INFLUENCES OF 50HZ ELECTRIC FIELDS ON GROWTH AND MULTIPLICATION OF SOME MICROORGANISMS

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Abstract: By dielectric spectroscopy and specific microbiological techniques have been studied the development (cell multiplication) of *Saccharomyces cerevisiae* and *Aspergillus niger* growing and multiplication - both on culture media with sucrose and on those with starch. The experimental results have been revealed that the biochemical processes of the studied biomasses have significant changes in certain frequencies in the range 1 - 160Hz, characteristic of the different species/metabolized carbon source, respectively species/ metabolized carbon source/ development phase. It has also been found that in the case of *Saccharomyces cerevisiae*, the electric field of 50 Hz up to 20V/cm reduces the length of the LAG time and increases the growth rate of intensive increasing phase. Cultures of *Aspergillus niger* on culture medium with sucrose are stimulated (increasing the spores production by approx. 50% and reducing the maturity time) of 50Hz signals up to 15V/cm. Electric fields higher than 30 V/cm in all media and investigated cultures carry on to the growth reduction / multiplication - up to a complete inhibition of growth at approx. 50V/cm.

Key words: 50Hz electric field, electromagnetic pollution, *Aspergillus niger, Saccharomyces cerevisiae,* dielectric spectroscopy

1. INTRODUCTION

The impact of anthropogenic electric and magnetic fields on living matter is an interdisciplinary issue, theoretically complex and of great practical importance.

The theoretical complexity of the issue is arising both from its interdisciplinary and from the many biochemical processes whose mechanism and kinetics involves transport phenomena and transfer of groups carrying electrical charges, condensation equilibrium / hydration, redox reactions (by load transfer) which are influenced by the electric field applied to the reaction medium.

Living matter has been developed and evolved in the specific conditions of natural electric and magnetic field of the Earth, in which case any field of anthropogenic origin applied on growth environment can distorts, influences, perturbs the natural processes of metabolism, growth and reproduction. Consequently it can be considered that, any electric or magnetic field of anthropogenic origin is a disturbing factor for the natural progression of the life, therefore a pollutant (electromagnetic pollution) [1]. The influences of electromagnetic fields on living matter are highly complex, effects are determined by species, influenced biochemical processes, by the amplitude and shape of the disturbing signals applied etc. - situation which may be beneficial or harmful to the development of living matter [2]. Therefore, the application of perturbing signals on living cells can produce changes in: the cell proliferation [3-5], the enzymatic activity [6, 7], gene expression [8, 9], cell permeability and ions homeostasis [10-12], also oxidative stress [13-15] and response to heat shock [16, 17] etc.

Recent studies have been shown that the 50Hz electric field produces major perturbations in behavior and metabolism of the *Saccharomyces cerevisiae* yeast [18-20]. Laboratory studies have been revealed that the synergistic action of the 50 Hz electric field and of filamentous fungi, the corrosion of carbon steel is substantially enhanced [21].

Also, several field investigations (case analysis) have been revealed that filamentous fungi from the soil have a decisive role in the degradation of external polymeric jacket [22-27], fact which can be explained by enhancing the metabolism of the fungi under 50 Hz field action [28] which is established between the metallic shield of the cable and the soil. Interferences issue caused by overhead high voltage power lines on vegetation has been addressed in [29].

Given these considerations, the aim of the paper is the experimental determination of the influence of extremely low frequency electric fields, particularly of the 50Hz on the growth and multiplication of microorganisms.

2. EXPERIMENTAL – WORKING PROCEDURE

In order to evaluate the influence of extremely low frequency electric fields on the micro-organisms have been carried out investigations by dielectric spectroscopy (in the range 1 - 160Hz), both on the suspension of the *Saccharomyces cerevisiae* yeast with 5% sucrose and 5% starch, and on gelled culture medium type CZAPEK-

DOX inoculated with *Aspergillus niger* having the sucrose and starch as easily assimilable carbon source.

It has also been made microbiological determinations, comparative on suspension of *Saccharomyces cerevisiae* with 5% sucrose and on CZAPEK-DOX gel medium inoculated with Aspergillus niger, both under natural conditions (without exposure to electric field) and exposed to 50 Hz electric fields with intensities up to 70V/cm.

