EFFECT OF MICROWAVES ON MOULDS ISOLATED FROM SURFACES

— short communication —

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Abstract: In this paper, the combined effect of microwaves (MW) on mould isolated from buildings surfaces was investigated. Three microbial species were selected for the tests: *Penicillium sp., Alternaria sp., Aureobasidium sp.* The influence of three variables on the spores inactivation rate was analysed: the exposure time to MW (x_1), MW power radiation (x_2) and the dilution of spores (x_3) by using a 2^3 full factorial design. Three models of the spores inactivation rate were obtained:
IR *Penicillium* = $61.723 + 5.973\ x_1 + 33.938\ x_2 + 5.241\ x_3 - 7.674\ x_1\ x_2 - 5.877\ x_2\ x_3$
IR *Aureobasidium* = $54.375 + 7.625\ x_1 + 31.250\ x_2 + 8.375\ x_3 - 8.375\ x_1\ x_3$
IR *Alternaria* = $54.12 + 7.96\ x_1 + 29.28\ x_2 + 12.94\ x_3 - 8.95\ x_2\ x_3 - 7.33\ x_1\ x_2\ x_3$
The result showed that the viability of studied moulds differed depending on their strains, power of MW radiation, time of exposure, fungal spore concentration.

Keywords: microwaves, fungi, surface, full factorial design, model

INTRODUCTION

Fungal spores are abundant in the atmosphere and settle onto surfaces they can grow and form colonies or even biofilms, depending on the physicochemical environmental conditions (Christofi et al., 2008). Important surfaces become contaminated; therefore it is a requirement for effective decontamination (Christofi et al., 2008).
A considered approach to surface disinfection is the use of microwaves (MW) radiation. That is because microwave surface treatment technique consists of converting electromagnetic field energy within the range of MW frequencies (2.5 MHz-300 GHz) into a thermal energy targeted at an exposed

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environment (Rafal et al, 2007). Such a technique is an alternative or supplement to the traditional methods of removing molds such as UV lamps or chemical cleaners, because it is relatively easy to use, it has low costs and is a non-invasive method (Mironescu et al., 2008).
Some mechanisms have been studied extensively since the 1970s by a number of researchers. (Culkin and Fung, 1975) reported earlier studies that suggested MW heating at 2450 MHz caused greater destruction of bacteria *Escherichia coli* and *Salmonella typhimurium*. (Baysan et al., 1998) used MW energy to disinfect a long-term soft lining material contaminated with a fungus (*Candida albicans*) or a bacteria (*Staphylococcus aureus).* MW is capable of inactivating microbial contaminants on the exposed surfaces and thus it results in reducing stopping colonization of microbiologically contaminated surfaces. Consequently, it can decrease the number of agents contributing to the adverse health effects in the outdoor environments (Rafal et al, 2007).
The effects of microwave radiation on microorganisms as a physical phenomenon are still not fully explained. 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 microbiologic material (Rafal et al, 2007).
This paper investigates the action of MW radiations on three fungal spores isolated from Buildings in Sibiu, Romania. The study aims to observe and analyze if the microwave action can be differentiated depending on the mould type and to model the relation between three MW parameters (fungal spore concentration, power of radiation and exposure time) and the destruction efficiency for each type of mould.

MATERIALS AND METHODS

The fungal species used for this experiment are important biological agents with respect to health hazards and have strong allergenic properties. Three microbial species were selected for the tests: *Penicillium sp., Alternaria sp., Aureobasidium sp.* Fungi had previously been isolated from contaminated building materials and were identified by using identification keys (Dan et al., 1999). These three fungal genera were chosen because they commonly occur outdoors in various climate zones worldwide (Rafal et al, 2007).
Before the experiments, pure cultures of microorganisms were growth on malt liquid substrate.

