

# **THESIS OF DOCTORAL (PHD) DISSERTATION**

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**„Precision Plant Production Methods” applied Doctoral  
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**MICROWAVE EFFECT ON BAKER’S YEAST  
(*SACCHAROMYCES CEREVISIAE*)**

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Mosonmagyaróvár  
2011

# 1. INTRODUCTION AND AIMS

## *1.1. Introduction*

The electromagnetic and microwave emission of widely used devices has unknown and unexplored effects on their direct biological environment. There are conflicting results and interpretations in the literature concerning the biological effects, the hazardous character, and the different technological advances of non ionizing electromagnetic (EM) and radiofrequency (RF) microwave irradiation effects. The research of these effects is therefore essential. It is extremely important to draw attention to biological effects, because they may be hazardous on health and on their direct environment. Due to an unexpected effect of certain microwave irradiation, toxic materials or molecules, which are unable to penetrate under normal physiological conditions, can be transported into the cell. This can cause changes in living organisms. The biological effect appears in case, when there is a response on cellular level in the electromagnetic space. This is different whether the living organism is able to sense it or not. When the generated vibrations exceed a certain limit, the molecular structure of the membrane and consequently ion permeability will be modified. Ionic compounds, micro-, and macromolecules of dipole character are the primary targets of microwave irradiation effects. We examined the irradiation effect on cellular level and on liquid aqueous media, which is present in all living biological system.

## ***1.2. Aims***

The aim of our research was to examine the 2,45 GHz frequency microwave irradiation effect on yeast cell (*Saccharomyces cerevisiae*), first of all on cell membrane, then on water, which is an essential component in all liquid biological media.

In order to answer our questions our tasks were as follows:

- Examining the 2,45 GHz frequency at 37 °C constant temperature irradiation effect on *Saccharomyces cerevisiae*, and testing its biological effect.
- Determining changes occurring in the cell membrane that is the first basic barrier covering the living cell from outside.
- To detect the 2,45 GHz frequency continuous microwave irradiation effect on aqueous media, and to determine the occurring changes.

## 2. MATERIALS AND METHODS

We examined the microwave's effect on cellular and subcellular level under sterile conditions in the Institute of Biosystems Engineering of University of West Hungary, Faculty of Agricultural and Food Sciences.

### 2.1. *The examination of Yeast (Saccharomyces cerevisiae) Irradiation*

*Microorganism, strain:* we applied *Saccharomyces cerevisiae* M-26 strain in all of our experiments. This strain was prepared from industrial Baker's yeast from Budafok.

*Experimental liquid cell culture:* The experimental cell system was inoculated from primary culture of definite density value. It was inoculated at the same time with the same inoculum quantity in the flasks applied for the experiments, so that irradiation experiments became comparable with each other. Liquid cell cultures were incubated at 37 °C by shaking (160 rpm).

*Constant temperature microwave irradiation protocol:* Experimental liquid cell cultures were irradiated in Model MARS<sup>®</sup> (Microwave Accelerated Reaction System, CEM Corporation, Matthews, North Carolina 28106) microwave equipment. The conditions of the irradiation protocol were the following: 2,45 GHz, intermittent irradiation, 50 Watt (12% of 400W), 0–45 minutes, 37 °C constant temperature, ambient pressure. Irradiation was carried out in the exponential phase after a 120-minutes incubation time. To examine the

non-thermal effect, we applied constant 37 °C temperature during the time of irradiation and incubation.

*Incubation and detectation of irradiation effect on experimental liquid cultures:* All cultures were incubated for 24 hours, at 37 °C by shaking (160 rpm). At certain time intervals (120 min) samples were taken for optical density measuring. In each phase of growth cell morphology was examined under light microscope and it was controlled, whether there is no contamination.