Dielectric spectroscopy measurements were performed on specialized equipment - Ametek Solartron Analytical 1260A Impedance/Gain-Phase Analyzer. Evolution of the *Aspergillus niger* has been evaluated by microbiological observations (optic microscopy) and the production of spores by counting in Bürker-Türk chamber. The rate of multiplication of *Saccharomyces cerevisiae* cells was determined by periodic counting in Bürker -Türk chamber.

3. EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

The results of the dielectric spectroscopy, respectively the evolution of dielectric losses *tgδ* made on suspension of *Saccharomyces cerevisiae* with 5% sucrose are presented in Fig. 1.

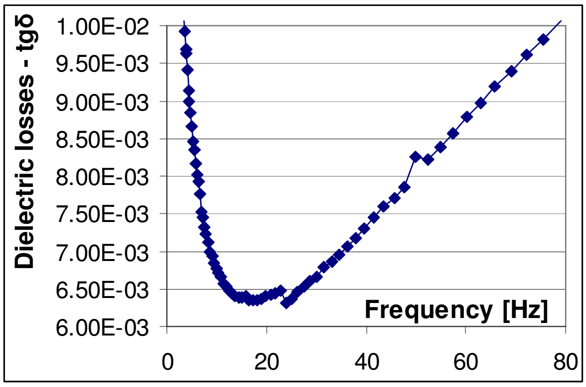

Fig. 1. The evolution of the dielectric losses depending on frequency on the suspension of *Saccharomyces cerevisiae* with 5% sucrose.

The analysis of the Fig. 1 has been revealed, that the function *tgδ* = *f* (frequency) presents a continuos evolution except the neighborhood of 22.85Hz and 50Hz frequencies, fact which suggests that the electric field applied to these frequencies produces perturbations in the metabolism / bio-chemistry of *Saccharomyces cerevisiae*.

The results of the dielectric spectroscopy, respectively the evolution of dielectric losses *tgδ* made on suspension of *Saccharomyces cerevisiae with* 5% starch are shown in Fig. 2.

The analysis of Fig. 2 shows that, in contrast to Fig. 1, the evolution of *tgδ* function for starch metabolism shows discontinuity at 18.1Hz, 43.5Hz and 50.0Hz.

These findings from Fig. 1 and Fig. 2. comparison suggest that the biochemical processes which take place in the metabolism of sucrose and starch by *Saccharomyces cerevisiae* presents a common phase, namely that influenced by 50 Hz electric field.

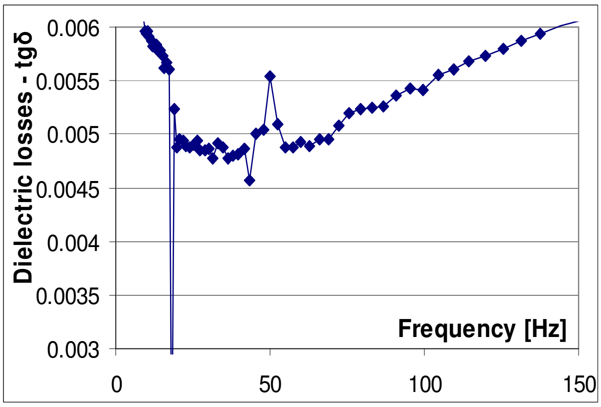

Fig. 2. The evolution of the dielectric losses depending on frequency on the suspension of *Saccharomyces cerevisiae* with 5% starch.

The results of microbiological determinations, made by counting in Bürker-Türk chamber, achieved on samples of *Saccharomyces cerevisiae* suspension with sucrose exposed to an electric field of 50Hz of different intensities and at different times, both in the LAG phase and the phase of intensive growth (Monod growth model) are summarized in Fig. 3 and Fig. 4.

