Dariusz SZTAFROWSKI[1], Zbigniew WRÓBLEWSKI[1], Marcin ŁUKASZEWICZ[2,3]

Institute of Electrical Power Engineering, Wrocław University of Technology, Wrocław, Poland (1)
Faculty of Biotechnology, University of Wrocław (2), Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland (3)

Survey identification of 50 Hz magnetic field impact on selected biological processes in the model organism *Saccharomyces cerevisiae*

Abstract. *The paper presents results of a research to determine the impact on selected biological processes in a model organism Saccharomyces cerevisiae of 50 Hz variable frequency magnetic field. The investigations have been performed on testing stand constructed at the Institute of Electrical Power Engineering, Wroclaw University of Technology.*

Streszczenie. *W artykule przedstawiono wyniki badań wpływu przemiennego pola magnetycznego 50 Hz na wybrane procesy biologiczne organizmu modelowego Saccharomyces cerevisiae. Badania przeprowadzono na zaprojektowanym w Instytucie Energoelektryki Politechniki Wrocławskiej stanowisku badawczym. (**Wyniki badań wpływu przemiennego pola magnetycznego 50 Hz na wybrane procesy biologiczne organizmu modelowego Saccharomyces cerevisiae**)*

Keywords. magnetic field 50 Hz, fluorescent probes, *Saccharomyces cerevisiae*
Słowa kluczowe: pole magnetyczne 50 Hz, badania, sondy fluorescencyjne, *Saccharomyces cerevisiae*

Introduction

Alternating current of power frequency (50/60Hz) has been used in industry and human households for approx. 120 years and its operation is accompanied by emission of many physical and chemical agents into the atmosphere. People more and more often ask a question whether being within immediate vicinity of electromagnetic fields sources has an impact on their health or can have negative consequences in the future. Insofar experimental researches described in the literature concerning impacts of lowest frequency electromagnetic field (ELF) with living matter describe existence of various biological phenomena taking place under its influence [1-6]. Due to slight knowledge about ELF impacts on living organisms, building of new power engineering facilities, including high voltage overhead lines, quite often triggers anxiety and protests of people living in the vicinity. At the same time, mass media usually broadcast negative or sensation-coloured information about threats related to electromagnetic fields (e.g. cancerous diseases), which, in inaccurate forms, get to the society and thus evoke a feeling of a threat. Such unfortified information creates a belief that life and work nearby facilities emitting power frequency electromagnetic field is dangerous for humans and the environment in which they live. This creates manifold difficulties while building new power engineering facilities or modernizing the already existing type of facilities. An example of this can be rebuilding of a single power line 220 kV to a 4-way power line (2x220 kV, 2x400kV) in Kamionki Borough near Poznan - figure.1. The construction was protested against by people living in one of the boroughs in the immediate vicinity of the executed investment (Kamionki Borough). The said inhabitants reasoned their objection by landscape destruction and negative impact of the line being rebuilt on the natural environment surrounding it and a threat to human life resulting from emitting by the line higher levels of electric and magnetic field in comparison to conditions generated by the previous line facility existing there. The anxiety is because of insufficient knowledge what causes a threat that new conditions will be less favourable from previous ones. At the same time it must be stated that the line, after rebuilding, will be generating comparative and, in some cases, lower values of electromagnetic and magnetic fields intensity 50Hz. This is possible, because in the vicinity of a multiple power line the electromagnetic field value at a given point is influenced by such factors as: line voltage and current intensity, diameter of wires and, in the case of contactors, also their distance in a bundle, distances between various phase wires, geometric configuration of phase wires, possible compensation of particular field constituents resulting from mutually located wires of the same phase in various lines (in the case of multiple lines).