Effect of different irradiation times, and different concentration of antibiotics (chloramphenicol, gentamycin, and neomycin-sulfate) added to fluid media culture were examined as follows:

Physiological growth profil of Yeast's strain applied for irradiation was taken. In the first phase of the research we examined whether microwave effects the normal physiological growth of liquid yeast culture or not.

Wide spectral antibacterial chloramphenicol, gentamycin and neomycin antibiotics were added in certain concentrations to prevent the contamination occurring often in cultures. Yeast growth was followed in cultures containing antibiotics without irradiation and after 45 min irradiation.

After we recognized the antibiotic induced growth inhibitory effect when irradiating the yeast, we examined the effect at different antibiotic concentrations by constant (30 min) duration of irradiation, and also different duration (5–45 min) of irradiation at constant antibiotic concentration.

## ***2.2. Irradiation experiment of aqueous media***

Water electrolysis was carried out to detect microwave effect. Samples of 1% NaCl water (200 mL) were used for examination. Irradiation was performed in a PANASONIC NNF 653 WF inverter type microwave oven with a FISO MWS-4 optic thermometer. Conditions of the irradiation setup were following: 2.45 GHz, 100W, continuous exposure, duration of 50 minutes, temperature scale: 12 °C – 45 °C. By measuring the speed of electrolysis we could determine the microwave effect on liquid samples. Immediately, 24 and 48 hours after irradiation electrolysis of constant fluid quantities were carried out in Hofman-voltameter equipment at 24 and 12V.

In preliminary experiments electrolysis of microwave irradiated water media and that of unexposed control samples was compared.

To examine the non thermal effect of microwave, two heating methods, so microwave and warming up on hotplate were compared with each other so that temperature couldn't exceed the 45 °C limit. The irradiated sample and the control sample treated on hotplate were warmed up beginning from the same temperature value at the same time with the same scale of warming up, so that samples were totally comparable.

It was examined the duration of the effect, that is how long after the irradiation is the sample able to store changes caused by microwave. Irradiated water samples were kept for 24 and 48 hours at room temperature after exposure and then the electrolysis was performed.

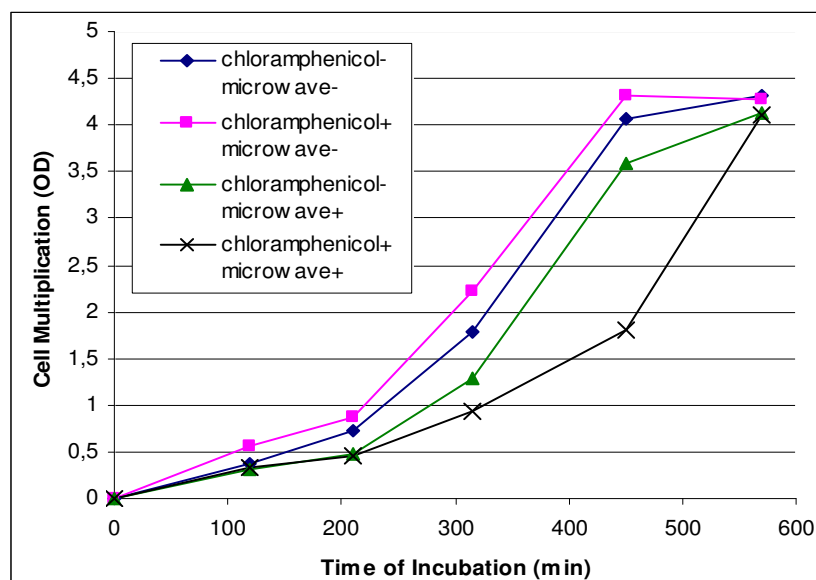
### 3. RESULTS AND DISCUSSION

#### 3.1. *The 2,45 GHz microwave effect on Yeast*

The optimized irradiation protocol (2,4 GHz, 37 °C, 50 Watt, 5–45 minutes) enabled normal physiological growth of liquid cell cultures and didn't inhibit the *Saccharomyces cerevisiae*. Our stable experiment cell system was appropriate to perform all irradiational experiments in a reproducible way under stable conditions.

