ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 4, Issue 7 , July 2017

Effect of Low Density Magnetic Field Generated by Helmholtz Coil on α-amylase Activity and Antibiotic Sensitivity of B.cereus

Morteza Haghi, Yigit Terzi, Salar Hassanzadeh, Zaka Abbaszade, Ismail Karaboz

Department of Biotechnology, Ege University, Izmir, Turkey & Young Researchers Club Azerbaijan province, Iran
Department of Industrial Microbiology, Ege University, Izmir, Turkey
Department of Industrial Microbiology, Ege University, Izmir, Turkey
Department of Biotechnology, Ege University, Izmir, Turkey
Department of Industrial Microbiology, Ege University, Izmir, Turkey

ABSTRACT: In recent years different forms of magnetic field systems have been applied to study their either harmful or useful effects on eukaryotic or prokaryotic cells and their industrial as well as medical potentials. To observe the possible effects of static magnetic field on *B.cereus* antibiotic susceptibility, growth rate and α-amylase activity a pair of Helmholtz coils supplied with DC power were designed to generate uniform low-density magnetic field varying from 1mT to 3mT. For antibiotic susceptibility test, Ampicillin, Penicillin, Tetracycline, Gentamicin, and chloramphenicol were used. Growth curve of each microorganism was plotted at 600nm. α-amylase activity of *B.cereus* was carried out using Dinitrosalicylic acid method. *B.cereus* which was resistant to Penicillin and Ampicillin became sensitive under 1.5mT and 2mT magnetic field. Under these densities, expotential phase of microorganism increased in contrast to control. The α-amylase activity of B.cereus decreased under 1.5mT.

KEYWORDS: Helmholtz coil, Static Magnetic Field, Antibiotics, α-amylase, Bacillus cereus

I. INTRODUCTION

Living things, particularly human beings are being exposed to various types of magnetic fields due to unavoidable application of modern life advanced technological tools such as cell phones, computers, and household appliances.(1-2) In recent years interest in research about the effects of magnetic fields on living systems has been raised.the effects in different fields such as cancer research, waste water treatment, antibiotic sensitivity, drug delivery, and sterilization have been studied. (3-7)

The magnetic field could have several biological effects on ion transportation, DNA synthesis and transcription. DNA repair acceleration by stimulation of heat shock protein synthesis. Bacterial cell wall, antibiotic resistance mechanism as wall as enzymatic activity.(8-11).Several studies have been carried out on prokaryotic and eukaryotic microorganism by using different magnetic and electromagnetic field effects basically depend on magnetic intensity and exposure time. It has been suggested that earth's geomagnetic field also could interfere analysis of results. (12)

It has been revealed that bacteria can have stress response to magnetic field (Perez et. al. 2010).It has also been reported that the magnetic field can change morphological characteristics of bacteria and also causes mutation in *S.typhimurium*.(13-14)

Antibiotic resistant bacteria which can cause major infectious diseases and death in human are becoming great danger for human health. There have been several studies on antibiotic resistance and growth rate of bacteria to understand either positive or negative effects (15).

Several experiments have been carried out to study the effect of magnetic field on different enzymes for instance catalase, cellulase, cytochrome oxidase but α-amylase is poorly studied.(16-17).

In this study we aim to analyze the effect of low density magnetic field(10,15 and 20 Gauss) on growth curve , antibiotic sensitivity and α-amylase activity of *B.cereus*.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 4, Issue 7 , July 2017

II. MATERIALS AND METHODS

A. Helmholtz Coil

The magnetic field generating system consisted of a pair of Helmholtz coils, DC power supply 24V,ampermeter,voltmeter, ohmmeter and gauss-meter. Radius of cores was 12 cm made from pure iron, number of turn was 144 in each coils with wire diameter of 1 mm. Resistance of each coil was 13.5±0.2Ω , total resistance of coils system after serial attachment of coils measured by ohmmeter and calculated by using the values of voltmeter and ampermeter according to Ohm theory. Distance between the coils was equal to their radius. The frequency was 0. And magnetic field flux values was varied between 1-3mT. Coil is placed inside incubator. Target group was placed at zero point (x=0) of coil system inside of incubator perpendicular to central axis of coils. Control group placed inside incubator in absence of coils.