In order to test the destructive action of the biocidal formulations on moulds, spore suspensions were obtained, as follows:

- *Penicillium sp.* was grown on Czapex-Dox solidified medium in Petri dishes, which were then incubated at 25° C for 10 days. The resulted spores were shaked on the Petri dish cover (by turning the box and applying small strokes) and then switched with sterile distilled water in sterile tubes. For analysis, the resulting spore suspension was used.
- For the *Alternaria sp.* and *Aureobasidium sp.*, another technique was applied to obtain spores. As observed practically, they grow much better on liquid broth than on solidified substrate. In addition, for these two types of moulds, spores are formed along the hyphae (*Aureobasidium*) or in conidia (called porospores) (*Alternaria*) (Dan et al., 1999), which is harder to separate from the solid substrate. Also, *Aureobasidium sp.* produces mucilage surrounding the hyphae and making it difficult to separate. For these reasons, the two types of moulds were grown on malt liquid broth at 25°C for 10 days with agitation. Then, the mycelium was disintegrated by intense shaking on magnetic stirrer. For analysis, the clear liquid obtained after filtration on sterile filters (pore size 50 μm) was used.

For the experiment, three spore concentrations were used. They were obtained as follows: undiluted, sporal suspensions diluted 1:10 and diluted 1:20. After obtaining these concentrations, Petri plates were inoculated in the centre with the suitable fungal spore suspension using the incorporation method in plates. Next, the samples were submitted to the microwave treatment. Concomitant with the execution of the samples subjected to the treatment, three blind samples were made, without any disturbing factors.

A 2^3 full factorial design with three variables (concentration, exposure time and radiations powers) was carried out (Table 1). This approach offer the advantage of using a reduced number of experiments (8), thus reducing the time needed for results and the consumables.

Table 1. Full factorial design used

Description			Level		
			-1	0	1
Input variables	x_1	Exposure time, s	20	40	60
	x_2	Power radiation, W	180	540	900
	x_3	Dilution of spores	1:20	1:10	0
Output variable	y	Spores inactivation rate, IR, %			

IR was calculated with the formula:

IR = (initial spores –spores after MW treatment) / initial spores

The data obtained using the factorial design was used to build a polynomial model with the form:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{123} x_1 x_2 x_3$$

Building steps of mathematical model were as it follows:

1. Calculation of the model coefficients;
2. Determination of the coefficients significance using the t - "Student" test;
3. Calculation of y with the new function in which it must be taken into account only the significant factors (Kafarow, 1976).

RESULTS AND DISCUSSIONS

The inactivation rate IR obtained for all three analysed moulds under the influence of MW is presented in Table 2.

Table 2. Inactivation rates IR obtained experimentally for each mould type

Exp.	**x_0**	**x_1**	**x_2**	**x_3**	**IR_*Alternaria***	**IR_*Aureobasidium***	**IR_*Penicillium***
1	1	1	1	-1	94.12	100	95.06
2	1	1	1	1	91.59	92.5	92.86
3	1	1	-1	-1	0	24	32.1
4	1	1	-1	1	62.62	31.5	50.77
5	1	-1	1	-1	64.71	56	97.53
6	1	-1	1	1	83.18	94	97.19
7	1	-1	-1	-1	5.88	4	1.23
8	1	-1	-1	1	30.84	33	27.04
9	0	0	0	0	96	96.15	100
9 `	0	0	0	0	88	84.62	96.43
9 ``	0	0	0	0	80	96.15	100

In order to analyse the influence of the three factors (retention time, power radiation and spores concentration) on the destruction rate for three moulds isolated, the results of the experimental cultivations were statistically analysed. The regression coefficients and the results of the Student t –test for the coefficients significance analysis are presented in Table 2. The results show that the MW radiations have a different action on the spores produced

by different fungi. As observed in Table 2, in all cases the free term has the bigger value, this result suggesting the existence of another factor which strongly influences the destruction rate.

Table 2. Parameters obtained at the model building

Penicillium sp. y_1

t – Student test	t_0	t_1	t_2	t_3	t_{12}	t_{13}	t_{23}	t_{123}
	84.67	8.19	46.55	7.19	10.53	1.54	8.06	0.90
Effect matrix	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
	61.723	5.973	33.938	5.241	-7.674	-1.126	-5.877	0.659
Action	↑↑↑	↑	↑↑	↑	↓	0	↓	0

Aureobasidium sp. y_2

t – Student test	t_0	t_1	t_2	t_3	t_{12}	t_{13}	t_{23}	t_{123}
	23.09	3.24	13.27	3.56	1.27	3.56	0.32	1.27
Effect matrix	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
	54.375	7.625	31.250	8.375	3000	-8.375	0.750	-3000
Action	↑↑↑	↑	↑↑	↑	0	↓	0	0

Alternaria sp. y_1

t – Student test	t_0	t_1	t_2	t_3	t_{12}	t_{13}	t_{23}	t_{123}
	19.13	2.82	10.35	4.57	0.53	0.74	3.17	2.59
Effect matrix	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}
	54.12	7.96	29.28	12.94	1.49	2.08	-8.95	-7.33
Action	↑↑↑	↑	↑↑	↑	0	0	↓	↓

Arrows indicate: ↑↑↑- strong positive influence; ↑↑ - middle positive influence; ↑- low positive influence; 0 - no influence; ↓ - negative influence.

