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SACCHAROMYCES CEREVISIAE MAYASININ ÜREMESİNE PULSLU ELEKTROMANYETİK ALANIN ETKİSİ

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ÖZET

Bu çalışmada, *Saccharomyces cerevisiae* maya hücrelerinin büyümesine 15 Hz'li pulslu elektromanyetik alanın etkisi araştırılmıştır. İstatistiksel incelemeler sonunda uyum (inkübasyon periyodunun ilk 6 saati) ve durgun (16-29 saatler arası) döneminde deney ve kontrol grupları arasında anlamlı fark bulunamamıştır ($P>0.05$). Ekimden sonraki 6. saat ile 26. saat arasında, manyetik alanın etkisinde büyüyen hücrelerin sayısının kontrol grubunun hücre sayısından daha düşük olduğu ve sonuçların istatistiksel olarak anlamlı olduğu bulunmuştur ($P<0.001$). Ayrıca deney ve kontrol gruplarının büyüme eğrileri matematiksel olarak incelenip, büyüme denklemleri oluşturulmuştur. İstatistiksel incelemeler sonucunda, deneysel ve teorik büyüme eğrileri arasında bir korelâsyonun olduğu bulunmuştur ($r_C=0.998$, $r_{PEMF}=0.99$).

Anahtar Kelimeler: Elektromanyetik alan büyüme eğrisi, Maya

EFFECTS OF PULSING ELECTROMAGNETIC FIELD ON THE GROWTH OF *SACCHAROMYCES CEREVISIAE*

ABSTRACT

In this study, the effects of 15 Hz-pulsing electromagnetic field (PEMF) on the growth of *Saccharomyces cerevisiae* yeast have been investigated. After statistical assessments, there was no significant difference between control and experimental group on the base of adaptation (first 6 hours of incubation period) and static phase (after 26 hours) ($P>0.05$). After inoculation (between 6 and about 26 hours), count of the cells growing under the effect of the magnetic field was lower than that of control group, and the results were statistically significant ($P<0.001$). In addition, growth curves of experimental and control group were mathematically studied and growth equations were formed. After statistical measurements a considerable accordance was found to be between experimental and theoretical growth curves ($r_C=0.998$, $r_{PEMF}=0.99$).

Key words: Electromagnetic field, growth curve, yeast

1. INTRODUCTION

Various studies aiming the effects of magnetic field on micro-organisms were carried out. In these studies, different micro-organisms and magnetic field parameters were employed. Depending on these parameters, it was concluded that the magnetic field did not increase or decrease the growth of micro-organisms. It was found that the magnetic fields of 2, 460 and 1500 mT decreased the growth of *Escherichia coli, Saccharomyces cerevisiae* and *Serratia marcescens,* respectively [1, 8, 15]. However, upon applying magnetic fields of 0.8 and 2.5 mT to *Bacillus subtilis* , an increase was observed in the cell count, on the other hand it was not the case with the magnetic field of 1500 mT to *Saccharomyces cerevisiae* and that of homogenous 1100 mT to Burgundy wine yeasts [11, 12, 17]. Kimball (1938) also reported that, depending on the application period of 0.4 mT heterogeneous magnetic field, the budding of Burgundy wine yeasts were not affected or decreased. In this study, we investigated the effect of Pulsing Electromagnetic Field (PEMF) on the growth of *Saccharomyces cerevisiae* yeast.

The population growths of micro-organisms are expressed in mathematical equations. One of the simplest models of population growth is that of Thomas Malthus [3], who observed at the end of the eighteen century that populations tend to grow in geometric progression. In his opinion that the means of subsistence could only increase in arithmetic progression would sooner or later inadequate. The point is that throughout nature most living things do have a propensity for geometrical growth which is only held in check by a sufficient degree of competition, disease, death, and destruction. A typical population grows rapidly at an increasing rate if it starts in an environment with an adequate food supply and a relative absence of predators. As time elapses the food supply becomes less adequate, overcrowding leads to less healthy conditions, fertility declines, and the death-rate increases. Under certain circumstances equilibrium is achieved, and the population remains more or less constant. It is clear that knowledge of the precise relationship to be expected between population size at different times and the birth- and death-rates is a matter of considerable interest.

