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VIABILITY OF FUNGAL AND ACTINOMYCETAL SPORES AFTER MICROWAVE RADIATION OF BUILDING MATERIALS

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Abstract: The effects of microwave radiation on viability of fungal and actinomycetal spores growing on agar (medium optimal for growth) as well as on wooden panel and drywall (common building construction/finishing materials) were studied. All materials were incubated at high (97-99%) and low (32-33%) relative humidity to mimic "wet" and "dry" environmental conditions. Two microwave power densities (10 and 60 mW/cm^2) and three times of exposure (5, 30, and 60 min) were tested to find the most effective parameters of radiation which could be applied to non-invasive reduction or cleaning of building materials from microbial contaminants. Additionally, a control of the surface temperature during the experiments allowed differentiation between thermal and microwave effect of such radiation. The results showed that the viability of studied microorganisms differed depending on their strains, growth conditions, power density of microwave radiation, time of exposure, and varied according to the applied combination of the two latter elements. The effect of radiation resulting in a decrease of spore viability on "wet" wooden panel and drywall was generally observed at 60 min exposure. Shorter exposure times decreased the viability of fungal spores only, while in actinomycetes colonizing the studied building materials, such radiation caused an opposite (supporting growth) effect.

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INTRODUCTION

Microwave surface treatment technique consists of converting electromagnetic field energy within the range of microwave radiation frequencies (2.5 MHz–300 GHz) into a thermal energy targeted at an exposed environment. Such a technique offers a convenient modeling of the area exposed to microwaves, is relatively easy to use and is a non-invasive method (no destruction of the induced material) enabling simultaneous desiccation and sterilization of building materials. In construction engineering, microwave generators are usually used in wall, ceiling and floor desiccation tasks at two frequencies – 2.5 MHz or 2.5 GHz, and have a power output from 800 W to several

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kW [56]. From the safety point of view, an application of microwave radiation requires a proper orientation of the electromagnetic field and a control of temperature in exposed building material. Taking into account the efficiency of such radiation, i.e., a possible 2.5 m penetration into the exposed building material, the microwaves should be capable of inactivating microbial contaminants not only on the exposed surfaces but inside them as well. It can result in reducing or stopping colonization of microbiologically contaminated surfaces and, thus, can decrease the number of agents contributing to the adverse health effects in the indoor environments.

The effects of microwave radiation on microorganisms as a physical phenomenon are still not fully explained. The microwave sterilization effectiveness has been well established by numerous studies [5, 18, 33, 54, 68, 78, 88, 94, 96, 99, 113]. The exact nature of the sterilization effect, however, has been a matter of controversy for decades [2, 10, 62, 63, 86, 92, 106, 107]. It is not clear whether the inactivation is due solely to the thermal effects (i.e., to the heat generated by adsorption of microwave energy by the water medium, or by organic complex systems characterized by a permanent or induced polarization – the heat is generated by the friction of dipole molecules due to oscillating electromagnetic field), or to the microwave non-thermal effect (i.e., direct energy transfer from the electromagnetic field to the vibrational modes of macromolecules altering their conformation, e.g., an induction of DNA covalent bond breakage by microwaves). The experiments carried out generally fall into two categories: with controlled temperature and with a "dry" medium. The controlled temperature experiments performed with bacterial vegetative cells (e.g., *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sofia*, *Salmonella enteritidis*, *Listeria* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*) [4, 14, 25, 30, 33, 45, 51, 59, 60, 62, 69, 87, 108, 113], bacterial spores (e.g., *Bacillus subtilis*, *Bacillus stearothermophilus*, *Clostridium sporogenes*) [83, 99, 111], and yeasts (*Candida albicans*, *Saccharomyces cerevisiae*) [7, 18, 32, 109] revealed no additional lethality caused by microwaves that could not be accounted for by conventional heating itself [31, 108, 112]. All these experiments were carried out using bacterial suspensions or moist yeast colonies, i.e., when water (or moisture) was freely available in the experimental medium. The experiments with a "dry" medium showed that for the cells exposed to microwaves, the killing effect was significantly decreased (mainly due to the lack of a component enabling transfer of microwave energy into heat), or was not present. In the dry environment, biocidal effects usually appeared if the time of exposure to microwave radiation was sufficiently long [51, 108]. The lack of thermal effect was observed by Carroll and Lopez [8], Culkin and Fung [14], Shin and Pyun [98], Kozempel *et al.* [64] as well as by Hadjiloucas *et al.* [44]. The destruction of microorganisms by microwaves at a temperature lower than the thermal destruction point [15, 19, 58, 60, 64] and even statistically significant enhanced the growth rate of *Saccharomyces cerevisiae* yeast cells on dry media exposed to a microwave radiation of 200-350 GHz [44] have also been observed.

As can be seen, the majority of the experiments were conducted on bacteria and yeasts. So far, the studies on the effects of microwaves on fungal spores have been very limited and their conclusions are contradictory regarding the influence of such radiation on spore viability [10, 17, 50, 79]. To the best of our knowledge, no such data are available for actinomycetes. It is well known that biological agents in indoor environments are most dangerous in the airborne state, as bioaerosols [1, 6, 13, 23, 57, 65, 75, 76, 95, 102]. Fungi and actinomycetes, which have the ability to grow on surfaces producing aerial mycelium with spores, are capable of colonizing the surface of building materials [29, 37-39, 49, 90, 110, 114], thus becoming a source of emission of immunologically reactive propagules into the air [39, 93] and causing a variety of health effects ranging from allergic reactions (sensitization and immune responses, i.e., asthma, allergic rhinitis, allergic alveolitis or hypersensitivity pneumonitis), to infections (growth of the fungus in or on the body, e.g., aspergillosis), toxic responses (mainly connected with the secondary fungal metabolites, i.e., mycotoxins, or fungal cell wall components) to nonspecific reactions described as "sick building syndrome" (headache, eye, nose, throat irritation, fatigue, etc.) [1, 3, 12, 21-23, 27, 40, 48, 53, 57, 70, 72, 73, 80, 81, 91, 100-104].

