

ELECTROMAGNETIC EXPOSURE INFLUENCE ON PROTEIN SYNTHESIS IN CELLULOLYTIC FUNGUS – AN ENVIRONMENTAL ISSUE

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Experimental investigation focused on electromagnetic pollution influence on fungi degrading wood is presented in this paper. 970 MHz low power microwaves were used to irradiate *Phanerochaete chrysosporum* cells combining two exposure times and two specific absorption rates – experimentally determined by the scattering parameter method. Total protein content estimation and electrophoretic fractions assay have evidenced diminished biosynthesis with up to 25%, following energy absorption from microwave source, but no qualitative modifications in protein fraction array.

Key words: Cellulolytic fungus, microwave exposure, protein biosynthesis.

1. INTRODUCTION

Thermal microwaves are known for their applications in domestic activities while non-thermal subtle bioeffects of microwaves are related to extensive telecommunications and intensive traffic control raising the preoccupation for electromagnetic pollution risk [1–3].

Considering the impact of electromagnetic radiation on biosphere and also the challenges of modern genetics, the microwave effects in eukaryote microorganisms like fungi growing in the environmental waters and soils were carefully studied during last decades. Early in the 70's and 80's years non-thermal microwaves were found not able to elicit genetic responses in such living systems. For example, in [4] experiments with low power densities microwaves in various frequency ranges acting on the yeast *Saccharomyces cerevisiae* – a model organism representative for fungal species – showed no modifications in the growth of microorganism cells as long as the temperature was kept at constant, physiological level; also the widely studied *Aspergillus* fungus was found genetically stable under microwave exposure [5]. Later, when the concern of microwave side effects was increased due to intensification of radiation use in telecommunications and medicine,

new experimental attempts were done and some bioeffects were clearly detected. So, the stimulation of *Saccharomyces* cell growth under 900 MHz radiation applied within Transverse Electromagnetic Cell device was reported in [6]. Using also 905 MHz electromagnetic field that closely matched the Global System for Mobile Communication (GSM), other authors [7] reported evident responses at the level of *Saccharomyces* yeast proliferation with distinct behavior for different strains, some responsive and some others inert to microwaves impact. Lately, biotechnology development attired the scientists' attention on some fungi with potential applications in controlling wood waste resulted from industrial delivery in the environment. The application of microwaves was reported in [8] as non-conventional method to inhibit *Aspergillus* fungi growth and its mycotoxin activities which are related mainly to protection of materials that are processed in food industry.

In [9] microwaves were reported as useful in mutagenesis of cellulolytic fungus *Trichoderma viridis* for increase cellulase activity with the target to enhance the ability of wood waste decomposing. In [10] the effects of microwaves on viability of fungal spores growing on wooden substrate were evidenced for power densities of 10 to 60 mW/cm² with distinct inhibitory action for 1 h exposure time. Less extensive research was focused on microwave indirect action on microorganisms [11] where water exposed to electromagnetic waves was supplied to cell cultures resulting in different responses depending on radiation power density.

Chaetomium globosum cellulolytic fungus was previously investigated by us [12] using exposure to low power density microwaves with focus on cellulolytic enzymes that were found sensitive to 10.75 GHz electromagnetic radiation with less than 1mW/cm².

Phanerochaete chrysosporium is known as a white-rot fungus growing on woody tissue and degrading cellulose. Like other cellulolytic microorganisms it is able of cellulose substrate modification due to its various enzyme equipments – highly specialized proteins that are known to be sensitive to radiation and heat. Since no literature reports dedicated to its response to non-thermal microwaves could be found up to date, in the present work the search for microwave effects in this fungus protein synthesis was investigated by applying low thermal and very low thermal microwaves. The experiment was planned to simulate electromagnetic pollution on eukaryote cellulolytic microorganisms with known benefits in environment remediation through wood waste decomposing.

2. MATERIALS AND METHODS

2.1. MICROWAVE EXPOSURE

(A) The exposure system was composed by a Transverse Electromagnetic Cell (TEM), a microwave/radiofrequency signal generator and an amplifier, with a

vector signal analyzer connected between the ports for the scattering parameters measurement (Fig. 1).