A mathematical form for this typical S-shaped curve of population growth was first devised by Verhulst [3], a contemporary of Quetelet, using the following kind of approach. First, it is convenient to treat the population size N as a continuous variable, which is accurate enough if N is fairly large. Secondly, we work with continuous time t instead of with discrete generations. Let us suppose that the average rate of growth of the population under favourable conditions is μ per individual, so that in time dt there is an increase of μNdt in the population size. This means that $dN=\mu Ndt$. The behaviour of the population is therefore described by the differential equation:

$$\frac{dN}{dt} = \mu N \qquad (1)$$

with solution:

$$N = N_o e^{\mu t} \qquad (2)$$

if we suppose that initially there are N_o individuals when t=0. The exponential growth of a continuous population in continuous time, exemplified by Eq.2, is equivalent to geometrical growth for discrete numbers over discrete generations [3].

Verhulst's idea was to impose on the exponential growth of Eq.2 some kind of retardation factor which would increase as the population grew. The simplest assumption to make is that the retardation-rate per individual is proportional to the population size, i.e. that the net growth-rate is not μ but μ-rN, where r is the retardation constant. The basic differential equation is now:

$$\frac{dN}{dt} = \mu N - rN^2 \qquad (3)$$

with solution:

$$N = \frac{\mu}{r + \left(\frac{\mu}{N_0} - r\right)e^{-\mu t}} \qquad (4)$$

starting, as before, with $N=N_0$ at t=0. This is Verhulst's logistical curve. Eq.4 is in fact a neat mathematical description of an S-shape curve which rises at an increasing rate to start with, like the exponential curve, but which gradually slows down and finally flattens out to approach the horizontal line $N_s=\mu / r$ as t becomes very large. The value N_s is the equilibrium value to which the population size tends [3].

Equation. 4 can be determined:

$$N = \frac{N_s}{1 + ke^{-\mu t}} \qquad (5)$$

where k is expressed as:

$$k = \frac{N_s - N_o}{N_o} \qquad (6)$$

Also, the generation time of population during logarithmic growth phase can be determined as:

$$T = \frac{t'}{n} \qquad (7)$$

where n, generation count and t', time during n generation [16].

2. MATERIAL AND METHOD

In this study, electromagnetic field generator was prepared in our laboratory. Out put of the electromagnetic field generator was connected to a pair of Helmholtz coils (20 cm in diameters). The coils were separated 15 cm and connected to the side of test tubes rack. PEMF was applied parallel to the earth's magnetic field. Yeasts cultures were located in the region within test tubes rack where fields were homogeneous. The induced mean of PEMF in centre of coils was 1.1 mT. Magnetic field excitation waveforms and amplitudes were monitored by an oscilloscope (Kikusui Cos 5020 TM) and field intensity was measured by used to dedection coil (Hall Effect). Amplitude of electromagnetic field was puls peak to peak 4 mV. The experiments were performed at 1.1 mT and 15 Hz.

In this study, *Saccharomyces cerevisiae* isolated in the our laboratory from beaker's yeast (Pak Food Produced Com.). Dry yeast cells were incubated in the Sabouroud's dextrose (SD) agar (Oxoid CM41) at 30^{o}C, for 24 hours. Isolated yeast cells were inoculated in Sabouroud's liquid medium (Oxoid CM147) and cells were counted per millilitre of growth medium (used as stock culture). Taking approximately equal numbers of cells (5.48 ± 0.09 x10^{5}) from the stock culture obtained, the test tubes containing 6 ml of liquid medium were inoculated. PEMF group was placed into coil; control group was placed into the metal case. Both of the groups were placed on to shakers (Nüve SL350) and incubated in the incubator (Nüve EN400) at 30^{o}C for 29 h at 100 rpm.

While a magnetic field was not applied to one of the groups (control group) consisting equal numbers of cells, the other group was affected by the magnetic field and at certain intervals cell counts of control and PEMF groups were determined. In determining the periods of experiments, adaptation phases of the cells (the first 6 hours of incubation period) were taken into consideration. In order to determine the changes in population increase in time, incubation periods were increased up to 29 hours. Cell counts were determined using a Thoma lam having special count areas for the liquid samples (Haemocytometric method). In the samples in which counting becomes too difficult due to cell population, a dilution process of 1/10-1/100 was performed using 10% (v/v) acetic acid (Merck) solution [9]. All growth cultures were tested for culture purification and morphology by Gram staining [4].