Therefore, the main goal of this study was to test the effects of microwave radiation on the viability of fungal (*Aspergillus versicolor* and *Penicillium brevicompactum*) and actinomycetal (*Thermoactinomyces vulgaris* and *Streptomyces albus*) spores growing on three different "wet" and "dry" surfaces, i.e., on agar representing the medium optimal for the growth of microorganisms, on wooden panel and drywall, which are common materials used in building construction and/or finishing. Two microwave power densities (10 and 60 mW/cm^2) and three times of exposure (5, 30, and 60 min) at microwave frequencies of 2450 MHz were tested in the study to find the most effective parameters of radiation which could be applied to non-invasive reduction of colonization or cleaning of building materials from microbial contaminants, e.g., after environmental disasters such as flood or inundation. The proposed study aimed to answer the following questions: how will the viability of tested fungal and actinomycetal spores be changed after exposure to microwaves, to what degree does the microwave radiation sterilize fungal and actinomycetal spores growing on building materials, and what is the influence of microwave effect compared to thermal effect of such radiation on viability of microbial spores? While the investigations of the influence of microwave on fungal spores have been performed before in different experimental settings, the experiments with actinomycetal spores are a novelty in the field.

MATERIALS AND METHODS

Tested materials. Three different materials were tested in this study: agar, wooden (pine wainscot) panel, and drywall. The Petri dishes were filled with proper agar nutrient medium, i.e., malt extract agar (MEA) (Emapol, Gdańsk, Poland) for cultivation of fungi; ISP Medium 2 agar (Difco/Becton-Dickinson Microbiology Systems, Sparks, MD, USA) for cultivation of *Streptomyces albus*; and half-strength trypticase soy agar (Emapol, Gdańsk, Poland) for cultivation of *Thermoactinomyces vulgaris*. Drywall and pine wainscot panel samples had the same round shape and the same dimensions as the plastic Petri dish: active surface (which was inoculated) 59.42 cm^2, height 1.4 cm, and diameter 8.7 cm. All three tested materials were sterilized before being prepared for the experiments. The agar plates were prepared according to the microbiological procedure recommended by the media manufacturers. Precut pieces of drywall and wooden panel were sterilized by dry heat at 150°C for 90 min After sterilization, the agar plates, drywall, and wainscot panel samples were inoculated with specific fungal and actinomycetal strains.

Fungal and actinomycetal species. Four microbial species were selected for the tests: fungi *Aspergillus versicolor* and *Penicillium brevicompactum* as well as actinomycetes *Streptomyces albus* and *Thermoactinomyces vulgaris*. Fungi had previously been isolated from contaminated building materials for our earlier studies [38, 39, 97]. These two fungal genera were chosen because they commonly occur indoors in various climate zones worldwide [9, 26, 35, 36, 48, 57, 66, 71, 89, 91]. Both of them represent a group of xerophilic fungi, i.e., they are able to grow when water activity is below 0.80 [28, 41, 49, 95, 114]. They can easily grow on different building materials utilizing the organic matter of substratum [38, 39, 90] and, hence, they are counted as first invading moulds (primary colonizers) [28, 41]. The selected actinomycetes have previously been isolated from the air of Upper Silesian dwellings [34]. Both are characterized by their resistance to heat and have an ability to grow at a temperature of 45°C (*S. albus*) and 55-60°C (*T. vulgaris*). The spores of *T. vulgaris* may survive 30 min heating at 90°C without any damage of their biological structure [47]. In the proposed project, where the control and monitoring of the temperature was a crucial factor, the heat resistance played an important role. *S. albus* has the ability to grow well on building materials [37] and is considered to be an indicator organism of indoor microbial contamination [84, 95]. Both actinomycetes species selected for this project are important biological agents with respect to health hazards and have strong allergenic properties [11, 23, 24, 46, 47, 65, 67, 74, 82, 102].

Growth conditions. Before the experiments, pure cultures of microorganisms were stored on an agar nutrient medium, suitable for each specific strain (see "Tested materials"). Before inoculation of tested materials, four selected microbial strains were grown first: fungi – on MEA plates at room temperature for 7 days, *S. albus* – on ISP Medium 2 agar at 37°C for 14 days, *T. vulgaris* – on half-strength trypticase soy agar at 55°C for 14 days. The growth of *T. vulgaris* was held at a relative humidity of 60-80% to protect the agar medium against drying. Fungal and actinomycetal spore suspensions were prepared by washing microbial colonies from the agar plates using deionized and sterilized water (Fresenius Kabi Poland Sp. z o. o., Warsaw, Poland). The spore concentrations in the initial water suspensions were checked using a bright line hemacytometer counting chamber (Blaubrand®, Brand GMBH + CO KG, Wertheim, Germany) and the concentrations were adjusted to 10^6-10^7 spores per 1 ml (see Tab. 1). The surfaces of tested materials were inoculated with 0.2 ml (for agar plates) and 5 ml (for drywall and wooden panel samples) of microbial spore suspensions. After inoculation, all samples were incubated in separate (for each material and microorganism) chambers at a room temperature and at a relative humidity of 97-99%. This humidity was achieved by placing a saturated K_2SO_4 solution (150 g per liter) (POCH, Gliwice, Poland) at the bottom of the incubation chamber [42, 61]. The agar plates were incubated in the above-mentioned conditions for 14 days, drywall samples for 31 days, and wainscot panel samples for 8 months, which resulted in abundant fungal and actinomycetal growth on all tested surfaces [37-39, 114]. During the entire incubation process, temperature and humidity in the chambers were monitored by a thermo-hygrometer (model 06917, Termometerfabriken Viking AB, Eskilstuna, Sweden). After incubation, all samples were divided into two groups. Half of the samples ("wet" samples) were incubated in the same chambers with a saturated K_2SO_4 solution to preserve the same humidity conditions. The second half ("dry" samples) were moved into the new chambers containing a hydrated $MgCl_2$ solution (3000 g/l) (POCH, Gliwice, Poland) [61], and were conditioned at room temperature for decreasing the tested material humidity to the level typical for dry materials, i.e., 32-33% [28]. The chambers with $MgCl_2$ solution were placed inside a class II biosafety cabinet (Aura 2000 MAC, bio air s.c.r.l., Opera, Italy) to prevent contamination. The conditioning was carried out until the agar surface became no thinner than 1 mm, or the humidity level of drywall and wainscot panel samples reached a value equal to that in the chambers with $MgCl_2$ solution.