The system was able to generate 970 MHz quasi-uniform electromagnetic field, in continuous wave. Petri dishes with agarized fungi cultures were placed inside the TEM device cell for two irradiation time durations: 1 hour and 4 hours.

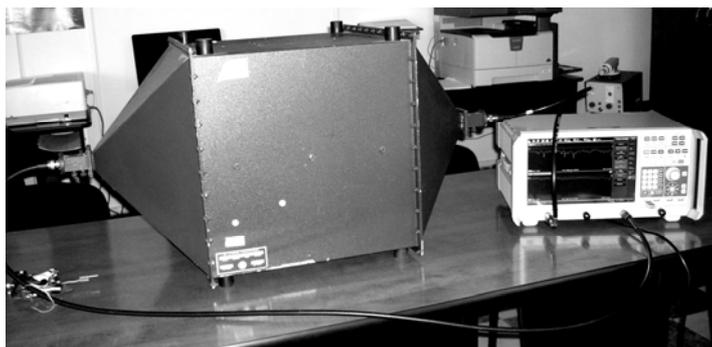


Fig. 1 – TEM cell with the vector signal analyzer connected between ports for the scattering parameters measurement.

(B) Dosimetry: Based on absorbing sample geometry and its physico-chemical characteristics as well as on the irradiation device parameters two average SAR levels were designed and calculated [13]: SAR1 = 2 W/kg representing very low thermal level and SAR2 = 8 W/kg, *i.e.* low thermal level. According to ICNIRP Guidelines [14]: very low MW doses range in 1–4 W/kg and results in less than 1 degree Celsius temperature rise in the absorbent sample while low-thermal MW doses deliver a bit more than 4 W/kg with about 2 degrees Celsius heating contribution.

2.2. ORGANISM AND CULTURE CONDITIONS

P. chrysosporium fungus was purchased from the Institute Scientifique de Santé Publique, Belgium (HEM no. 5772).

The cellulolytic microorganism was cultivated in Petri dishes on agarized Sabouraud medium (peptone 10 g/l, glucose 35g/l, agar 2g/l, distilled water up to 1.0 l [15]).

When fungi cultures were 7 days old the Petri dishes were introduced in the TEM device to be exposed to 970 MHz microwaves. Afterwards 3 cm diameter mycelia discs were withdrawn to be inoculated in Erlenmeyer flasks with liquid Sabouraud medium (3.5 g glucose, 1 g peptone, 2 g bran and 100 ml distillate water). All samples were incubated at 28 °C in INCUCCELL room for other two weeks. All reagents were purchased from Merck.

2.3. PROTEIN CONTENT ASSAY

The assay of soluble protein content was carried out using 50 mM Tris-HCl buffer, pH 7, according to Bradford method [16] for extraction and bovine serum albumin as reference or standard. The measurement is based on sensing the binding of Coomassie Brilliant Blue G-250 to aromatic amino acid radicals through color intensity change at 595 nm. The soluble protein content is given in mg per g of mycelium mass. The Coomassie Brilliant Blue G-250 was purchased from Fluka. Shimadzu spectrophotometer type 1800 Pharmaspec with quartz cells was used for color intensity readings.

2.4. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) AND STAINING OF GELS

Gel electrophoresis test was accomplished using SDS-PAGE method according with Laemmli protocol [17], with gradient gels (5–20 %) under denaturing conditions – using a SciePlas TV400 device. Sample preparation involved protein extraction in 0.1 M phosphate buffer pH = 7. Fungal tissue intended for protein extraction was disintegration within a mortar with pestle and few grains of silica sand. Prior to electrophoresis samples were thermally treated at 95 °C with a denaturing buffer solution containing sodium dodecyl sulfate (SDS) and beta-mercaptoethanol. Aliquots of 10 µl of protein extract were loaded onto the gel and electrophoresed with Tris-glycine buffer (pH 8.3) at a constant current intensity of 30 mA / gel; after the bromophenol blue reached the bottom of the gel briefly washing with deionized water was carried out and then dying with Coomassie Brilliant Blue R250 was applied. Colored gels were photographed with a high resolution camera (Canon 550D). Images were analyzed with GE ImageQuant 8.1 software. Reagents came from Carl Roth and/or Sigma.