B. Antibiotic susceptibility test

Antibiotic susceptibility test was performed using Mueller-Hinton Agar medium according to Kirby-Bauer Disk Diffusion technique. Ampicillin(10µg), penicillin (10µg), Gentamicin(10 µg), chloramphenicol(30 µg),tetracycline(10 µg),antibiotic discs used.The control group was incubated at 37 ° C for 24 hours. The study group was exposed to 10g, 15g and 20g magnetic fields under the same incubation conditions as the control group. At the end of the incubation results were compared.

C. Growth curve

For the growth curve, the organism was inoculated to LB medium and incubated at 37 ° C. OD values were taken at 600nm at every 2 hours from both control and study groups.

Effect on amylase enzyme activity

Bacillus cereus was inoculated in 1% starch media (K_2HPO_4 . $3H_2O$: 0.26, KH_2PO_4: 0.8 g, $MgSO_4$. $7H_2O$: 0.41 g, $CaSO_4$. H_2O: 0.1, $NaMoO_4$: 0.0039 g , $FeSO_4$. 7H2O: 0.005 g ,$(NH_4)_2SO_4$: 0.3 g) and incubated at 37 °C without magnetic field effect. The extracellular enzyme solutions were obtained by centrifugation at 5000 rpm for 10 min using centrifuge. The supernatant obtained was collected and used as enzyme source. The supernatant was divided into 3 control groups and 3 study groups. The samples which contain enzyme were mixed with 1% starch substrate. The test group incubated at 37 ° C for 30 minutes in the magnetic field. Control group was excluded from the magnetic field under the same conditions. The enzyme activity at the end of the incubation was measured spectrophotometrically(546nm) using 3, 5-dinitrosalicyclic acid (DNSA) method and the enzyme activity was calculated by absorbance values.

III. RESULTS

Under various magnetic field antibiotic susceptibility test on *Bacillus cereus* indicated various effects. There was no effect on the sensitivity of the magnetic field to Gentamicin. The activity of chloramphenicol antibiotic was increased under the magnetic field and the maximum effect was determined at 15G. The tetracycline sensitivity of *Bacilluscereus* was not changed at 10G but increased at 15G and 20G. The most noticable effect of the magnetic field was seen in the Ampicillin and penicillin antibiotic susceptibility.However *Bacillus cereus* was resistant to penicillin, became sensitive at 10G and 15G values. There is no effect at 20G. While the control group was resistant to amp, it became sensitive at 10G,15G,20G.(Figure 1).

Growth curve of *Bacillus cereus* influenced under 10 and 15 Gauss but no significant effect was observed at 20 G. (Fig 2,3 and 4).Under the magnetic field α– amylase enzyme activity produced by *Bacillus cereus* showed lower activity comparing to the control group . Enzyme activity decreased by 5% at 10G, 18% at 15G, and 5% at 20G.(Figure 5).