After incubation, at least two samples of each investigated material were used for testing the initial spore concentrations. A 2 cm^2 piece of the contaminated material was cut using a sterile scalpel and suspended in 25 ml of deionized and sterilized water in a centrifugal test tube. The spores were then extracted from the material by 10 min vortexing using programmable rotator-mixer (Multi RS-60, Biosan, Riga, Latvia). Their concentrations in the resulting suspension were examined using a bright line hemacytometer counting chamber. Because an inactivating effect of

microwave radiation on tested spores was expected, the fungal and actinomycetal spore concentrations were similar to the other biological indicators routinely used in sterilization control experiments (see Tab. 1), i.e., about 10^6-10^7 spores obtained from 1 cm^2 of inoculated material [52].

Microwave generator and radiation parameters. The schematic overview of the experimental setup is presented in Figure 1. To build the system, a microwave generator emitting radiation at a frequency of 2,450 MHz with a power of 1,300 W was used (MBO MW, produced for Le Cygne Sportif, Praha, Czech Republic). Such parameters are typical for microwave radiators being used to desiccate dry walls and studies revealed an inactivating effect on bacterial spores [56, 83]. Two power densities of 10 and 60 mW/cm^2 [10, 16, 79] and three times of exposure – 5, 30 and 60 min [68, 79, 83, 85] were applied. The desired power densities were achieved by varying the distance between the growth materials and the generator. The distance was calibrated using the microwave meter (Microwave Survey Meter 1500, Holaday Industries, Inc., Hopkins, MN, USA) to achieve the two desired power densities evenly spread above whole tested surfaces. The microwave system was shielded to prevent exposure to people conducting the experiments. During the experiments, the emission of microwave radiation was controlled to prevent the increase of temperature above 30°C for *P. brevicompactum* samples, 40°C for *A. versicolor* samples, 45°C for *S. albus*, and 90°C for *T. vulgaris* samples, i.e., within the borders in which particular spores can retain their abilities to grow and develop [41, 47, 95, 105]. Moreover, a precise control of the temperature allowed observing the non-thermal effect of microwave radiation on spores. The control of temperature was achieved using a non-contact infrared thermometer with 1°C accuracy (Raynger ST20 Pro, Raytek, Santa Cruz, CA, USA).

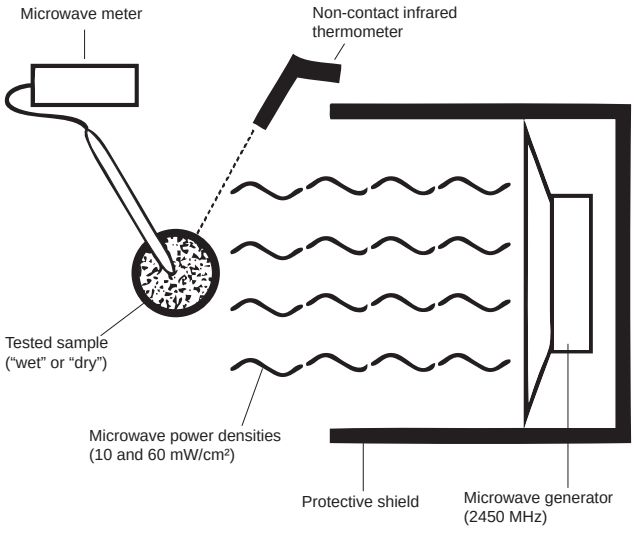

Figure 1. A schematic diagram of the experimental setup.

Viability testing. For each "dry" and "wet" sample, for each microorganism, the viability of spores was tested before and after exposure to microwave radiation. A 2 cm^2 piece of the contaminated material was cut using a sterile scalpel and was treated according to the procedure applied in evaluation of efficiency of the incubation process (see paragraph "Growth conditions"). From each of the received fungal and actinomycetal suspensions, the serial dilutions in deionized sterile water were prepared and each of these samples was cultivated on an agar media suitable for specific fungi or actinomycetes. The temperature and time of

Table 1. Average concentration of microbial spores in suspensions used for inoculation of tested material surfaces and recovered from the "wet" and "dry" samples before and after microwave experiments.

Microorganism	Material	Average concentration (spores/ml)				
		Initial suspension for inoculation	After cultivation			
			Before experiments		After experiments	
			"Dry" sample	„Wet" sample	"Dry" sample	"Wet" sample
A. versicolor	Agar	3.4×10^6	2.4×10^7	2.2×10^7	2.7×10^7	1.9×10^7
	Wooden panel	6.0×10^6	7.3×10^5	4.0×10^6	5.3×10^6	5.0×10^6
	Drywall		8.7×10^6	1.4×10^6	5.1×10^6	7.2×10^6
P. brevicompactum	Agar	2.8×10^6	7.0×10^6	3.3×10^7	8.9×10^6	2.7×10^7
	Wooden panel	9.2×10^6	3.8×10^6	2.1×10^6	1.4×10^6	2.1×10^6
	Drywall		6.3×10^6	5.1×10^6	1.3×10^6	3.5×10^6
T. vulgaris	Agar	2.0×10^7	1.3×10^6	1.3×10^8	3.2×10^7	8.5×10^7
	Wooden panel	1.1×10^7	3.8×10^6	6.2×10^7	4.2×10^6	4.8×10^6
	Drywall		5.3×10^6	2.9×10^7	2.1×10^6	3.1×10^6
S. albus	Agar	2.7×10^7	5.9×10^7	6.4×10^7	5.5×10^7	8.8×10^7
	Wooden panel	3.9×10^7	3.7×10^7	3.5×10^7	6.3×10^6	5.7×10^6
	Drywall		4.6×10^7	2.2×10^7	3.1×10^6	2.4×10^6

incubation were adjusted to the specific microbial strain. After incubation, the number of colony forming units (cfu) on agar plates was counted and the comparison of cfu values for samples before and after exposure to specific microwave radiation parameters were performed. The difference in cfu numbers before and after radiation experiments were tested to determine the inactivating effect of microwave radiation on tested fungal or actinomycetal spores.