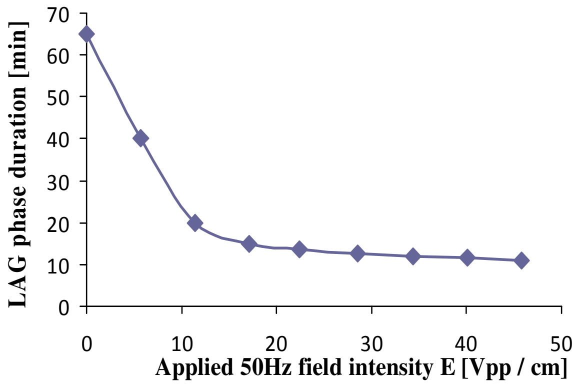

Fig. 3. The time evolution of the adaptation phase LAG depending on 50 Hz electric field applied to the suspension of *Saccharomyces cerevisiae* / sucrose (5%)

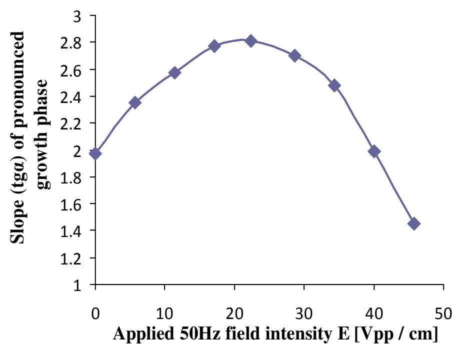

Fig. 4. Evolution of the growth rate in intensive growth phase, depending on the electric field of 50 Hz applied to the suspension of *Saccharomyces cerevisiae* /sugar (5%)

The analysis of the Fig. 3 shows that to the increasing of perturbing field intensity applied from 0 to 22,4V/cm, the LAG phase decreases from 65 to 25 minutes. At higher intensities of 22,4V/cm, the LAG phase does not change significantly.

The analysis of Fig. 4 has been revealed that the progressive increasing of the applied field intensity of 50Hz to E = 22,4V/cm the multiplication rate of cells increases, and to E = 22,4V/cm the multiplication rate decreases. These findings suggest that the 50 Hz signal applied, stimulates both the phase of adaptation phase and the processes through which the metabolism of sucrose by *Saccharomyces cerevisiae* take place, up to a maximum level which is reached at E = 22,4V / cm, and at values higher then 22,4V / cm, the metabolic processes are inhibited.

The results of measurements by dielectric spectroscopy carried out on CZAPEK-DOX mineral gel with the addition of 30 g / 1000mL sucrose and inoculated with *Aspergillus niger* are presented in Fig. 5.

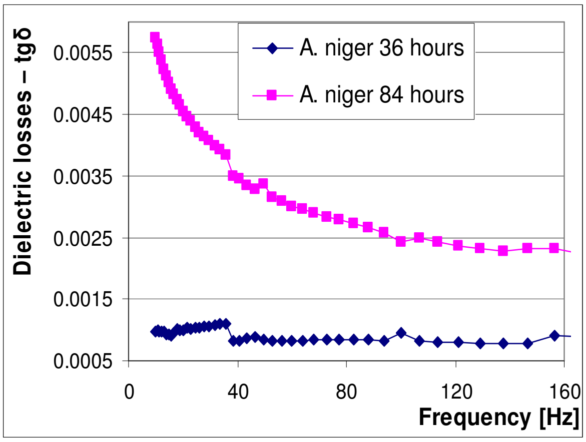

Fig. 5. Evolution of the dielectric losses depending on frequency on CZAPEK-DOX gel with sucrose in various phases of growth of *Aspergillus niger*

The analysis of Fig. 5 has been revealed that, on the biomasses resulted after 36 and 84 hours of incubation, the evolutions of $tg\delta$, present discontinuities at various frequencies, which suggests that under the action of the measurement signal, at those frequencies, in biomass take place biochemical processes which change significantly the electrical conductivity of the reaction products compared to reactants entered into the process - for example enzymes synthesis from aminoacids.

Thus, at 36 hours after inoculation when the primary mycelium formation is predominant the discontinuities are found to 16.7Hz, 35.9Hz and 50Hz and (less pronounced), and at 84 hours of incubation the fructifications growth and maturation are predominan, the discontinuities are found to 35.9Hz, 50Hz and 100Hz.

The results of observations on the *Aspergillus niger* growth on Czapek-Dox medium with sucrose exposed to an electric field of 50Hz have been revealed that at 5 ÷ 20V / cm the fructifications maturation time is reduced from 3 days (in the absence of applied electric field) to 2 days - or approx. with 33%. This reduction of the time for fructifications maturity was not highlighted on the culture medium with starch.

The results of microbiological determinations on spores production on CZAPEK-DOX medium with sucrose, respectively starch exposed to 50 Hz electric field of different intensities are shown in Fig. 6. [23].