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 4, Issue 7 , July 2017

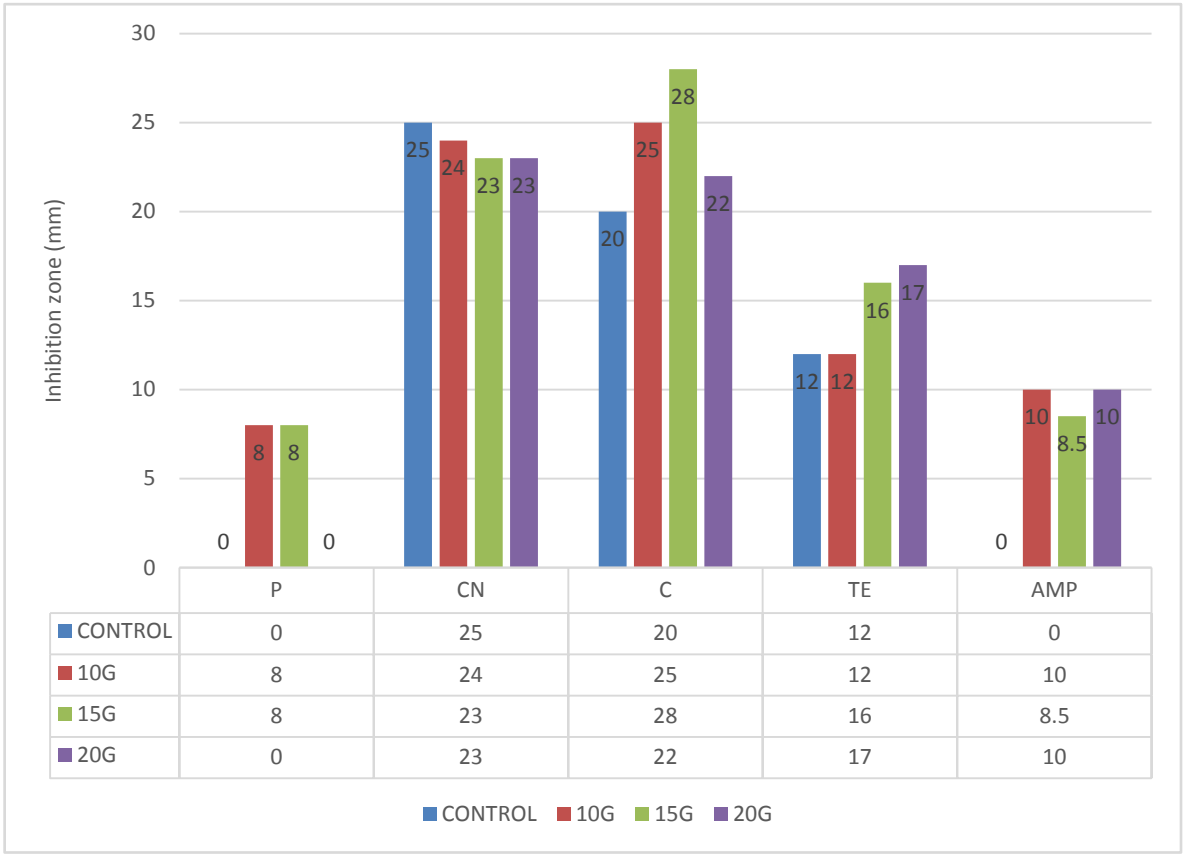

Fig.1. Effect of magnetic field on antibiotic sucsceptibility of *B.cereus* at 10,15 and 20 GaussAMP:Ampicillin(10µg), P: penicillin (10µg), CN: Gentamicin(10 µg), C:chloramphenicol(30 µg), TE:tetracycline(10 µg)