The following models were obtained:

$$y_1 = 61.723 + 5.973\,x_1 + 33.938\,x_2 + 5.241\,x_3 - 7.674\,x_1x_2 - 5.877\,x_2x_3$$

$$y_2 = 54.375 + 7.625\,x_1 + 31.250\,x_2 + 8.375\,x_3 - 8.375\,x_1x_3$$

$$y_3 = 54.12 + 7.96\,x_1 + 29.28\,x_2 + 12.94\,x_3 - 8.95\,x_2x_3 - 7.33\,x_1x_2x_3$$

In all cases, the irradiation power influences positively the destruction rate, this factor affecting especially the spores produced by *Penicillium sp*. In the case of the spore concentration, the most affected sample was the one containing *Alternaria sp.* spores and the time of exposure has the smallest influences upon every samples.

The combined action of all medium variables resulted in having the most high inactivation rate of spores.

CONCLUSIONS

The three analysed parameters: exposure time, power radiation and spore concentration influence the destruction rate of fungal spores under the action of microwaves. Their action is very different for spores derived from different types of fungi. So, the first step at the decontamination of buildings attacked by fungi, it is very necessary to analyse the most abundant genus.
For an efficient destruction of fungal spores, next step is to find the proper conditions of spore inactivation using MW treatment. As the models obtained in this paper show, surfaces contaminated with *Penicillium sp., Aureobasidium sp.* or *Alternaria sp.* spores are better disinfected when MW with increased power radiation is used. Both exposure time and spores concentration shows lower influence on the spores inactivation rate. The combined action of all three parameters tested is negative or is not influencing the fungal spores destruction by MW.
As the very high value of the free coefficient b_0 shows, other factors influence very strongly the process, for all the analysed fungi.

REFERENCES

1. Baysan A., Whiley R., Wright P., Use of microwave energy to disinfect a long-term soft lining material contaminated with *Candida albicans* or *Staphylococcus aureus*, J. Prost. Dentistry, 1998, 79 (4), 454-458
2. Christofi N., Misakyan M.A., Matafonova G.G., Barkhudarov E.M., Batoev V.B., Kossy I.A., Sharp J., UV treatment of microorganisms on artificially-contaminated surfaces using excimer and microwave UV lamps, Chemosphere, 2008, 73 (5), 717-722
3. Culkin K. A., Fung D. Y. C., Destruction of *Escherichia coli* and *Salmonella typhimurium* in microwave-cooked soups, J Milk Food Technol., 1975, 38 (1), 8-15
4. Dan., V., Kramer, C., Bahrim, G., Nicolau A., Zara, M.: Memorator de mucegaiuri, ed. Evrika, Brăila, 1999
5. Kafarow, V., Cybernetic methods in chemistry and chemical engineering, MIR Publishers, Moscow, 1976
6. Mironescu M., Oprean L., Mironescu I.D., Pacala M-L., Mathematical modeling of the sporicidal effect of UV radiations on fungi isolated from the industrial wastewater sludge, 18-th International Congress of Chemical and Process Engineering, 2008, Prague [1638], www.chisa.cz/2008
7. Rafal L., Mainelis G., Wlazło A., Niesler A., O Lis D., Marzec S., Siwińska E., Łudzeń-Izbińska B., Harkawy A., Kasznia-Kocot J., Viability of fungal and actinomycetal spores after microwave radiation of building materials, Annals of agricultural and environmental medicine AAEM, 2007, 14 (2), 313-324
8. Zhang H., Zheng X., Su D., Postharvest control of blue mold rot of pear by microwave treatment and *Cryptococcus laurentii*, Journal of Food Engineering, 2006, 77 (3) 539–544