Data analysis. The data were analyzed using analysis of variance (ANOVA) followed by Scheffe's test, as well as t-test and correlation analysis using Statistica (data analysis software system) version 7.1 – 2006 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

After agar, wooden panel, and drywall samples were incubated, the microbial spore concentration was checked in duplicates by cutting 2 cm^2 pieces of each of the investigated materials and suspending them in sterile distilled water. After extraction, their concentrations were subsequently counted using hemocytometer and the obtained results of this analysis are presented in Table 1. The number of spores developed on investigated materials were between 7.3×10^5 and 2.7×10^7 spores per ml for *A. versicolor*, 1.3×10^6 and 3.3×10^7 spores per ml for *P. brevicompactum*, 1.3×10^6 and 1.3×10^8 spores per ml for *T. vulgaris*, and between 2.4×10^6 and 8.8×10^7 spores per ml for *S. albus*. Such a high number of spores obtained from 1 cm^2 of inoculated materials, confirming successful recovery after cultivation conditions, was in accordance with our expectations.

The temperatures of "wet" and "dry" sample materials before and after microwave experiments are presented in Table 2. The performed tests with the two desired power densities of 10 and 60 mW/cm^2, combined with three exposure times of 5, 30 and 60 min, showed that the registered temperature of both tested surfaces after microwave radiation did not exceed the levels which can destructively influence the growth of investigated microorganisms. For agar, wooden, and drywall panels, the average increase in temperature of the "wet" and "dry" surfaces after microwave experiments compared to the conditions before the radiation of the samples were 2.1°C and 1.9°C, 4.3°C and 4.1°C, as well as 3.5°C and 4.1°C, respectively. Such a small elevation of material temperature confirmed the theoretical assumption (see "Materials and Methods") that the investigated combination of microwave power densities and exposure times did not cause destruction of the studied surfaces, and that the tested parameters can be applied to an inactivation and/or destruction of microbial contaminants on building materials. The correlations between the number of viable spores on tested materials and surface temperature were not statistically significant ($p>0.05$), hence, the increase in contaminated material temperature enabled differentiation between thermal and microwave effects of radiation.

Figures 2-5 present the results of viability testing for *A. versicolor*, *P. brevicompactum*, *T. vulgaris*, and *S. albus* "wet" and "dry" spores growing on agar, wooden panel, and drywall samples before (control) and after microwave radiation experiments. The obtained results showed that the effect of microware radiation on the viability of studied microorganisms depended on the following: strain of the microorganisms, growth conditions, power density of microwave radiation, time of exposure. The effect was observed to vary depending on the applied combination of these two latter elements. For a specific microbial strain, our results revealed the following:

Table 2. Temperature of the sample materials before and after microwave experiments performed with "wet" and dry" samples.

Microorganism	Material	Temperature (ºC)							
		"Wet" sample				"Dry" sample			
		Before		After		Before		After	
		Average	SD	Average	SD	Average	SD	Average	SD
A. versicolor	Agar	18.6	2.1	23.1	4.5	26.9	2.3	28.9	3.6
	Wooden panel	18.9	0.7	21.6	1.9	19.1	1.0	22.3	1.3
	Drywall	23.0	0.6	27.1	2.9	23.8	0.3	27.1	2.1
P. brevicompactum	Agar	23.7	1.6	26.1	3.0	24.8	1.8	27.6	2.7
	Wooden panel	21.6	1.1	27.6	5.0	20.5	0.8	25.3	2.1
	Drywall	22.5	0.4	26.1	2.0	24.2	0.3	29.2	3.2
T. vulgaris	Agar	24.8	0.5	25.3	1.9	26.2	0.5	26.5	1.3
	Wooden panel	23.8	0.8	28.2	2.5	24.9	0.5	29.7	3.4
	Drywall	24.1	0.3	26.9	2.1	23.3	0.7	26.7	1.9
S. albus	Agar	26.5	0.4	27.5	2.5	24.7	0.3	27.2	2.6
	Wooden panel	23.6	1.4	27.6	4.4	23.8	1.2	27.4	4.6
	Drywall	26.1	2.1	29.4	5.2	23.2	0.8	27.9	5.4

SD – standard deviation

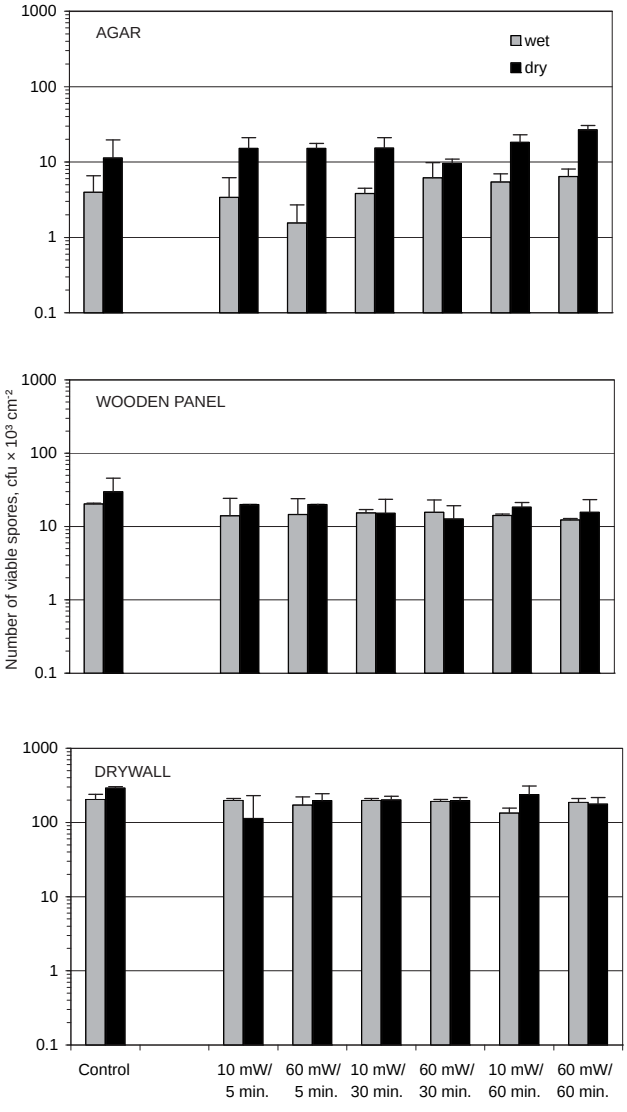

Figure 2. Viability (as a colony forming units, cfu) of *Aspergillus versicolor* "wet" and "dry" spores growing on agar, wooden panel, and drywall samples before and after microwave radiation. The error bars represent standard deviation of 18 and 3 repeats for the samples before and after microwave experiments, respectively.