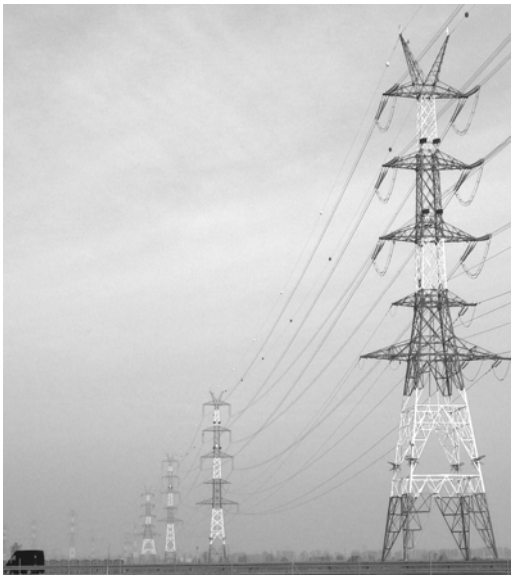

Fig. 1. Rebuilding of a single power line 220 kV to a 4-way power line (2x220 kV, 2x400kV) in Kamionki Borough near Poznan

At one hand such situations result in huge material losses related to lengthened completion period of the investment and increased cost of its execution, due to changing the route of the transfer line existing for years. On the other hand, considering this problem, one must not disregard the fact that the impact of electromagnetic fields on human organism is not neutral and analysis of available literature does not provide an unambiguous answer as to all health-related potential side effects resulting from operating these types of facilities located in direct vicinity of human habitats. Low-frequency magnetic field, in intensity values present in the neighbourhood of some industrial facilities (e.g. welding machines) may cause metamorphopsia, amaurosis (lost of sight vision when the field is active - from

several mT induction value - own research) [7], but it may also have a positive impact on human organism adding to, for instance, fight with some conditions. (e.g. facilitated knitting of bons, wound-healing). Between such extreme situations there is a number of cases in which, owing to professional, health and other reasons, the problem of safe exposition levels to the aforelisted factors should be considered. The issues are yet not well-examined and researches need to be made in this field. That is why concrete and objective analysis of this type of issues should be a subject of scientific analyses by experts representing various fields of science. Objective results can only be obtained at a level of interdisciplinary research works [8,9,10].

The impact of this factor can be examined at various organization levels of biological live organisms. It is possible to conduct the research exposing entire live organisms and such type of research is classified as medical research, which can be divided into two basic types: epidemiological and medical examinations. Yet different type of examinations is *in vivo*. The term is usually used when describing biological examinations and it refers to something which takes place inside a live organism - in a living cell. Research conducted on live cells or substances isolated from an organism are classified as *in vitro* experiment. At each biological organization level the research is of a slightly different character. Methods of application differ and way of drawing concussions in different, pursuant to obtained results.

The paper described 50 Hz magnetic field impact on *Saccharomyces cererevisae* live organism. The research on impact caused by 50Hz magnetic fields of 60-68 /m intensity on eukaryotic cells survival rate in *Saccharomyces cerevisiae* organism was conducted using fluorescent probes. The applied magnetic field range is at the limit value of allowable electromagnetic fields of 50Hz frequency that can be present in the environment inhabited by people on the territory of Poland. The level was defined, in detail, in the Decree of the Minister of Environment of October 30th 2003. For a magnetic constituent (H) of electromagnetic field the maximum allowable value of field intensity in places accessible for people, without time restrictions, is 60 A/m, what matches the intensity level of 50Hz magnetic constituent, generated by lines and power engineering facilities in natural environment [11].