To prevent bacterial contamination, wide spectral antibiomatic chloramphenicol was added to the fluid media culture. Chloramphenicol hasn't any antifungal effect under normal conditions, so we weren't afraid of any inhibition of yeast. However we observed a growth inhibition effect of this strain, when chloramphenicol was present in the cell culture during irradiation. This effect obviously failed, when there was no irradiation. After repeating the irradiation experiment in the presence of chloramphenicol many times, our results could be reproduced and a newly observed real phenomenon is determined. Thus, no expected circumstance lead us to get know the surprising phenomenon not published in the literature till now.

The phenomenon occurred, when further antibacterial antibiotics like gentamicin and neomycin belonging to the aminoglycosid group were applied. The microwave induced antifungal effect of the tested antibacterial antibiotics is confirmed (Fig.1.).



**Fig.1. The together effect of 45 minutes microwave irradiation and chloramphenicol ( $20 \text{ mgL}^{-1}$ ) on the growth of liquid yeast cultures**

It was determined, that the growth inhibitory effect raised in correlation with antibiotic concentration. We examined different durations of constant irradiation conditions (2,4 GHz, 37 °C, 50W) and constant antibiotic concentration on liquid cultures. It was detected, that in case of all the three tested antibiotics, microwave's inhibitory effect cannot be raised after certain duration of irradiation (40 min).

It was clarified, that microwave irradiation induced the sensitivity of yeast strain against the tested antibacterial antibiotics. The different durations of irradiation and the different antibiotic concentrations in the fluid media affect the uptake of antibiotics by yeast and the extent of the induced changes. The target of chloramphenicol, gentamicin and neomycin are the procaryotic ribosomes. These type of ribosomes in eukaryot cells are located in the mitochondria. The endosymbiont theory is the declaration for it, that is antibacterial antibiotics are protocellular protein synthesis inhibitors able to penetrate



through the protocellular membranes. They can pass easily through the cell membranes of protocellular organisms, so through the mitochondrial membrane as well, because this cell organelle together with its covering membrane has prokaryotic origin. The tested antibiotics aren't effective against yeast under normal physiological conditions, because they cannot get into the cell. However in our case they inhibited the multiplication of the yeast cells.

Declaration for this newly observed phenomenon is possibly a reversible membrane permeability change induced by microwave. Antibiotics able to get into yeast mitochondria inhibit the protein synthesis and so block the energy metabolism of the yeast cells. We suppose that microwave can cause a transient permeability change in living membranes changing the motility of cell membrane's phospholipids molecules in the lipid bilayer. The reason for that may be an induced rotation movement occurring in the chain of molecules. We can determine, a real biological effect existing in these mechanisms.

The experiment system seems to be appropriate for examining the biological effects of microwave irradiation.

The applied irradiation protocol can be an effective tool for cellular uptake of molecules unable to get into the cell under normal physiological conditions.

Further research is to be done to understand and clarify the cellular changes and the exact mechanism and of the observed phenomenon.

### 3.2. The 2,45 GHz microwave effect on aqueous media

The nonionizing radiation has effect on water molecules due to their dipolar character and we were able to measure it. In speed of electrolysis at 24 V there was an obvious difference (13%) between the microwave irradiated NaCl solution sample and the untreated control sample.