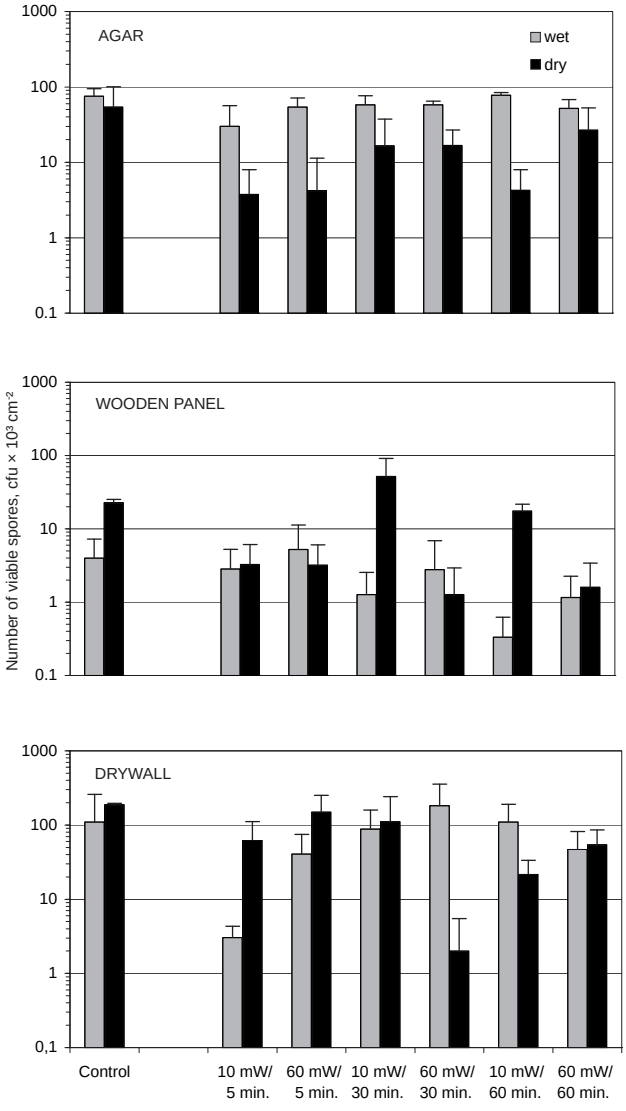

Figure 3. Viability (as a colony forming units, cfu) of *Penicillium brevicompactum* "wet" and "dry" spores growing on agar, wooden panel, and drywall samples before and after microwave radiation. The error bars represent standard deviation of 18 and 3 repeats for the samples before and after microwave experiments, respectively.

for *A. versicolor* (Fig. 2):

a) differences in viability of spores among studied materials were observed for "wet" and "dry" samples both before and after microwave experiments (in both cases ANOVA: $p<0.000001$). While viability on agar and wooden panel samples was similar to each other, viability of spores on drywall samples were significantly higher for "wet" and "dry" surfaces (Scheffe test: $p<0.05$). Evaluating apparent differences in spore viability for "wet" and "dry" samples, it is necessary to consider different incubation times for investigated materials after their inoculation with a specific fungal strain (see "Materials and Methods"). Nevertheless, it should be stated that *A. versicolor* growing on drywall in favourable conditions was able to produce within a 1 month period from 1.7×10^2 to 3.2×10^5 viable spores on the surface of 1 cm^2. With such growth, *A. versicolor* can colonize a wide area of contaminated surface, delivering to the environment a substantial number of immunologically reactive particles [38, 39];

b) viability of spores on agar: in "dry" samples (independently on applied microwave power density in the experiments) viability of spores significantly decreased ($p<0.05$) after exposure. For "wet" samples, such a difference was not statistically significant ($p>0.05$). For these spores present on agar, it is possible that an insufficient amount of water in a medium, which can convert microwave energy into thermal energy, created a situation in which the "microwave effect" of radiation had a more substantial influence on spore viability than simple heat transfer. In terms of time factor, the performed

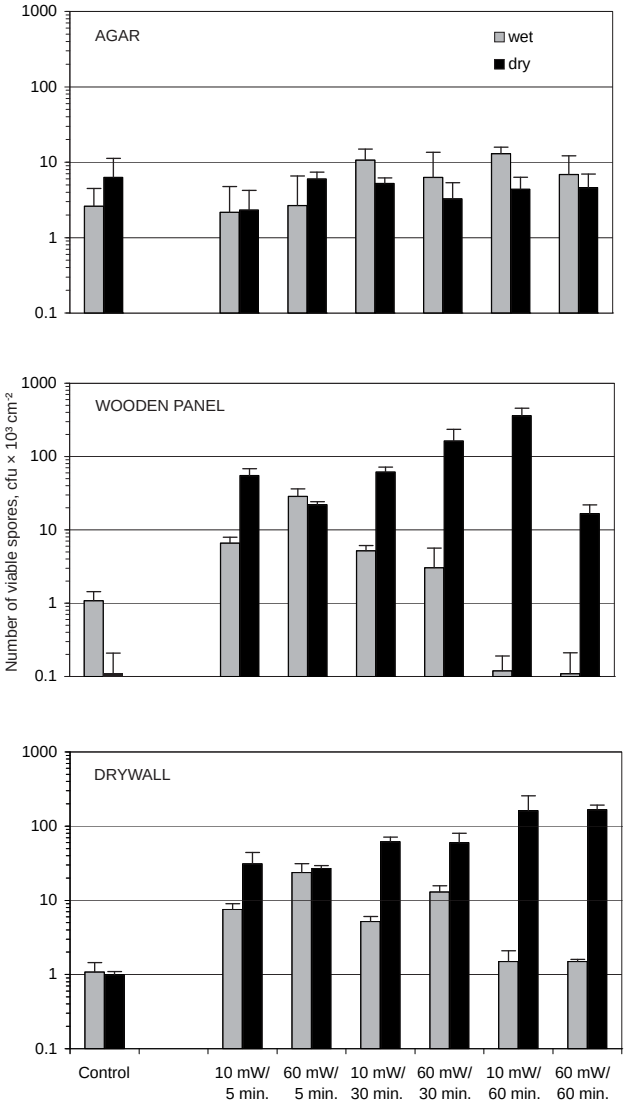

Figure 4. Viability (as a colony forming units, cfu) of *Thermoactinomyces vulgaris* "wet" and "dry" spores growing on agar, wooden panel, and drywall samples before and after microwave radiation. The error bars represent standard deviation of 18 and 3 repeats for the samples before and after microwave experiments, respectively.