Measurement by using fluorescent probes

In this type of research it is very difficult to observe short-time fluctuations which go back to the initial condition owing to normal regulation and adaptation cell mechanisms. That is why many modern methods base on fluorescent probes. Apart from substrate specificity, the probes can preferentially accumulate in certain compartments thus enabling examination of reactions taking place in certain organelle, such as mitochondria or chloroplasts. Fluorescence cam also depend on subtle changes in hydrogen ions concentration, which, theoretically, enables examination of such subtle changes as membrane polarization, being the basis for power change taking place in a cell. A great advantage of fluorescent probes is high sensitivity of the measurement method. one particle of the fluorescent probe can, if exposed to a wave of suitable length, emit many photons. Unfortunately, this is not an infinite process. Probes are subject to photo-deactivation (damage of chemical structure due to absorbed light). That is why researches on new probes are still being conducted. The greatest advantage of fluorescent probes is possibility of using them *in vivo* and conduct practically real-time measurements. To make this possible it is necessary to insert the probe into cells (overcoming the barrier created by selectively permeable biological membranes). As far as biology is concerned, the probes must be relatively safe for metabolism so that the observed changes reflect the impact of the examined factors, not the probe itself. Until present the fluorescent probes meant to examine the impact of electromagnetic field on live organisms have been used in few research works. One of aims presented in the paper was verification of the probes' usability in these types of research [12].

Materials and methods used in the research

In the conducted research a conventional exposition stand was used to expose biological materials in alternating magnetic field of power frequency. Building this exposition stand was based on so-called Helmholtz coils system, which enables achieving a highly uniform magnetic field in a significant volume of inter-coil space - figure 2. This is one of simpler and, at the same time, reliable structures, not requiring special supply systems and thus it constitutes one of the best solutions for exposition purposes of biological materials. The stand guaranteed possibility of adjusting the magnetic field intensity within a range from 0 to 5000 A/m.

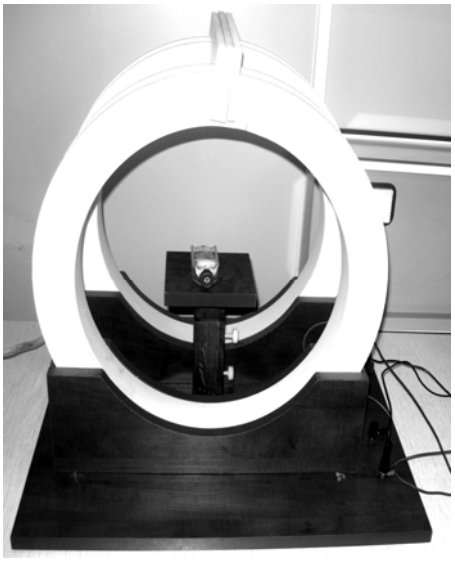

Fig. 2. The research station (Hemholtz coils system)

In the conducted research the following measuring devices were used to monitor field conditions: Emdex II, Holaday HI-3627, Power Quality analyser Fluke 43, Multimeter Fluke 45, Multimeter Fluke 8846A, a fluorescent microscope by Zeiss model Axiovert 40, Axiocam camera and Axiovision 4.6 computer program.

Research on the magnetic field impact on the model organism *Saccharomyces cerevisiae*

Research on the 50Hz magnetic field impact on increased mortality rate among *Saccharomyces* cerevisiae strain and verification of usability of the designed laboratory stand for observation of biological processes taking place during exposition in the 50Hz magnetic field was conducted. The induced magnetic field was within a range 60-68 A/m. The temperature value during exposition period was 24-26^{o}C. Samples of the examined strain were divided into three groups: positive group (not subject to the field impact), examined group and negative group (with cells thermally killed by autoclaving). For the purpose of further differentiation between dead and live cells, during observation under the fluorescent microscope, *Saccharomyces* cerevisiae cells, after exposition in the

