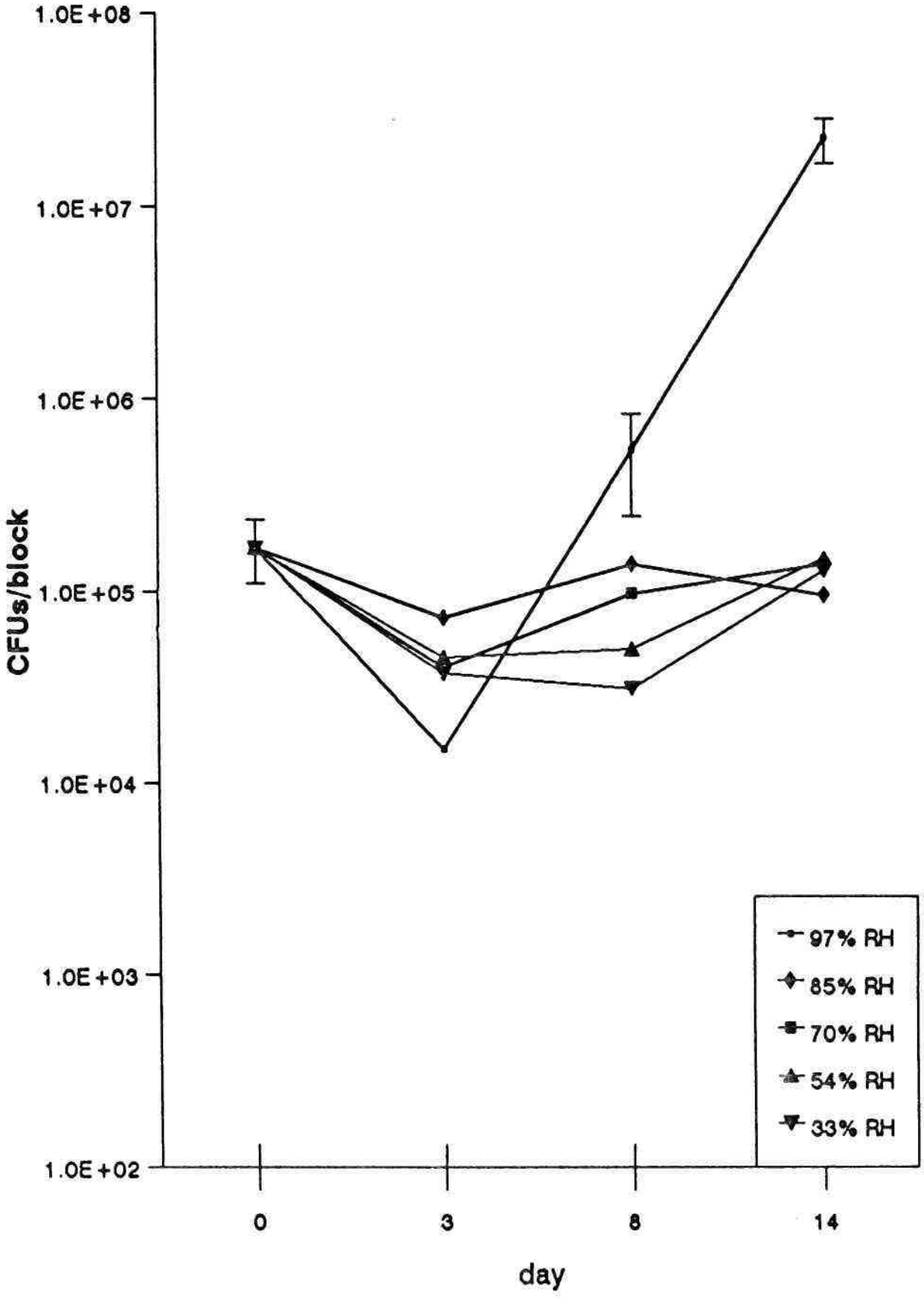

Figure 3. Penicillium Growth on Wetted Blocks (No Fan)