3. RESULTS

The growth curves of PEMF applied and control groups are shown in Fig. 1. The first 6 hours following the inoculation of growth medium was determined as adaptation phase. In this period of growth curve there was not statistically significance ($p>0.05$) observed between PEMF applied and control groups. A similar case at which nutritional sources starts to finish and cell count reached satiation after 27 hours, was observed ($p>0.05$). Although, in the exponential phase in between 6 and 26 hours, cell counts of the samples generated in a magnetic field were always less than that of control group, and the results were statistically significant ($p<0.001$).

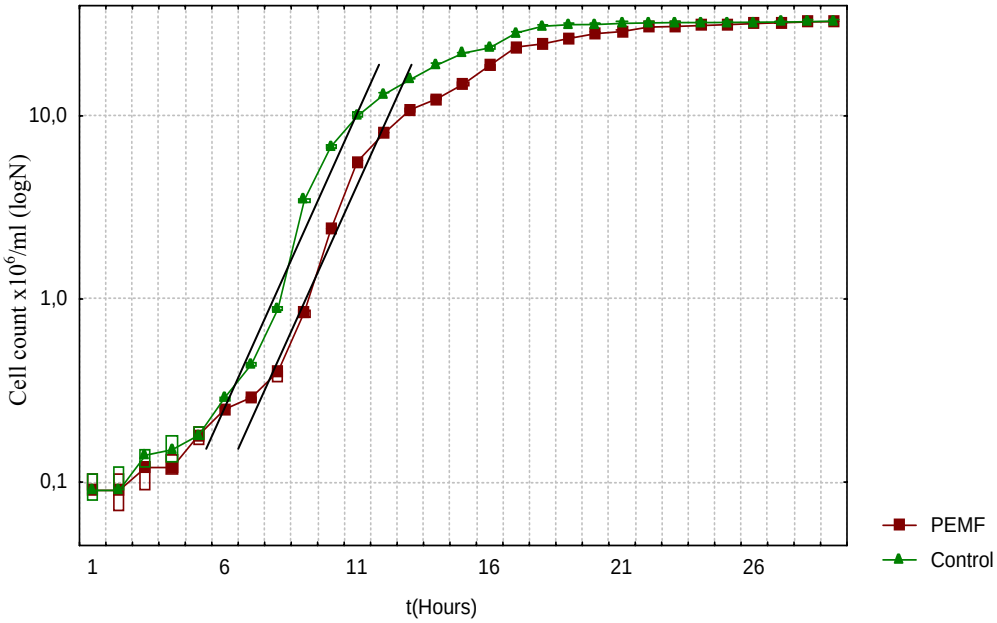

Figure 1. The growth curves in semilogarithmic scale.

For correlation between modelling and control groups, the growth curves used to paired samples test. This groups correlation found statistically significant (c=0.998). This results show that the modelling used to described growth of *S. cerevisiae*.

In order to assess these curves showing population increase in time, the changes of logarithms of cell counts in time are given in Figure 1. Under careful observations the introductory parts of graphs (6-26 hours) seem to be linear. This results show that cell reproduction in the related time interval can be expressed by exponential function, and values of the m parameters obtained from the Fig. 1 are given in Table 1. Generation time can also be measured using the area called logarithmic growth phase. The resulting generation time are shown in Table 3.1. Comparing with T and m parameters in Table 1 revealed that there were no significant statistical difference observed between the data of control group and PEMF applied group. Thus, cell increase in the magnetic field, apart from a shift in time scale, shows the same change as that of control group. Therefore, the growth curves of control and PEMF applied group are parallel to each other as shown in Figure 1.

Table 3.1 Generation time and mean growth rate of PEMF applied and control groups.

		Control Group	PEMF Applied	Test statistics
T (hours)	Equation. (4)	0.89±0.05	0.94±0.05	P>0.05
m	Equation. (1)	0.78±0.05	0.75±0.02	P>0.05

Also all cultures were carefully examined under the light microscope for any morphological disorder which could cause by the magnetic field. In this examinations there was not observed any morphological disorders in both set of cultures.