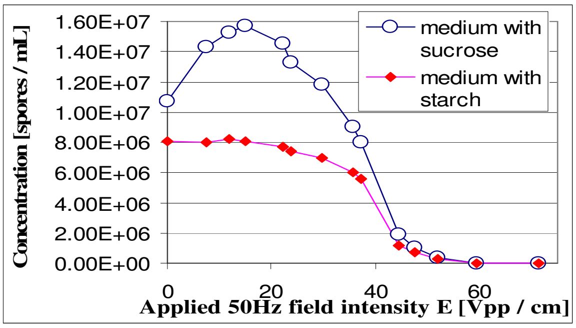

Fig. 6.The evolution of Aspergillus niger spores production on CZAPEK-DOX medium with sucrose, respectively starch, exposed to different intensities of 50 Hz electric field [23].

The analysis of Fig. 6. has been revealed that, on the medium with sucrose compared to the control sample (unexposed to 50Hz electric field action, but prepared and processed / incubated under the same conditions with samples exposed to various intensities of 50Hz field), with the increasing till 14.9 V/cm of applied field intensity, increases the concentration of spores in the extraction solution (thus mold productivity increases) - the maximum recorded increasing being from 10.7 x 10^6 (control) to 15.7 x 10^6 spores / cm^3, ie approx. 47%.

At values of applied electric field intensity of more than 14.9V / cm the spores production gradually decreases - reaching as approx. 30V / cm to be the same to that of the control sample, after which further decreases relatively quickly - and more than 50V/cm tends exponentially to zero.

These findings suggest that the perturbing signals of 50Hz stimulates the metabolic processes of sucrose by *Aspergillus niger* and thus growth and spores production, but electric field intensities higher than approx. 15V/cm start a process of gradual deterioration of the cell membrane - until its complete breakthrough at values more than 50 V/cm.

It is noted that, as shown in Fig. 5, the stimulation of the growth process on medium with sucrose is conditioned by the perturbing signal frequency (stimulates the biochemical processes which involve reactants with relaxation time corresponding to 50 Hz frequency), stimulation whose measure increases with increasing of the applied electric field intensities. At high field intensities, more than approx. 30V/cm (electrical stress) - regardless of the frequency of the applied voltage - the dielectric strength of the membrane is limited, the degradation takes place by breakthrough of the cell membrane.

Fig. 6 also shows that to the culture medium with starch, the growth stimulation does not takes place (concentration of spores is constant in the range of 0 - 15V/cm). At electric field intensities higher than 15V/cm, starts the damage process of the cells - up to their complete destruction from approx. 50 V/cm - a process that is determined, firstly, by the intensity of the applied electric field, and not its frequency.

4. CONCLUSIONS

Through specific microbiological tests it was found that:

- 50Hz electric field up to 20V/cm overlapped on aqueous suspension of *Saccharomyces cerevisiae* with 5% sucrose or starch, reduces the LAG phase time and accelerates cell proliferation in intensive growth phase; more than 20V/cm, the LAG time stop changing and cell multiplication rate decreases, which completely inhibits approx. to 50 V/cm;
- 50Hz electric field up to approx. 15V/cm overlapped on CZAPEK-DOX gel with sucrose reduces by approx. 33% the time of growth and maturation of *Aspergillus niger* and leads to an increasing in spores production of approx. 50%;
- 50 Hz electric field of approx. 15V/cm overlapped on CZAPEK-DOX gel with starch does not alter the growth and maturation time, namely production of *Aspergillus niger* spores;
- 50Hz electric field over 15V/cm gel overlapped on CZAPEK-DOX gel with sucrose or starch diminishes the growth of *Aspergillus niger* spores and reduces the spores production – at approx. 50V/cm the *Aspergillus niger* growth and multiplication is completely inhibited.

The measurements of dielectric spectroscopy in the extremely low frequencies have been revealed that the biochemical processes from biomass have significant changes in certain frequencies characteristic of the different species / metabolized carbon source, respectively species/ metabolized carbon source/stage of development.

Given these findings both to streamline certain industrial biochemical processes and to assess the impact of 50Hz radiations from the transmission and distribution networks of electricity on the biosphere, it is considered appropriate to continue investigations in order to determine the influence of perturbing 50Hz signals on other species of microorganisms.

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