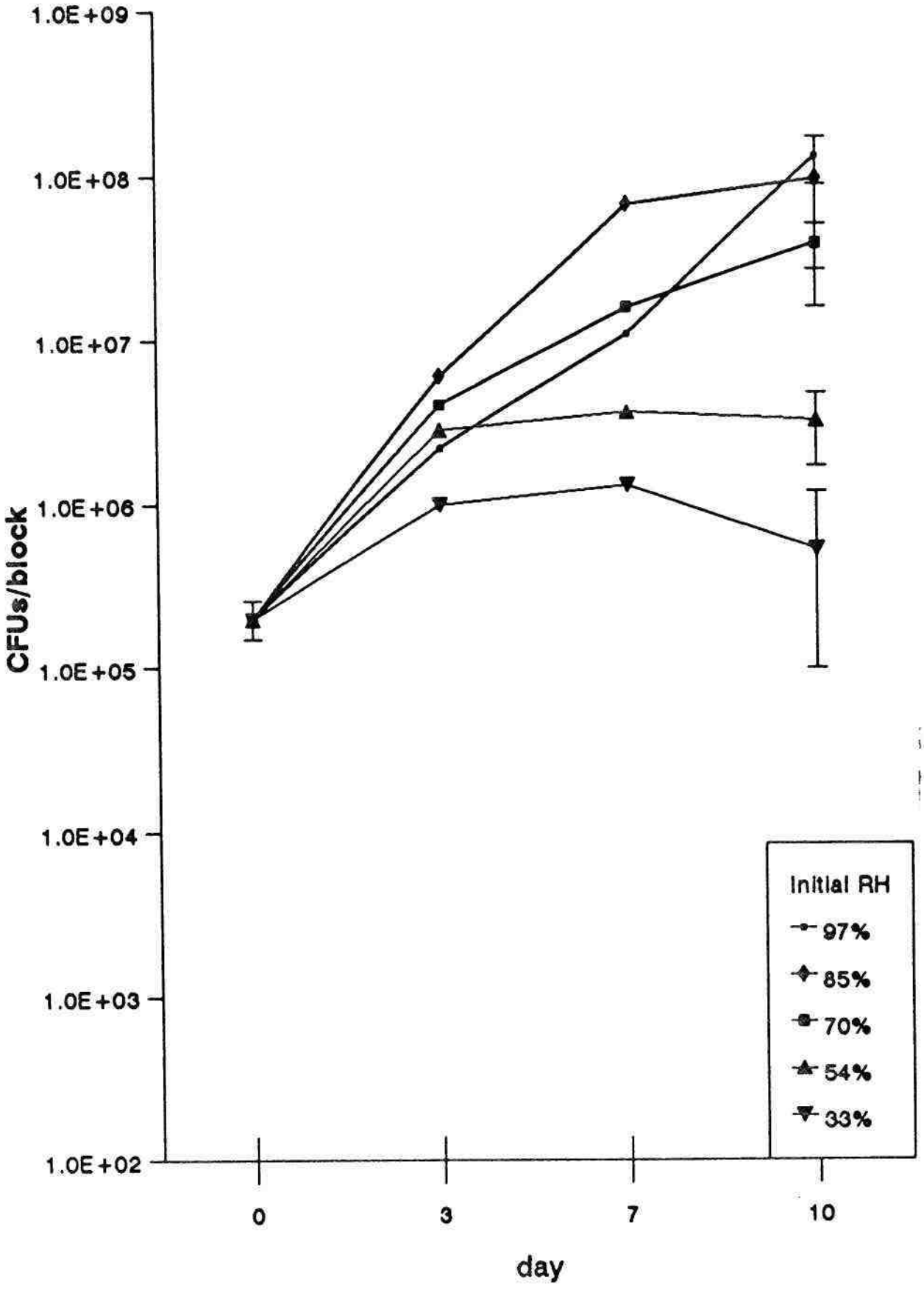

Figure 4. Penicillium Growth on Wetted Blocks (Fan)