50Hz magnetic field (or not exposed, in the case of positive control) were marked by the fluorescent probe. *LIVE*/*DEAD*® *Yeast* Viability Kit (Invitrogen). *was used for that purpose.* FUN1 probe was used in the conducted research. Affinity of this colorant to nucleic and its endogenic biochemical transformation process cause that as a result of that FUN1 probe, which constitutes a dispersed amount of colorant inside the cell, while the colorant shows green fluorescence, is converted to a shape forming cylindrical structures inside vacuole and emitting red light. This transformation requires both integrity of the cell membrane and metabolic activity of the cells. Therefore only metabolically active cells have red-colouring fluorescent structures inside their vacuoles, while dead cells show dispersed green-yellow fluorescence. To mark the cells they were whirled, supernatant was removed and cell deposit was suspended in 1 ml of GH buffer (10 mM HEPES, 2% glucose, pH=7,2). The same buffer was used to dilute FUN1 colorant to 12.5 μM concentration. 50 μl of probe and 50 μl of cell suspension in GH was pipetted to 1,5 Eppendorf-type test tubes obtaining 6.25 μM probe concentration. This was incubated in dark, at 28^{o}C, for 30 min, and after that microscope observations took place. From cerevisiae strain culture on a stable base YPG (1% yeast extract, 1% bacto-peptone, 2% glucose, 2% agar-agar) inoculum were sampled and 5 ml of liquid YPG culture medium was inoculated thus forming a pre-culture which was observed over the night at 28 ^{o}C. The next day 200 ml of fresh, liquid YPG culture medium (as above, yet without agar) was inoculated) to 4 ml of culture and left for the night at 28^{0} C. Optical density after the night cultivation was 2,92 (λ= 600). The culture was poured into 4 ml test tubes, which were subject to magnetic field exposition. Dead cells show green fluorescence in the entre volume. Live cells are characterised by red fluorescence of fine grainy structures inside vacuoles. The experiment was performed on both research stands. Cells in the control groups and groups subject to 50Hz magnetic field exposition during 60, 120 and 180 minutes were counted. Cultures used for the experiment were in a logarithmic phase of growth.

Table. 1. Results of the 50Hz magnetic field impact on increased mortality rate among *Saccharomyces* cerevisiae strain before and after exposition in 50Hz magnetic field. MF - data obtained after 50Hz magnetic field impact on the examined samples

Time	left-side Student test		right-side Student test	
	p-value		p-value	
60 & 60 MF	0,955		0,045	MF decreases the cell death rate
120-& 120MF	0,079		0,922	
180 & 180 MF	0,033	MF has an impact on the cell death rate	0,967	

Discussion about results of the 50Hz magnetic field impact on the model organism *Saccharomyces cerevisiae*

Results of the research (table 1) were subject to an analysis meant to determine, whether 50Hz magnetic field has an impact on the ageing process and survival rate of the model organism cells - *Saccharomyces cerevisiae* differentiated by FUN1 fluorescent probe at 5% significance level. The analysis makes it possible to formulate the following statements: Magnetic field of the considered parameters has a significant impact on the number of dead cells present in *Saccharomyces* cerevisiae population while the impact depends on exposition period of the examined organism in the field. In the case of short-lasting exposition, approx. 60 minutes, one can observe decreased percentage of dead cells in the group subject to magnetic field exposition in relation to the control group, on which the magnetic field had no impact.

After 120 minutes impact of 50Hz magnetic field on the examined parameters was not observed. For exposition periods being 180 minutes percentage of dead cells in relation to the control group increased.

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***Autorzy**: dr hab. inż. Marcin Łukaszewicz, Uniwersytet Wrocławski, Wydział Biotechnologii ul. Przybyszewskiego 63/77, 51-148 Wrocław, Politechnika Wrocławska, Wydział Chemii, ul. Wybrzeże Wyspiańskiego 27, 50-370 Wrocław E-mail: marcin.lukaszewicz@pwr.wroc.pl, prof. dr hab. inż. Zbigniew Wróblewski E-mail: zbigniew.wroblewski@pwr.wroc.pli, mgr Dariusz Sztafrowski, E-mail: dariusz.sztafrowski@pwr.wroc.pl Politechnika Wrocławska, Instytut Energoelektryki, ul. Wybrzeże Wyspiańskiego 27, 50-370 Wrocław.*