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EVALUATING THE EFFECTS OF NON-THERMAL MICROWAVE EXPOSURES ON THE PROLIFERATION RESPONSE OF SACCHAROMYCES CEREVISIAE YEAST

PROCENA EFEKTA NETERMALNOG MIKROTALASNOG ZRAČENJA NA PROLIFERACIJU ĆELIJA KVASCA SACCHAROMYCES CEREVISIAE

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Ključne reči
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proliferacija, ćelije kvasca

Abstract

This study evaluates the effect of non-thermal weak radiofrequency microwave (RF/MW) radiation on the proliferation response of the yeast Saccharomyces cerevisiae. S. cerevisiae strains type II (Sigma) were exposed to the microwaves at 900MHz and the selected powers of 13dBm, 3dBm and -7dBm using the Transverse Electro-Magnetic (TEM) cell. The average specific absorption rate (SAR) for a single cell was 0.12 W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells. In these experiments yeast cells were continuously exposed to the MW radiation. Changes in yeast culture growth were monitored using the spectrophotometry method. Measurements of the yeast cells' growth in control (sham-exposed) vs. irradiated samples were performed. Experimental data were collected and statistically analyzed. The results revealed that the rate of yeast growth was increased at the MW exposures at 900MHz and powers of 13dBm and 3dBm.

INTRODUCTION

In the last few decades, the use of microwave radiation has greatly increased in radar and communication systems, food-processing technology, and other industrial applications. The development of consumer and medical microwave devices for clinical diagnosis and therapy also has prompted widespread interest and has stimulated much research into the mechanisms of interaction between microwave radiation and living matter [1].

Experimental studies made in the millimeter band at very low microwave energy flux densities (no more than a few milliwatts per square centimeter) induced highly specific effects of applied radiation. The results revealed: (a) the effect of irradiation depends strongly on the frequency of the microwaves; (b) in certain microwave-power ranges, the effect of exposure depends weakly on variation of the power through several orders of magnitude; (c) the observed effects are significantly dependent on duration of exposure. A resonant effect of microwaves on the division rate of both the cell cultures and yeast was observed [2].

Two types of effects can be ascribed to microwaves, i.e. thermal and non-thermal. The heating effect of microwaves is already well known and documented [3], however, doubt remains on the existence of non-thermal biological effects. The thermal effects are related to the heat generated by the absorption of microwave energy by the water medium or by complex organic systems [4].

Non-thermal biological effects are measurable changes in biological systems that may or may not be associated with adverse health effects. It was demonstrated that extremely low power microwaves can affect enzymes activities [5],[6]. Little is known about the molecular mechanisms involved in putative non-thermal effects. One hypothesis is that microwave radiation can induce dipole oscillations in a protein's active site and thus, can alter its function [7].

Yeast cells are single celled eukaryotic fungi organisms that reproduce asexually by budding or division (Pic. 1) [8]. While yeast can vary in size, they typically measure 3-8 ìm in diameter. Saccharomyces cerevisiae is the most commonly used strain in scientific research, baking and fermentation

and has become synonymous with the term yeast. Yeast has been used for thousands of years to ferment alcohol.

Yeast Growth Phases:

When cultured for the fermentation of beer, yeast cells in culture follow a very predictable pattern of growth that can easily be divided into four phases: (1) lag; (2) log; (3) deceleration; and (4) stationary. During the lag phase, no growth occurs as newly pitched yeast cells mature and acclimatise to the environment (Fig. 1). This is followed by the log phase, where cells are rapidly growing and dividing. Nutrients are in excess relative to cell number and waste is being sufficiently diluted as to be insignificant. The growth rate in this phase will follow first order kinetics. As cell numbers increase, cell growth begins to slow as a number of parameters (e. g. substrate and waste), each with saturation effects, become significant. Eventually the yeast cells reach the stationary phase, where no growth occurs due to high waste concentration or complete substrate consumption (Fig. 1).

In this study we applied non-thermal low intensity microwaves at the 900 MHz and the powers of 13dBm, 3dBm and -7dBm to irradiate S. cerevisiae yeast for evaluating the hypothesis that the external electromagnetic field (EMF) of the specific frequency and power affects the proliferation response in yeast.