experiments revealed that only 30 min and longer exposure to microwave radiation resulted in a change of *A. versicolor* spore viability. For "dry" samples, a decrease in viability was noticed after 30 min of exposure at a power density of 60 mW/cm^2, or, after 60 min of exposure independent of the power density of microwave radiation (in both cases: $p<0.05$). For "wet" samples, time of exposure had no effect on the spore viability ($p>0.05$). Quantitative comparison of "dry" and "wet" spore viability showed that both numbers did not differ from each other before the experiments ($p>0.05$), but after the exposure, both duration of radiation and its power density had a significant effect on *A. versicolor* viability, especially on the samples incubated at lower relative humidity (statistical significance was between $p<0.05$ and $p<0.01$);

c) viability of spores on wooden panel: there was no statistically significant difference in the viability of spores after incubation for "wet" and "dry" samples ($p>0.05$). Microwave experiments showed that, independent of power density applied, the presence of water and time of exposure were the key factors determining viability. It seems that water is a major factor for transferring energy from microwaves onto spores, and, when present in sufficient quantity in "wet" samples, was responsible for the observed destructive effect of radiation. This was especially noticeable after 60 min exposure at power densities of 10 mW/cm^2 ($p<0.001$) and 60 mW/cm^2 ($p<0.0001$). In the case of „dry" samples, such a relationship was not statistically significant. Based on that, it can be concluded that in the case of *A. versicolor* spores growing on pine wainscot panels, the thermal effect of microwave radiation was responsible for decrease of their viability;

d) viability of spores on drywall: in the case of drywall, even before microwave experiments, the differences in viability of spores were clearly visible. On "dry" surfaces, viability was significantly higher than on "wet" surface of drywall ($p<0.05$). After radiation, although viability of spores on both ("wet" and "dry") tested surfaces decreased, such a difference was not statistically significant. Based on this result, it can be speculated that in case of drywall, a microwave effect has a more pronounced influence on microbial viability than the thermal effect of radiation. Also, it seems that power density of such radiation is less important, but time of exposure of microbiologically contaminated surface is a more predominant factor. An application of 60 min exposure at power densities of 10 mW/cm^2 and 60 mW/cm^2 caused statistically significant 18% ($p<0.01$) and 39% ($p<0.05$) decrease in viability of spores growing on the "dry" drywall surface, respectively;

for *P. brevicompactum* (Fig. 3):

e) the only difference in viability of these spores among studied materials was the difference in number of *P. brevicompactum* spores in "dry" samples of respective materials tested before microwave experiments (ANOVA: $p<0.01$). Similar to *A. versicolor*, *Penicillium* produced a majority of spores on a "dry" drywall (Scheffe test: $p<0.01$). The differences in viability of *P. brevicompactum* after microwave experiments were close to the above-described results for *Aspergillus* spores, i.e., for "dry" samples statistically significant differences (ANOVA: $p<0.001$) were the result of poor production of spores on a wooden panel in comparison to the agar and drywall (Scheffe test: $p<0.05$ and $p<0.001$ respectively). For "wet" samples (ANOVA: $p<0.01$), the difference came from abundant spore production on drywall (Scheffe test: $p<0.01$);

f) viability of spores on agar: for "wet" and "dry" samples, viability of spores determined immediately after their incubation did not differ statistically. Comparison of spore viability for "wet" and "dry" samples revealed a statistically significant decrease after microwave radiation in the number of viable spores growing on both "wet" and "dry"

surfaces ($p<0.05$ and $p<0.01$, respectively). After analyzing combinations of microwave parameters applied in this study, it can be stated that for "wet" and "dry" samples cultivated on agar a significant decrease in viability of spores was already observed after 5 min of exposure to radiation with a power density of 60 mW/cm^2 ($p<0.05$). At longer times of exposure, a decrease in viability, still observed, was not statistically significant;

g) viability of spores on wooden panel: analysis of spore viability on "wet" and "dry" surfaces before the microwave experiments showed a statistically significant difference. Although *P. brevicompactum*, like *A. versicolor*, belongs to the same xerophilic group of fungi, it behaved differently on the wooden panel at low water availability conditions, i.e., produced significantly more spores ($p<0.01$). After microwave experiments, difference was no longer observed ($p>0.05$). Hence, the number of spores which were inactivated on a "dry" surface was significantly higher than the number of spores which lost their viability on the "wet" panel. In the case of viability of *P. brevicompactum* spores on "dry" samples, taking into account the absence of water which converts microwave energy into increased surface temperature, a microwave effect was more pronounced compared to a thermal effect of radiation. Regarding the studied parameters of microwave exposure, independently of the time of radiation, the biggest statistically confirmed effectiveness in decreasing of spore viability had a power density of 60 mW/cm^2 ($p<0.001$ for 5, 30, and 60 min);

h) viability of spores on drywall: for "wet" and "dry" samples, viability of spores before the exposure did not differ ($p>0.05$). Viability of "wet" spores after radiation was not different as well. For "dry" samples, a combination of radiation parameters caused a decrease in the number of viable spores ($p<0.05$). It was especially noticeable when exposure to microwaves lasted for 60 min at both tested power densities of 10 and 60 mW/cm^2 ($p<0.001$ and $p<0.05$, respectively), or 30 min at 60 mW/cm^2 ($p<0.0001$). Taking into account the degree of hydration of this surface together with a slight increase of the surface temperature during microwave experiments, it can be concluded that, as in the case of the wooden panel, microwave effect played a major role in determining of *P. brevicompactum* spore viability;