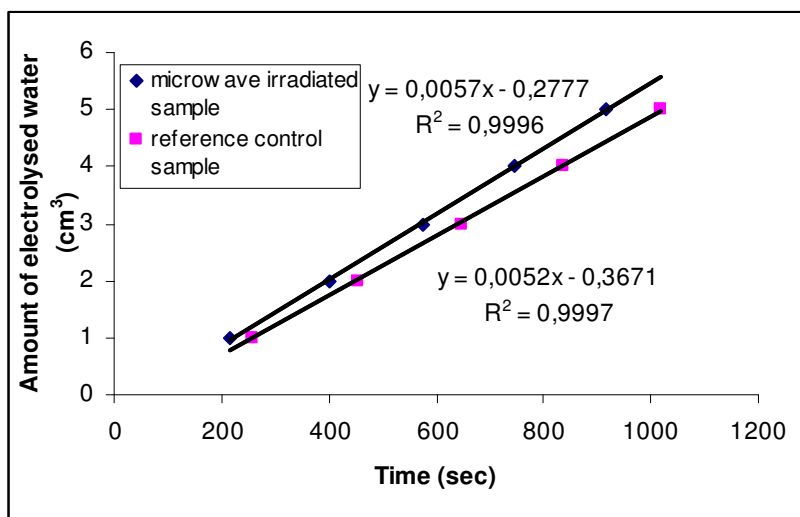


Fig. 2. Speed of electrolysis of irradiated and of reference control sample (non-irradiated, unheated)

Speed of 12V electrolysis was 10% higher of the irradiated samples than that of control sample heated on hotplate with same heating parameters, which can certify the non thermal effect.

Irradiated samples 24 and 48 hours after irradiation had higher speed of electrolysis, compared to that of controls. The experiments suggest that water was able to store the effect of low frequency microwave field 48 hours after irradiation. The results conclude that microwave irradiated samples are obviously faster when done the electrolysis, than control samples heated on hotplate or untreated control samples.

As conclusion it was determined that microwave effects among the different materials the water media aswell. The non ionizing radiation has effect on water molecules because of their dipolar character and inhomogeneous charge distribution. The effect induced microwave doesn't disappear immediately after irradiation, it remains detectable for a certain time. We suppose that under certain conditions of irradiation, water molecules acquire a transient oriented structure, which remains for certain time, but after a while they will be reorganized. Water is an essential and basic component of the different media, so it's presence can have an indirect effect on all microwave irradiated objects.

## 4. NEW SCIENTIFIC FINDINGS

Based on the results outlined previously, the novel scientific findings of this research can be summarized as follows:

1. The widely used microwave irradiation is permitted and strictly regulated by WHO (*World Health Organization*) and ICNIRP (*International Commission on Non-Ionizing Radiation Protection*) and has a lot of unknown advantageous and hazardous effects. In our research it was observed a phenomenon not known till now.
2. The application of optimized irradiation protocol (2,45 GHz, 37°C, 50W, 0-45 min) could prove a specific biological effect on Yeast cultures. The applied constant temperature nonthermal irradiation didn't inhibit the growth and multiplication of *Saccharomyces cerevisiae* cells.
3. Irradiation induces the transport of materials of certain molecular size and character from outside into the inner cellular space of the yeast cell. The applied irradiation protocol seems to be an effective tool for facilitating the uptake of those compounds into cells, which cannot penetrate under normal physiological conditions through the cell membrane. Monitoring the effect in case of *S. cerevisiae*, antibacterial antibiotics with low molecular weight: chloramphenicol, gentamicin, and neomycin were suitable.

4. The combined effect of irradiation and of antibacterial antibiotics, non inhibiting the yeast cells under normal conditions produces well detectable growth inhibition. The reason for the newly observed phenomenon might be in case of the lacking previous information the consequence of a transitory, reversible change in plasma membrane permeability upon irradiation. The experimental system seems to be suitable to study the different microwave effects.
5. Irradiating different aqueous media, microwave has effect on the water as well. It was detected by electrolysis, that water as storing media is able to keep changes after irradiation for certain time, even for 48 hours. It has been confirmed, that the reason for that is not the heating, but microwave effect.
6. Comparing the irradiation within definite temperature range and the heating on hotplate, the non thermal microwave effect of 2,4 GHz frequency in water media was determined. Beside the thermal effect of microwave there is the non thermal microwave effect. Further research is to be done to clarify and understand the exact molecular mechanism changes caused by radiofrequency irradiation.

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