MATERIALS AND METHODS

A. Microwave exposure system

As a source of microwave radiation, we used a TC-5062AUHF TEMCell (100 kHz–3 GHz) from TESCOMLtd (Unitechvill, Goyang,Korea). Through the input port, an external signal was applied to generate a predictable field inside the Gigahertz Transverse Electro-Magnetic (GTEM) Cell. The GTEM was used to irradiate the yeast samples.

The GTEM was calibrated using a broadband electric field probe to determine the electric field produced at the sample position inside the camera for a given input power. We scaled the field values provided for 10 dBm (10 mW) input to the power levels applied, using the equation (TC-5060 Manual 2005):

$$E_1 = E_0\sqrt{\frac{P_1}{10mW}}$$

Where E1 is the exposure field of the sample, E0 is the calibration field we found using a test power of 10 mW, and P1 is the test power we used in our exposure.

The calibration test showed that the estimated uncertainty in the generated test field is ±1–3% depending on the input signal frequency. The calibration test was performed by TESCOM laboratory (Tescom Company Limited, Goyang, Korea).

The operation principle of the TC-5062A is essentially the same as the TEM Cell with the applied RF voltage on one port of the cell, while the other port is terminated with a 50 ohms resistor. The TEM Cell maintains 50 ohms characteristic impedance along the cell. The E–H field inside the test volume is proportional to the input voltage and inversely proportional to the cell height. The TC-5062 has a specific pyramidal geometry designed to extend the usable frequency range (Pic. 2a). Typically, this can be achieved by replacing one port of a two-port TEM cell with a wideband non-tapered hybrid discrete wave absorber termination. The GTEM cell, described thoroughly by Nothofe [10], is a similar pyramidal cell that produces a similar field configuration.

Since the TEM Cell produces the TEM waves, there is an orthogonal H-field (A/m) proportional to the E-field inside the TEM Cell. The relationship between the H- and E-fields is defined by the equation:

$$H(A/m) = \frac{E(V/m)}{377\Omega}$$

where 377 Ω is the free space wave impedance.

The electrical field inside the TEM cell can be calculated using the equation:

$$E(V/m) = \frac{V(V)}{L(m)}$$

For the model TC-5060 UHF TEM, the length (L) is 0.22 m (Pic. 2b). It is a wide band TEM Cell with absorber termination. The large absorber wall eliminates potential resonance inside the cell and produces a wide band of operation. If the radiofrequency (RF) test signal from the signal generator is injected into the TEM Cell input port, the predictable TEM mode field is generated at the test position. In the case of a direct cable connection, the percentage of the leaked RF power compared to the signal is very small. The TC-5062A is an accurate, broad band RF coupler with a high quality shielding wall.

In addition, the voltage standing wave ratio (VSWR) of the TEM cell was tested with the sample holder (no sample) using microwaves within the interval of 300 MHz–3 GHz by Measuring instruments for Wireless Communications''. It was reported that the VSWR has a maximal value of 1.7 for 3 GHz. Therefore, the power propagating through the TEM cell can be calculated as follows:

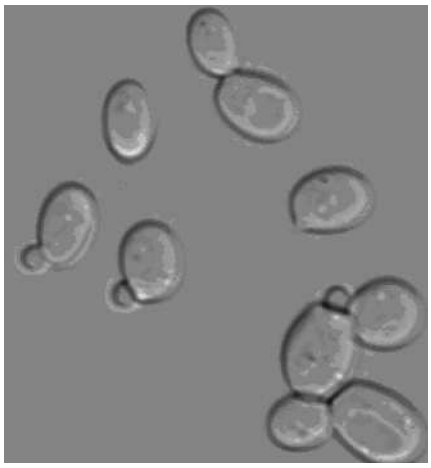
$$P_p = P_g\left(1-\frac{VSWR-1}{VSWR+1}\right)$$

where Pp is the power propagating through the cell, and Pg is the generator power. For VSWR = 1.7, the ratio Pp=Pg>0.93.