for *T. vulgaris* (Fig. 4):

i) differences in viability of spores between studied materials: before the microwave experiments, viability of "wet" and "dry" spores did not differ statistically. Except for the wooden panel (see below), no difference was noted for "wet" samples after microwave radiation. For "dry" spores, their viability after the experiments underwent a statistically significant change ($p<0.01$). Compared to the agar samples, viability of "dry" spores on the wooden panel and drywall was significantly higher ($p<0.01$ and $p<0.05$, respectively). Hence, for thermophilic microorganism, an increase of surface temperature on which it grew, actively maintained the spore viability level and masked the potential sterilizing effect of microwave radiation;

j) viability of spores on agar: *T. vulgaris* spores behaved differently compared to the fungal spores, i.e., the microwave radiation had less influence on the actinomycete spore viability. For both "dry" and "wet" samples, viability before and after exposure did not differ ($p>0.05$). However, inversely to the tested fungal spores, specific combinations of 10 mW/cm^2 power density with 30 and 60 min exposure resulted in a significant increase in the number of viable spores on the agar surface exposed to microwaves ($p<0.05$ and $p<0.01$, respectively). It is possible that an increase of the temperature of the exposed surface played a certain role here. As a thermophilic actinomycete, *T. vulgaris* has its own optimal growth conditions between 50-60ºC and surface heating due to the microwave radiation which can support an increase in spore production rather than their destruction;

k) viability of spores on the wooden panel: for "wet" samples before microwave experiments, viability of spores was significantly higher than for "dry" samples ($p<0.01$). This is in contrast to the observed fungal spore viability. It is likely that for fungi, lack of water in the environment or on the surface, mobilized their colony to survive an unfavourable period of growth, which is manifested by a more abundant production of conidia. For *T. vulgaris* colony on wood, a constant access to the water source seemed to be necessary for the spore production. Microwave radiation applied to the "wet" and "dry" samples affected differently the viability of spores. As is shown in Figure 4, the survival rate for "dry" spores was significantly higher than for the "wet" ones ($p<0.01$). This finding differed from that observed on the agar surface. Possibly, the main role played here was by the thermal effect of microwave radiation, which – at relatively good water availability and sufficiently long exposure – decreased the spore viability ($p<0.001$ for 60 min exposure at 10 and 60 mW/cm^2 power densities). Shorter exposure times can significantly favor spore viability on "wet" wooden panels ($p<0.05$ for 5 min exposure at 10 mW/cm^2 and for 30 min exposure at 10 and 60 mW/cm^2 power densities). For "dry" spores of *T. vulgaris*, each of the tested combinations of parameters acted pro-developmentally ($p<0.01$ for 30 and 60 min exposure at power density of 60 mW/cm^2; $p<0.001$ for 5 and 60 min at 10 mW/cm^2; $p<0.0001$ for 30 min at 10 mW/cm^2 and $p<0.00001$ for 5 min at 60 mW/cm^2);

l) viability of spores on drywall: a similar observation as for wooden panel was stated for spore viability on drywall. Although before radiation the differences between "wet" and "dry" samples were not prominent, after the experiments the number of spores, which were still viable in the "dry" samples, significantly exceeded that for the "wet" samples ($p<0.0001$). Observing viability on drywall, it could be concluded that for "wet" spores the increase in their viability was favoured by the short exposure times, irrespective of power densities applied ($p<0.01$ for 5 and 30 min exposure at 10 mW/cm^2 power density and 30 min exposure at 60 mW/cm^2 as well as $p<0.05$ for 5 min exposure at 60 mW/cm^2);

for *S. albus* (Fig. 5):

m) differences in viability of spores between studied materials: spore viability on tested surfaces before and after their exposure to microwave radiation was different for "wet" samples only (ANOVA: $p<0.05$ and $p<0.001$, respectively). In both cases, significantly higher viability was demonstrated by the "wet" spores growing on agar than those on the wooden panel and drywall (Scheffe test: $p<0.05$ and $p<0.01$, respectively);

n) viability of spores on agar: viability of "wet" spores was significantly higher than "dry" ones if compared in samples before and after microwave experiments ($p<0.05$). Exposure of "wet" spores for 60 min and "dry" spores for 30 min visibly decreased the viability of *S. albus* on agar (both cases: $p<0.05$). For "dry" spores, however, radiation extended to 60 min at a power density of 60 mW/cm^2 for "dry" spores significantly supported viability of this actinomycete spores ($p<0.05$). *S. albus* manifests its ability to grow at elevated (compared to the normal room) temperature (see "Materials and Methods") and it is possible that the observed increase of surface temperature during the microwave experiments was responsible for observed elevation in spore viability at described conditions;

o) viability of spores on wooden panels: there was no significant difference between viability of the "wet" and "dry" spores before the experiments. After the experiments, both types of spores increased their viability. "Dry" spores showed a significantly higher viability rate than "wet" ones ($p<0.01$), especially when samples were exposed to a power density of 10 mW/cm^2 during 5 and 30 min exposure, or to 60 mW/cm^2 during 5 min (in all cases: $p<0.05$). The latest combination also had the same positive influence on "wet" spores of *S. albus* ($p<0.01$);

p) viability of spores on drywall: similarly to the above described example, the "wet" and "dry" samples before the microwave experiments had the same level of viable spores. After microwave radiation, an increase of viability was observed. In this case, for both "wet" and "dry" samples, the most significant factor was the effect of exposure time and power density (5 min exposure at 60 mW/cm^2 for "wet" and "dry" samples: $p<0.05$ and $p<0.01$, respectively, as well as 60 min exposure at 10 mW/cm^2 for "dry" samples: $p<0.05$).