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 4, Issue 7 , July 2017

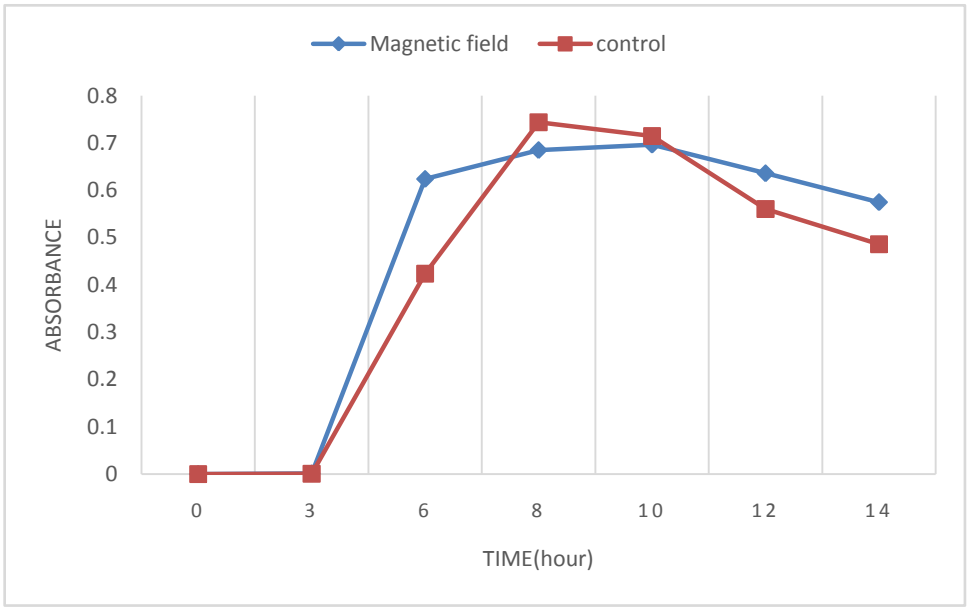

Fig.2. *Bacillus cereus* growth curve at 10 Gauss

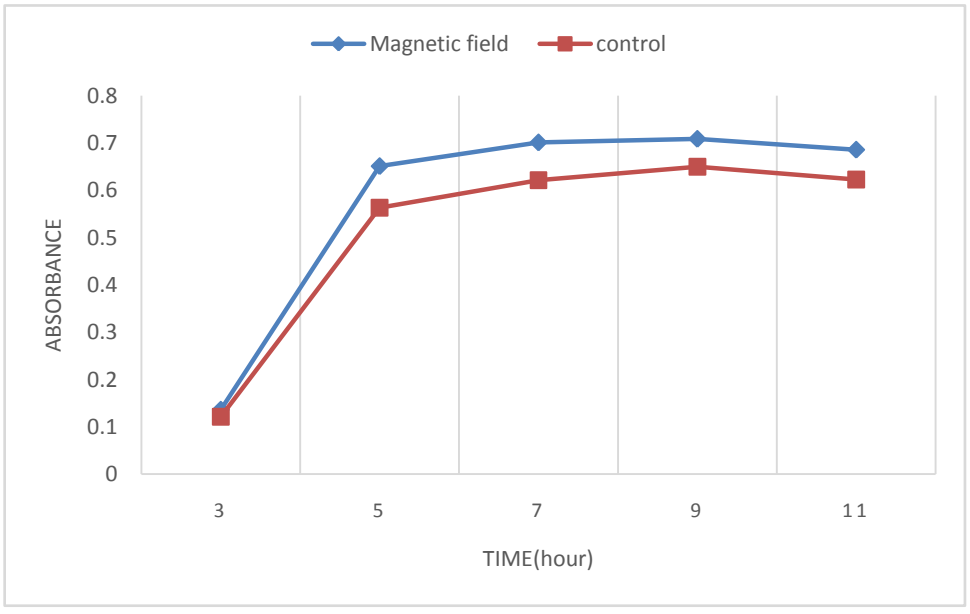

Fig.3. *Bacillus cereus* growth curve at 15 Gauss

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 4, Issue 7 , July 2017

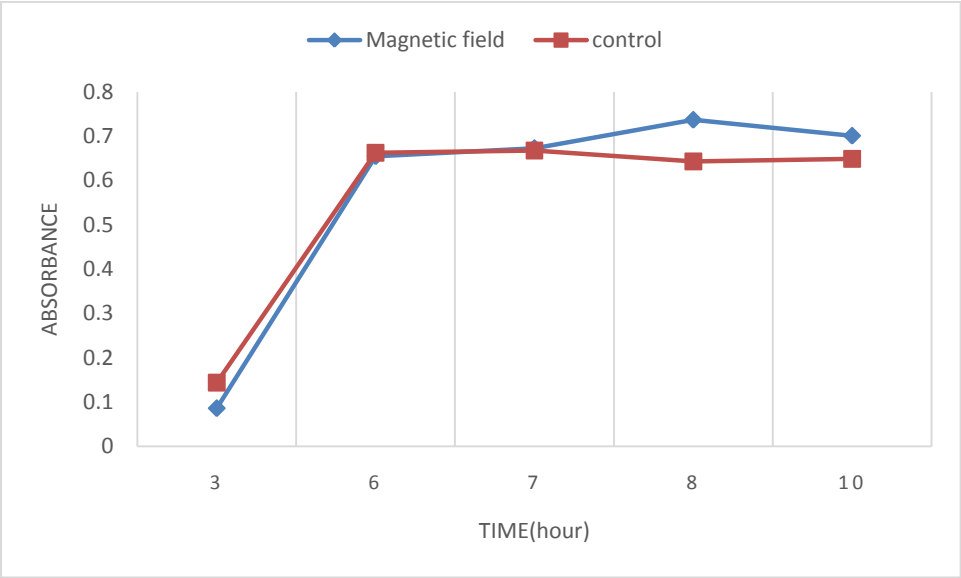

Fig.4. *Bacillus cereus* growth curve at 20 Gauss

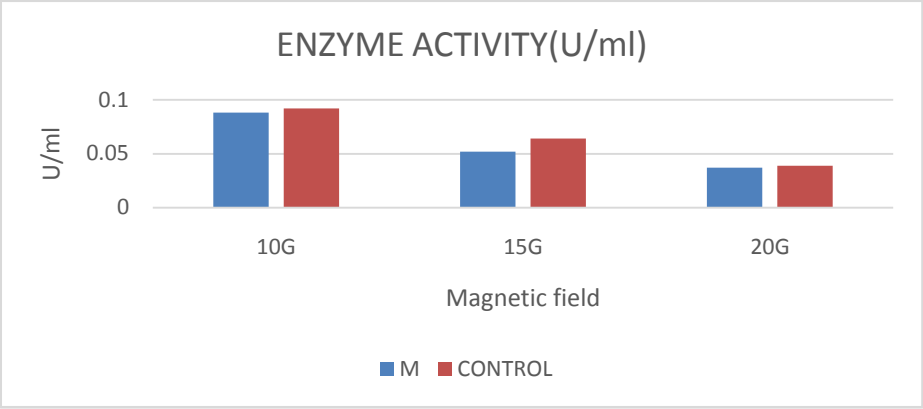

Fig.5. α–amylase activity under 10,15 and 20 Gauss.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 4, Issue 7 , July 2017

IV. DISCUSSION

The effect of magnetic field on bacterial antibiotic resistance and growth rate has long been studied. However the effects are variable based on type of microorganisim and magnetic field as well as exposure time.

Khaled A *et al*. showed that low static magnetic field ranging from 30 to 80 G increased growth rate of *B.subtilis* and decreased *E.coli* and *S.aureus* growth rate. (18). While another study reported that SMF ranging 0.5 to 4 T has no significant effect on growth rate and antibiotic sensitivity of *E.coli* and *S.aureus* (19).

It has been revealed that SMF increases the activity of gentamicin against biofilm forming *P.aeruginosa* which is limited to 5G-20G (20).