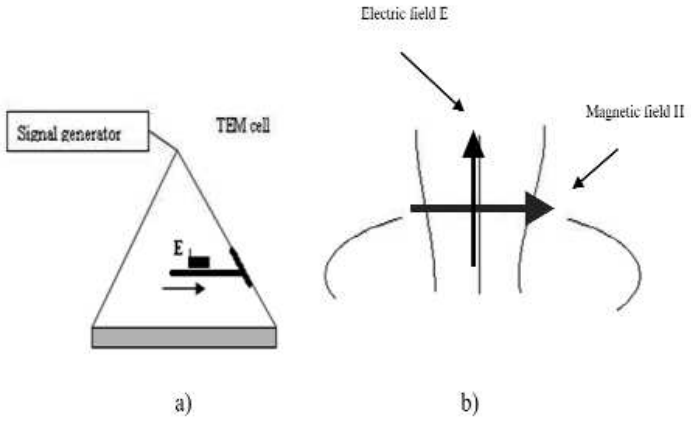
The signal generator used in this study was a Rhode & Schwartz (100kHz–1000MHz) SMX generator (Munich, Germany). The spectrophotometer was located outside the TEM cell.

B. Experimental study

The S. cerevisiae yeast powder was purchased from Sigma (Australia). The experimental solution was prepared as follows: 50 g/l of YPD broth (Sigma, Australia); 20 g/l of S. yeast, and ionized water. The solution was incubated at 24°C for 72 hrs. Then the solution was kept at 4°C. The yeast samples were prepared by diluting the experimental solution as 1 ml in 100 ml of the ionized water (1:100). The yeast samples were placed in the 20 ml bottles. The bottles (external dimensions are h=50mm; d=25mm, V=20ml) were



Picture 1. Saccharomyces cerevisiae[8]



Picture 2. a) Position of the sample and direction of the electric field inside the TEM cell. The vertical distance from the top of the cell to the sample is 22 cm. b) Field pattern at the position of the sample (top view).

filled with the aliquot (liquid's column height of 45mm) ands kept at 22 cm distance from the top of the TEM cell (Pic. 2).

In these experiments, three yeast samples were irradiated continuously for 6 hrs and other three control yeast samples were sham-exposed for the same time duration. The absorption characteristics of each (3 exposed and 3 non-exposed) samples were measured every 1 hr. Changes in yeast culture growth were monitored using the spectrophotometry method. Spectrophotometric analysis is based on turbidity and allows for indirect measurement of a number of yeast cells. The absorption coefficients of the yeast samples were measured using an Ocean Optics USB2000 spectrometer.

The experimental data obtained were collected and are presented in Fig. 2a-2c. The results obtained show the changes in normalized concentration of yeast cells in time.

Table I. Relative change in proliferation response of yeast cells under the influence of the MW at different powers

Microwave power	13dBm	3dBm	-7dBm
Irradiated vs Non-irradiated sample	14%	11%	-1%

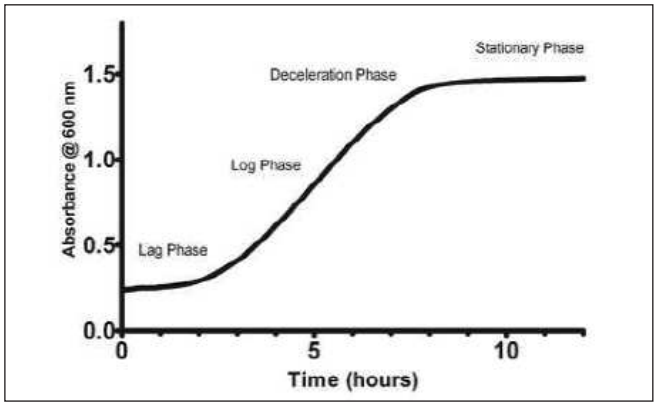


Figure 1. Typical yeast growth curve. Saccharomyces cerevisiae grown in YPD media at 30°C for 12 hours with data measurements every 2 minutes [9].