As shown by our results, microwave radiation can influence microbial spore viability using its both modes of action, i.e., through the thermal and microwave effects. Exactly which one of them turns out to be the most prevalent depends on the biological resistance of exposed microorganisms, their affinity to the specific genus or group of microorganisms, and environmental conditions, mainly the degree of hydration of the contaminated surface. For both *A. versicolor* and *P. brevicompactum* colonies growing on "wet" and "dry" samples, the highest survival rate of spores was noted for drywall samples compared to agar and wooden panel samples. For these fungi, the non-thermal microwave effect decreased the spore viability much more substantially than the thermal effect of such radiation,

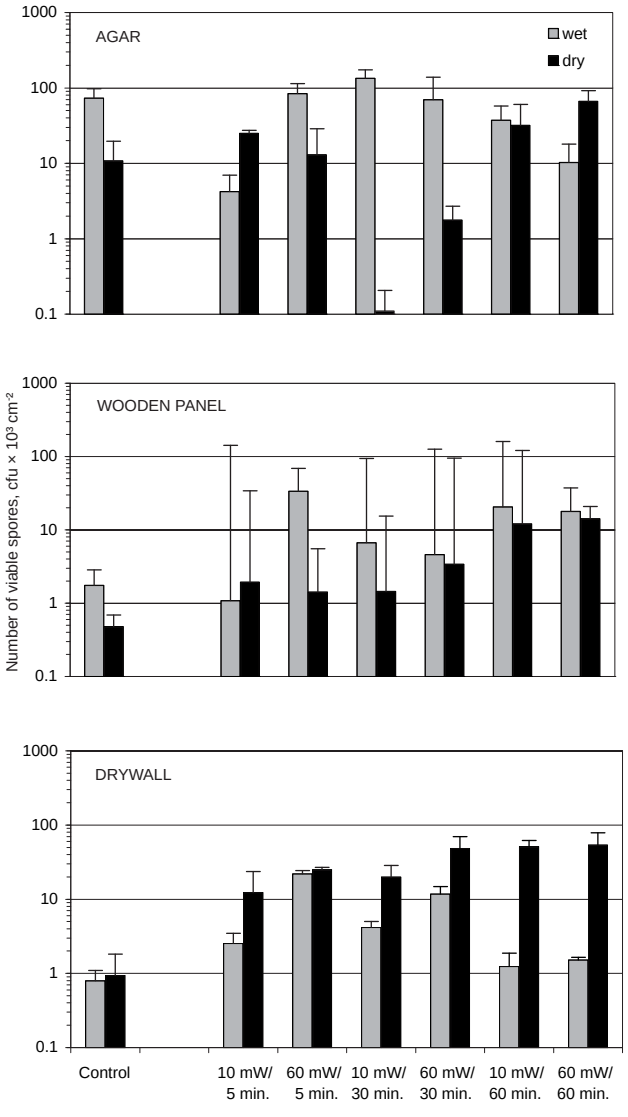

Figure 5. Viability (as a colony forming units, cfu) of *Streptomyces albus* "wet" and "dry" spores growing on agar, wooden panel, and drywall samples before and after microwave radiation. The error bars represent standard deviation of 18 and 3 repeats for the samples before and after microwave experiments, respectively.

except for *A. versicolor* spores growing on the wooden panel where the thermal effect of microwaves was the most prevalent. These results are contradictory to earlier studies by Dhahi *et al.* [17] and Mężykowski *et al.* [79] who, testing *Aspergillus nidulans* and *A. amstelodami* strains, did not observe a significant change in spore viability. This could be explained by the frequency of microwave radiation used in those studies. The authors applied 8.7175 GHz microwave frequency for radiation of the samples and, hence, no significant change in spore viability was revealed. As shown by Kakita *et al.* [55], the microwave effect at 2,450 MHz (the same frequency used in the present study) can be distinguishable from external heating [55, 63]; however, the decrease in viability for fungal spores (*A. niger*) was slight [10].

For tested actinomycetal spores, microwave radiation reinforced spore survival on tested surfaces instead of decreasing their viability. For the "dry" as well as "wet" samples, the majority of tested combinations of power densities and short (5 and 10 min) exposure times resulted in an increase of *T. vulgaris* and *S. albus* spore viability. Only the extension of the microwave exposure to 60 min caused a statistically significant decrease of actinomycete spores growing on the tested building materials. The same relationship was true for the fungal spore viability on wooden panel and drywall samples, where 60 min exposure at 10 or 60 mW/cm^2 power densities was most effective in decreasing the viability of *A. versicolor* and *P. brevicompactum* conidia.

CONCLUSIONS

The obtained results show that the effect of microwave radiation on the viability of studied microorganisms differed depending on the strain of microorganisms, growth conditions, power density of microwave radiation, time of exposure to microwaves, and varied depending on the applied combination of the two latter elements. For *P. brevicompactum* growing on wooden panel and drywall, as well as for *A. versicolor*, colonizing wood samples, the microwave effect of radiation had a more substantial effect on the viability of spores than the thermal effect. For *A. versicolor*, the highest decrease in viability of spores was noted for 60 min exposure at the microwave power densities of 10 and 60 mW/cm^2; for *P. brevicompactum* – depending on the surface – for 5 and 60 min at 60 mW/cm^2 and for 30 and 60 min at 10 and 60 mW/cm^2. An opposite behaviour of spore viability was revealed for tested actinomycetes. Both *T. vulgaris* and *S. albus* colonies, when exposed to microwave radiation, increased the viability of their spores.

For all tested microorganisms, the effect of microwave radiation resulting in a decrease of spore viability on "wet" wooden panel and drywall samples was usually observed at 60 min exposure only. Shorter exposure times decreased the viability of fungal spores only, while in actinomycete colonies contaminating the studied building materials, a short radiation caused the opposite effect – an increase in the viability of spores. Such information seems to be of great importance. The goal of this study was to check the most effective parameters of microwave radiation method of building material sterilization, which could be applicable, e.g., to the fight against microbial contamination during remediation procedures. Fungal and actinomycetal contamination of buildings is very often connected with environmental disasters. The latest examples are floods in Poland in 1997, in New Orleans in 2005, and in Great Britain in the summer of 2007. Taking only the Polish example into consideration, in consequence, 20% of civil parishes sustained significant losses, 500,000 hectares of urbanized area with 680,000 dwellings and several thousand factories and institutions were flooded [20, 43, 77]. In such massive number and scale, it is almost certain that there will be long term effects due to buildings damaged by moisture, leading to serious health outcomes for the families whose dwellings have not been rebuilt, dried or remediated against moulds. The use of proper microwave "cleaning" parameters could, in the majority of these cases, help to effectively protect people by preventing microbial contamination of the building materials and inactivating the contaminants of microbial origin already growing on the building envelope.

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