Statistical analysis. All experiments were carried out with four independent repetitions and the results were expressed as the mean values ± standard deviation (SD). Statistic significance was ensured by four replies of each SAR/exposure time combination and by *t*-test application.

3. RESULTS AND DISCUSSION

In Fig. 2 the SAR distribution within the TEM cell is presented according to the calculation performed in agreement with the technical details given above.

For each SAR level quasi uniform MW power density was obtained in the bottom zone where the Petri dishes were placed for fungi cell electromagnetic exposure.

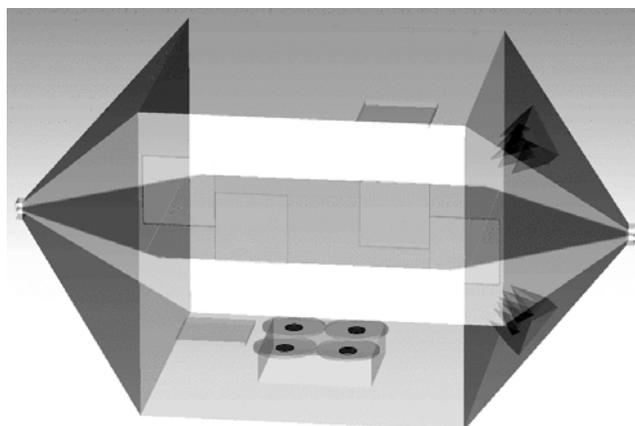


Fig. 2 – Modeled TEM cell with the four dishes exposed inside, at bottom part at cvasi-uniform irradiation.

3.1. EFFECT OF IRRADIATION ON PROTEIN CONTENT

For both fungal culture arrays (at 7 and at 14 days) the effect of SAR₁ microwaves was the diminution of the soluble protein level (Figs. 3, 4).

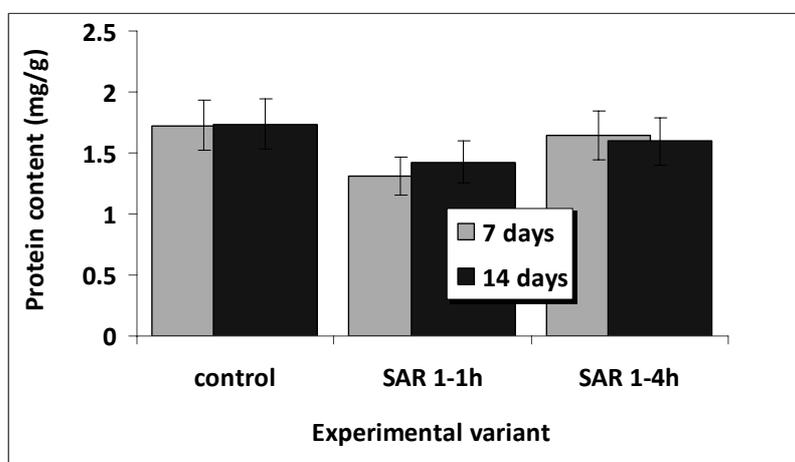


Fig. 3 – Protein content in fungus cells after electromagnetic exposure to SAR1.

Thus, at 7 days after inoculation, the reduction of soluble protein content was of 24 % for 1 h exposure ($p < 0.05$) and of only 4 % for 4 h (statistically non-significant) while at 14 days the decrease was of 18% for 1 h exposure and 8% for 4 h with similar statistical significance levels.