The Modelling of Growth Curve

Modelling was made to investigate on the growth curve of control group. Primary, a plot was created of changes of cell counts with time (dN/dt) (Fig. 2). As can be seen from Fig. 2, there are two different maxima. This finding revealed that, the growth curve of control group seems to have two different growth curves. Since this observation, we were determined with summarised of two different logistic equation of Eq.5 to our modelling. As can be seen from Fig. 2, the first maximum point was found approximately at 11. hours and at this time the cell count (N_{as}) was calculated as $10x10^6$/ml. Initial cell count (N_{ao}) was $0.29x10^6$/ml. We calculated to k constant with Eq.6 (k_a=33.5) and the average rate of growth of the population (μ_a) by Eq.2. (μ_a=0.78). It placed in Verhust Equation (Eq.5) to variable:

$$N_a = \frac{N_{as}}{1 + k_a e^{-\mu_a . t}}$$

$$N_1 = \frac{10}{1 + 33.5e^{-0.78.t}} \qquad (8)$$

In this equation placed to t 0 to 29 hours and found N_a curve as in the Fig. 2. N_b curve found to subtraction to the N_a curve from control group's growth curve (Fig. 2). Similarly from N_b curve, N_{bs}=22.9, k_b=20.2 and μ_b=0.51 were calculated. These variables were placed in the Eq.5 and second logistic equation was found:

$$N_b = \frac{N_{bs}}{1 + k_b e^{-\mu_b . t}}$$

$$N_b = \frac{22.9}{1 + 20.2e^{-0.51.t}} \qquad (9)$$

Our modelling total Eq.8 and Eq.9 were as follows:

$$N = N_a + N_b$$

$$N = \frac{10}{1 + 33.5e^{-0.78.t}} + \frac{22.9}{1 + 20.2e^{-0.51.t}} \qquad (10)$$

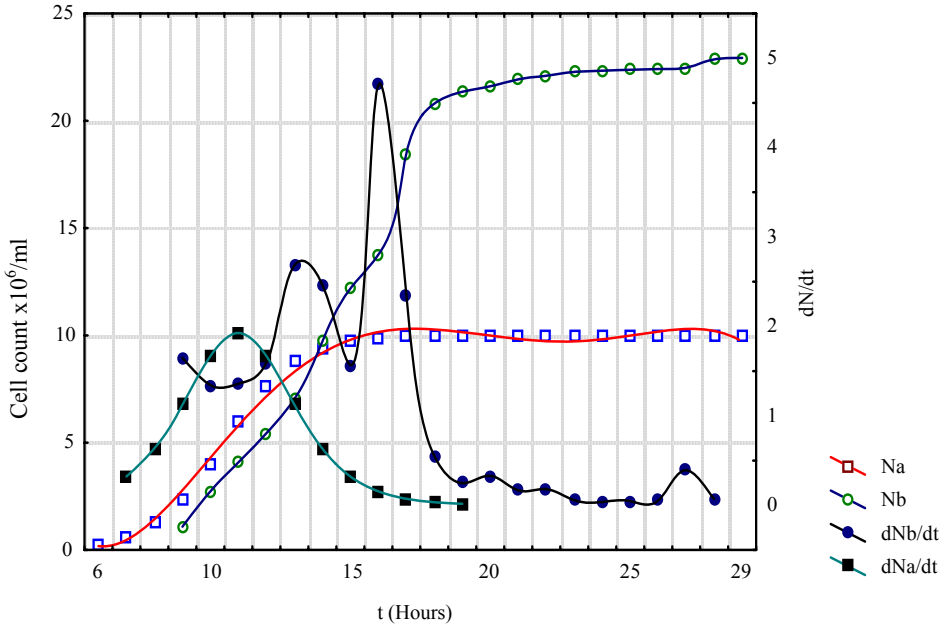

Figure 2. The yeast cell counts of PEMF and control groups and population growth rate.

The comparison of the modelling growth curve obtained by using Eq.10 with growth curve of control groups, a statistically significant relationship was observed (c=0.99, r_C=0.998). By taken in to consideration a shift 1,25 hours in time scale of electromagnetic field and by being used Eq.10, we obtained a second modelling growth curve. The comparison of this modelling growth curve with PEMF growth curve, a statistically significant relationship was observed (c=0.99, r_{PEMF}=0.99). The results of experimental data show that the electromagnetic field caused a shift with 1,25 hours in the time scale on the exponential phase of growth curve. This discovery was tested with the modelling which to take note of 1,25 hours shift (Figure 1). The statistically correlation between PEMF and modelling groups found significant (c=0.99).

4. DISCUSSION

In this study, 15 Hz with 1.1 mT pulsing electromagnetic field had an abating effect on the growth of *S. cerevisiae* cells have been observed. However, when generation phases of PEMF applied and control groups and m (mean growth rate) parameters were compared, any significant difference was not observed (p>0.05). These results show that PEMF applied and control groups generate at the same rate, but there was a certain shift in time scale. This statement is confirmed with the generation curves being parallel to each other in Figure 1.