Jihen *et al* reported that 200mT exposure increased sensitivity of *Salmonella* Hadar against gentamicin but no effect was seen in other antibiotics.

Fouad Houssein Kamel *et al*. also observed increased antibiotic resistance to Gentamycin, Ceftazidium, Tetracycline, Chloramphenicol, Rifampcin, Ceftriaxone after exposure of 400, 800, 1200 and 1600 Gauss magnetic field after 2, 4, 6, 8 and 24 hours (21).

In another study Ceon *et al*. (2005) found that static magnetic field increased antibiotic resistance of *E. coli*. (22). According to study done by Marion *et al*. that *E.coli* showed increased antibiotic resistance after exposure of magnetic field. We observed significant changes in antibiotic resistance of ampicillin and penicillin at 10G and 15G SMF exposure but no effect was observed in gentamicin . In contrast, Segatore *et al*. tested kanamycin, amikacin, ampicillin, cefazolin, ceftazidime, ceftriaxone, moxalactam, and levofloxacin antibiotics on *E. coli* and *P. aeruginosa*. But no significant difference found in growth and antibiotic susceptibility between exposed and unexposed groups (23). In our study 10G and 15G were more effective on *B. cereus* and seems to have increased the growth rate after 10 hours exposure but no significant effect was observed in 20G exposure.