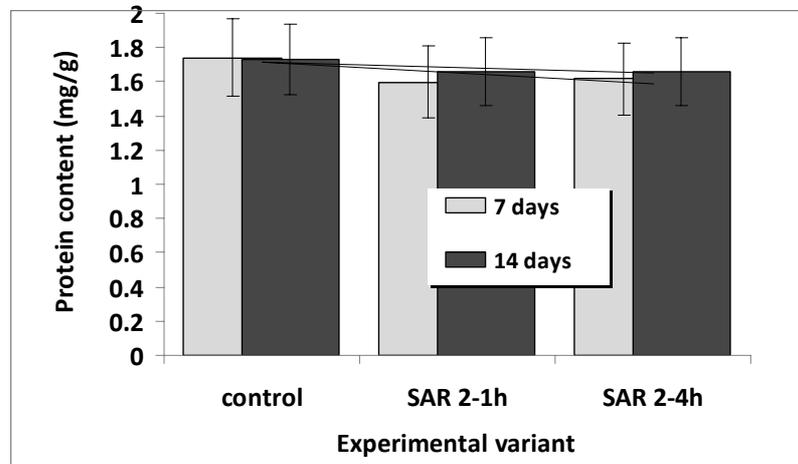


Fig. 4 – Protein content in fungus cells after electromagnetic exposure to SAR₂.

The picture of soluble protein content following exposure at SAR₂ looks different, with a diminution to the increase of exposure time at both ages of fungal culture: 7 and 14 days (Fig. 4). The highest decrease of soluble protein amount compared with control was observed at 7 days – of 4% and at 14 days – of 8% in case of long exposure duration (4 h). So the overall MW effect seems to be the biosynthesis inhibition for both SAR levels and exposure times – with linear trend for SAR₂. So, $Protein\ content = -0.0347 \times exposure\ time + 1.752$; correlation coefficient, $R = 0.8$ at 7 days; and $Protein\ content = -0.0605 \times exposure\ time + 1.772$; $R = 0.88$ at 14 days.

3.2. EFFECT ON PROTEIN FRACTION

It is not simple to estimate the sources of total protein content variation in the fungus cells where there is a large variety of proteins that could be affected by MW impact. The extracellular and intracellular protein expressions profiles in the fungus *P. chrysosporium* exposed to low power microwaves were studied by SDS-PAGE gel electrophoresis.

At 7 days the electrophoresis pattern shows, as expected, a multitude of small fractions in all the samples (Fig. 5). All these polypeptides that have been identified are distributed almost over the entire range of molecular masses comparatively with Sigma markers. Also there are several fractions heavier than 200 kDa but none with mass significantly smaller than 6.5 kDa. There are two representative electrophoretical fractions with molecular weight higher than 200 kDa (Fig. 5) with R_f of 0.400 and 0.435. There are no visible qualitative differences between the non-exposed samples and those exposed to microwaves - meaning no fractions missing nor additionally appeared.

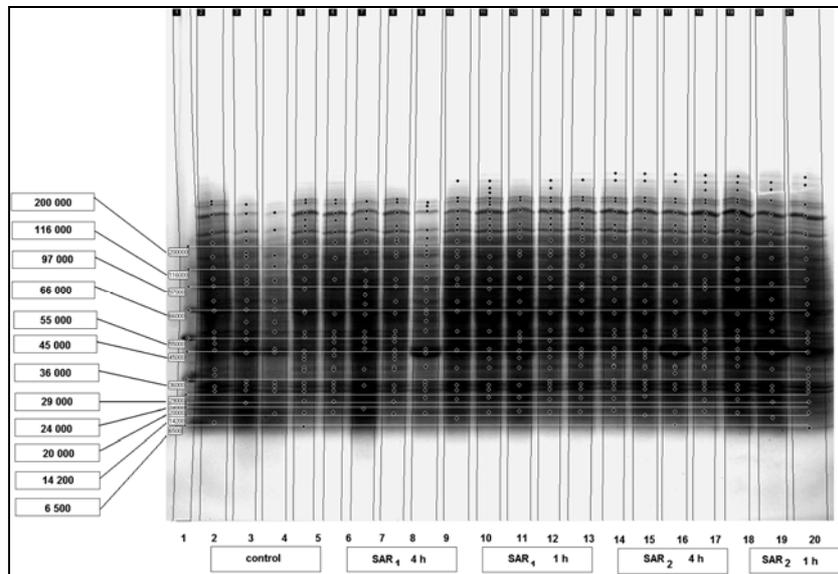


Fig. 5 – The extracellular expression protein profile of *P. chrysosporium* exposed at SAR irradiation after 7 days incubation. Lane 1: molecular weight marker; Lanes 2-5: control; Lanes 6-9: SAR₁ – exposed 4h; Lanes 10-13: SAR₁ – exposed 1h; Lanes 14-17: SAR₂ – exposed 4h; Lanes 18-20: SAR₂ - exposed 1h