The growth curves in Figure 1 appear to have different characteristics after 11-12 hours of incubation period. This change suggests that cell division gets into a different process.

Under favourable conditions yeast cells generate asexually, whereas when the generation conditions changes they generate sexually [6]. Ours hypothesis which is supported with mathematical expressions, also should be supported by these experimental results.

The magnetic field of 460 mT applied on to *S. cerevisiae* yeast cell, that of 1500 mT on to *S. aureus* and *S. marcescens* bacteria, the heterogeneous magnetic field 0.4 mT on to Burgundy wine yeast at certain time, that of 500-800 mT on to *Micrococcus denitrificans* , that of 200, 320, 420 mT on to *Trichomonas vaginalis* and those of 30, 60 mT on to various bacteria and yeasts were found to have an effect to slow down the growth, in accordance with the results obtained here [8, 11, 14, 15].

Increased cell population was found when the magnetic field of 4200 mT and 5.2 mT applied on the cell culture of *E. coli* and *S. cerevisiae,* respectively [5, 13]. In addition, it was indicated that the magnetic field have a stimulating effect on various cells of bacteria and yeast in some value. In the study, using *T. vaginalis* the magnetic field 46 mT and 120 mT and that 15 mT in some bacteria and yeast cells were found to have an effect to stimulate the growth [14]. However, magnetic field of 0.8 and 2.5 mT applied on *Bacillus subtilis* cells was stated to have an effect to increase on growth [17]. The magnetic field of 27 MHz frequency was repoeted to increase *Salmonella typhimurium* cells in high concentrations [10].

The magnetic field of 1100 mT formed with Burgundy wine yeast, in some interaction times that of 0.4 mT, *E. coli* cells of the pulsing electromagnetic field of 27.12 MHz frequency. *S. cerevisiae* yeast cells of 1500 mT magnetic field was shown to have no effect on growth [2, 11, 12, 18]. Moreover, 300 mT magnetic field applied on *E. coli* and 25 various bacteria were reported to have no effect on growth [14].

In this study, we found that the effect of 15 Hz pulsing electromagnetic field to decrease on growth was formed only during logarithmic phase. The inhibitory effect of the magnetic field of 460 mT on *S. cerevisiae* yeast and the inefficiency of *E. coli* cells of 27.12 MHz pulsing electromagnetic field occur in logarithmic phase have been reported [2, 15]. However, five different bacteria and yeast cells inhibitory and stimulating effects of various magnetic field values were observed during logarithmic phase by Moore (1979). However, there are some other studies that state the reducing effect in budding of yeast cells of 0.4 mT heterogeneous magnetic field to be seen only at the end of adaptation phase [11].

As a result of our evaluations we could not find a significant difference between generation time of experimental and control groups. But it was determined that magnetic fields of 50 Hz and 16.66 Hz, 0.48 mT, 0.8 mT and 1500 mT reduced mean generation time of *E. coli* cells [1].

In this study, generation period of *S. cerevisiae* was determined to be 0.89 ± 0.05 h. But, in some sources, the generation time of this yeast is stated to be between 1.73 and 2.42 h (during logarithmic phase) [7]. It is well know that yeast cells grow forming buds in several points. As we expected, we found that cells multiplicate being folded in two. Thus, the generation time being shorter should be the expected result which we concluded.

In the studies under light microscopy we observed that there was not any change in the morphology of cells under the magnetic field. In some other studies the magnetic field did not cause any change on the morphology of various bacteria and yeast cells [14].

5. CONCLUSION

In this study we found that the effect of 15 Hz pulsing electromagnetic field reducing growth was formed only during logarithmic phase. On the one hand, we finally concluded that, the magnetic field exerts a reducing effect on yeast cell growth, but it does not seem to be effective on the generation time of the cells and their mean growth rates. These results show that the magnetic field leads to a shift in growth curve of the cells under magnetic field, in time scale, and this shift was determined to be 1.25 h.

Studying growth curve characteristics of cells, we determined growth to form in two different ways during logarithmic phase. Depending on our results, we obtained an equational expression of cell growth. After plotting cell growth curve, we compared it with those of PEMF applied and control group and found a significant similarity between experimental and theoretical cell growth curves by using the equation. We have the opinion the change in quality of growth and this result is supported by experimental studies as well.

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