Other studies showed different results according to intensity and exposure time of magnetic field. *E.coli* and *P.putida* exposed to 17mT SMF in different time intervals and inhibitory effect was seen on growth rate and it was reversible after exposure. SMF also caused increased dehydrogenase activity and ATP levels (24). ELF EMF application altered growth rate negatively in both gram negative and gram positive strains after exposure of 1mT magnetic field after 3 hours in comparison to control group. (Ayse Inhan-Garip *et al*).

There are few studies which analyzed the effect of magnetic field on α-amylase activity,however the results are not conclusive.Shaoyi *et al*. reported that α-amylase activity increases considerably under 0.15T, 0.30T and 0.45T SMF exposure. They also found a change in kinetic parameters and α-amylase secondary confirmation.(25).

Our results showed that α-amylase activity was decreased significantly under 15G SMF. It has been reported that the activity of purified α-amylase which was immobilized onto chitosan increased by 19.53% under 0.15 T SMF exposure.(26).

M.Rochalska *et al*. reported that subjecting seeds of Triticum aestivum and T.dicocuni to 5mT alternating magnetic field lowered α-amylase activity by 21.7 % and 40.5 % respectively. Another study reported 50% increase in activity of α-amylase under 50Hz, 0.5mT (27). In conclusion, we suggest that further studies should be done to having profound understanding of effect of magnetic field on α-amylase activity.

REFERENCES

1. Masahiro, *et al*. (2000), Effect of Static Magnetic Fields on Bacteria: Streptococcus mutans,Staphylococcus aureus, and Escherichia coli. Pathophysiology. 7, 143–148.
2. Pérez, M.G., Zambrano, N. & Salas, A.R. (2010), Effect of Weak Magnetic Fields on Bacterium Staphylococcus aureus. REVECITEC. 1, 77-83.
3. C. D'Angelo,' E. Costantini,M.A. Kamal, M. Reale. Saudi J Biol Sci. 2015 Jan; 22(1): 75–84. Published online 2014 Aug
6. doi: 10.1016/j.sjbs.2014.07.006
4. Yavuz, H. and S.S. Celebi, Effects of magnetic field on activity of activated sludge in wastewater treatment. Enzyme and Microbial Technology, 2000. 26(1): p. 22-27.
5.Yadollahpour, A., M. Jalilifar, and S. Rashidi, Antimicrobial Effects of Electromagnetic Fields: A Review of Current Techniques and Mechanisms of Action. J PURE APPL MICROBIO, 2014. 8(5): p. 4031-4043.
6.ZM Saiyed, SD Telang,CN RamchandApplication of magnetic techniques in the field of drug discovery and biomedicine, Biomagn Res Technol. 2003; 1: 2.Published online 2003 Sep 18. doi: 10.1186/1477-044X-1-2
7. Ma Y. Deng Y., CHU J. (2003).Sterilization of watermelon juice with high voltage pulse magnetic field and its mechanism analysis, Transactions Chinese Soc. Agricult. Eng. 19(2): 163–166.
8. Robert P. Liburdy, Calcium signaling in lymphocytes and ELF fields Evidence for an electric field metric and a site of interaction involving the calcium ion channel, FEBS Letters, Volume 301, Issue 1, 1992, Pages 53-59, ISSN 0014-5793,
9. King-Chuen Chow, Wai Lin Tung, Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis, FEBS Letters, Volume 478, Issues 1–2, 28 July 2000, Pages 133-136, ISSN 0014-5793,