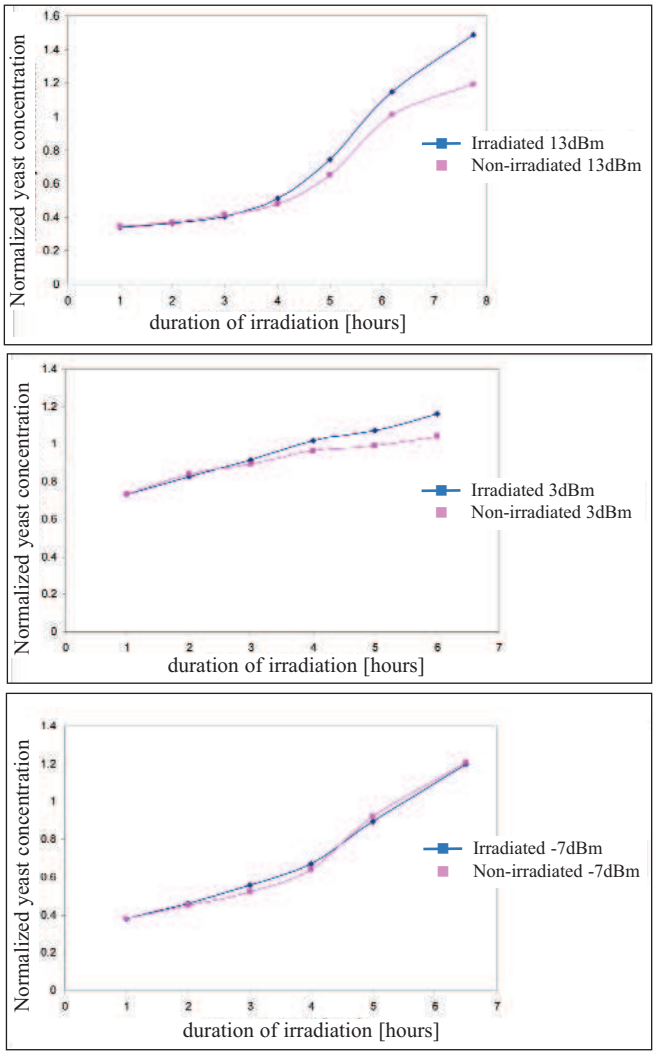


Figure 2. Changes in normalized concentration of yeast cells in time (non-irradiated vs. irradiated) at 900 MHz and the powers of: a) 13dBm, b) 3dBm and c) -7dBm

RESULTS AND DISCUSSIONS

In this study S. yeast solutions were exposed and sham-exposed to the MW radiation at 900 MHz and powers of 13dBm, 3dBm and -7dBm. Table 1 presents data on relative change (%) in proliferation response of yeast cells achieved upon irradiation of the yeast samples.

The results presented in Fig. 3a-3c indicate that the MW radiation at 900 MHz and powers of 13dBm and 3dBm affect significantly (14% and 11% respectively) the concentration of yeast cells and thus, it is concluded that the external irradiation can modulate the proliferation rate of the exposed yeast cells.

CONCLUSION

This experimental evaluation was aimed to test the hypothesis that the external low power MW radiation can affect the biological activity (proliferation rate) of the yeast cells. In this study we applied the MW exposures at 900 MHz and powers of 13dBm, 3dBm and -7dBm to the experimental yeast solutions.

The yeast samples were exposed and sham-exposed for 6 hours. The results obtained show that the MW radiation at 900 MHz and power of -7dBm produced no effect on the yeast samples growth. However, the MW at 900 MHz and powers of 13dBm and 3dBm affected significantly (14% and 11% respectively) the concentration of yeast cells in the experimental solution. These findings imply that the external non-thermal MW radiation at the selected frequency and powers can modulate the proliferation of the exposed yeast cells.

Apstrakt

Ova studija analizira efekat ne-termalnog mikrotalasnog zračenja malog inteziteta na proliferaciju kvasca Saccharomyces cerevisiae. Kvasac S. cerevisiae tip II (Sigma) je bio eksponiran mikrotalasnom zračenju na 900 MHZ za tri različita inteziteta zračenja 13 dBm, 3 dBm i -7 dBm koristeći transverzalnu elektromagnetsku ćeliju. Srednja specifična apsorpciona vrednost (SAR) za kvasce je 0.12 W/kg. SAR je procenjen usrednjavanjem individualnih parametara ćelijskih komponenti u saglasnosti sa delom zapremine koji oni imaju unutar ćelije S. cerevisiae. U svim eksperimentima je korišćeno kontinualno zračenje. Promene u brzini rasta kulture kvasaca su praćene spektrofotometrijskom metodom.. Izvršeno je merenje brzine rasta kontrolnih ćelija (neozračenih) u poređenju sa brzinom rasta ozračenih ćelija. Eksperimetalni podaci su sakupljeni i statistički analizirani. Rezultati merenja jasno pokazuju povećanje brzine rasta ćelija kvasca usled ozračavanja na 900 MHz na snagama od 13 dBm i 3dBm

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