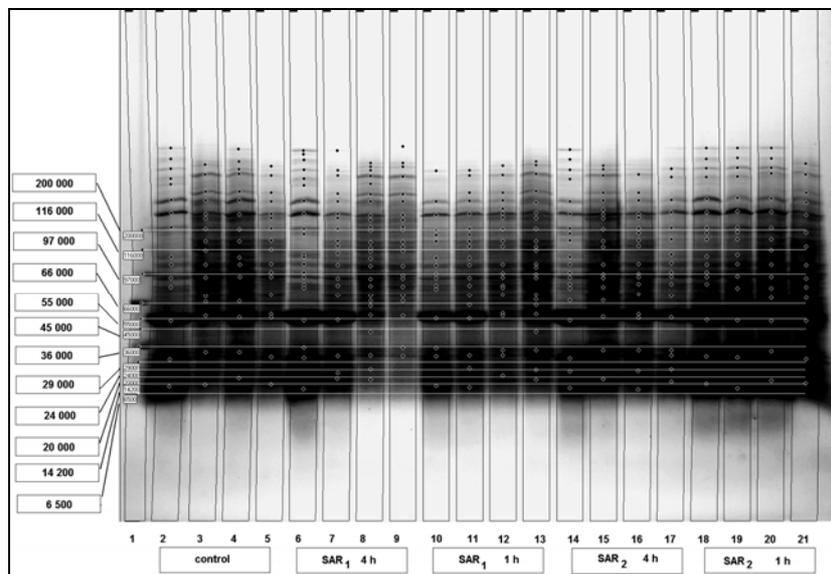


Fig. 6 – The extracellular expression protein profile of *P. chrysosporium* exposed at SAR irradiation after 14 days incubation. Lane 1: molecular weight marker; Lanes 2-5: control; Lanes 6-9: SAR₁ – exposed 4h; Lanes 10-13: SAR₁ – exposed 1h; Lanes 14-17: SAR₂ – exposed 4h; Lanes 18-21: SAR₂ – exposed 1h.

At 14 days there are fewer visible fractions than a week before but many of them tend to be slighter, with an intensified peak fraction around 55 kDa (Fig. 6), which suggests a correlation with the results of total protein assay. There has been a change in the distribution of protein fractions in the range of molecular masses with fewer fractions heavier than 200 kDa and with many smaller fractions with molecular mass lower than 6.5 kDa. This fact could be put in relationship with fungus metabolic evolution but not with electromagnetic exposure. Indeed, in 14 days old mycelium no significant changes between controls and exposed fungal samples could be seen – as in the case of 7 days old samples. In the older fungus cells there are additional protein fractions with molecular weight higher than 200 kDa characterized by electrophoresis parameter R_f of 0.379 (Fig. 6) compared to those found in 7 days old samples (Fig. 5). Therefore the total protein assay seems to be a better indicator on the microwave effects in fungus protein synthesis than protein fraction screening. Both SAR levels resulted in slight diminution of protein level. This result is important due to the fact that various biochemical parameters related to enzyme activity are estimated relatively to the protein content. Some of them are of particular interest in radiation biology when enzyme balance is difficult to determine and discuss as long as enzyme activity may be diminished following enzyme denaturation or the cell defense mechanisms may be activated to compensate damages induce by irradiation.

4. CONCLUSION

Low thermal microwaves as well as very low thermal ones induced slight but significant responses in the fungus cell metabolism. For low thermal radiation eventually slight thermal effect could be responsible for certain positive influence on the biosynthesis processes; the protein content diminution recorded in this experimental study revealed, on the contrary, subtle non-thermal effects with negative influence on cell metabolism. This could be assumed to occur also in natural environment under the atmospheric background of 900 MHz electromagnetic waves – intensively and extensively used in modern telecommunication system. Further study is planned to evidence if proteins with enzyme activity could be influenced following electromagnetic exposure - which is of interest for cellulolytic fungi ability of processing specific substrate through cellulolytic enzymatic equipment.

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