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 4, Issue 7 , July 2017

10.Ayse Inhan-Garip, Burak Aksu, Zafer Akan, Dilek Akakin, A. Nilufer Ozaydin & Tangul San.Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria.International Journal of Radiation BiologyVol. 87 , Iss. 12,2011
11.B. Segatore, D. Setacci, F. Bennato, R. Cardigno, G. Amicosante, and R. Iorio. Evaluations of the Effects of Extremely Low-Frequency Electromagnetic Fields on Growth and Antibiotic Susceptibility of Escherichia coli and Pseudomonas aeruginosa.
12.Ryan W. Hunt , Andrey Zavalin , Ashish Bhatnagar, Senthil Chinnasamy and Keshav C. Das. Electromagnetic Biostimulation of Living Cultures for Biotechnology, Biofuel and Bioenergy Applications. *Int. J. Mol. Sci.*2009, *10*(10), 4515-4558.
13. FOJT, Lukáš, Luděk STRAŠÁK a Vladimír VETTERL. 50 Hz magnetic field effect on the morphology of bacteria. In BES 2007 Toulouse, Book of Abstracts. Toulouse: The Bioelectrochemical Society, 2007.
14. Satoshi Nakasono, Masateru Ikehata, Takao Koana, Hiroshi Saiki, A .50 Hz, 14 mT magnetic field is not mutagenic or co-mutagenic in bacterial mutation assays, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Volume 471, Issues 1–2, 20 November 2000, Pages 127-134, ISSN 1383-5718
15. Stansell M.J., Winters WD., Doe RH., Dart BK. Increased antibiotic resistance of E. coil exposed to static magnetic field, and Bioelectromagnetics, 22(2), 129–37, 2001.
16. Zhang J, Wang S, Xu B, Gao M. Effect of alternating magnetic field treatments on enzymatic parameters of cellulase. J Sci Food Agric. 2012 May;92(7):1384-8. doi: 10.1002/jsfa.4711. Epub 2011 Nov 14.
17. Blank, M., and Soo, L., 2001. Optimal Frequencies in Magnetic Field Acceleration of Cytochrome Oxidase and Na,K-ATPase Reactions. Bioelectrochemistry 53: 171-174.
18. Khaled A. Al-Khaza'leh , Abdullah T. Al-fawwaz. The Effect of Static Magnetic Field on E. coli , S. aureus and B. subtilis Viability . Journal of Natural Sciences Research ISSN 2224-3186 Vol.5, No.24, 2015.
19. Grosman Z, Kolár M, Tesaríková E. Effects of static magnetic fields on some pathogenic microorganisms. Acta Univ Palacki Olomuc Fac Med. 1992;134:7–9.
20.Benson, Dianna E.; Grissom, Charles B.; Burns, Gregory L.; Mohammad, S Fazal . Magnetic Field Enhancement of Antibiotic Activity in Biofilm Forming Pseudomonas aeruginosa. ASAIO Journal: July/August/September 1994.
21. Fouad, H.K., Chiman, H.S. & Saleem, S.Q. (2014), Magnetic Field Effect on Growth and Antibiotic Susceptibility of Staphylococcus aureus . Journal of Al-Nahrain University . 17, 138-143.
22. Ceon R. and Martin J.T. (2005). Low-Level Magnetic field induced growth modification of Bacillus subtilis. J Bioelectromagnetics.8 (3):275-282.
23. B. Segatore, D. Setacci, F. Bennato, R. Cardigno, G. Amicosante, and R. Iorio, Evaluations of the Effects of Extremely Low-Frequency Electromagnetic Fields on Growth and Antibiotic Susceptibility of Escherichia coli and Pseudomonas aeruginosa, International Journal of Microbiology, vol. 2012, Article ID 587293, 7 pages, 2012. doi:10.1155/2012/587293.
24. Jasmina Filipič, Barbara Kraigher, Brigita Tepuš, Vanja Kokol, Ines Mandic-Mulec, Effects of low-density static magnetic fields on the growth and activities of wastewater bacteria Escherichia coli and Pseudomonas putida, Bioresource Technology, Volume 120, September 2012, Pages 225-232, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2012.06.023.
25 .Shaoyi Jia ,Yong Liu ,Songhai Wu, Zhibin Wang. Effect of static magnetic field on α-amylase activity and enzymatic reaction.Transactions of Tianjin University. August 2009, Volume 15, Issue 4, pp 272–275
26. Yong Liu, Shaoyi Jia, Jingyu Ran, Songhai Wu, Effects of static magnetic field on activity and stability of immobilized α-amylase in chitosan bead, Catalysis Communications, Volume 11, Issue 5, 25 January 2010, Pages 364-367, ISSN 1566-7367
27.M. Rochalska and K. Grabowska, Influence of magnetic fields on the activity of enzymes:α–and β -amylase and glutathione S-transferase (GST) in wheat plants Int. Agrophysics, 2007, 21, 185-188.