The Effect of MRET Activated Water on Microbiological Culture *Escherichia coli* K-12 and on Complex Microbiological Associations

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Abstract

The article relates to detailed observation of the effect of MRET activated water with the modified molecular structure (produced with the help of patented in the USA Molecular Resonance Effect Technology) on metabolic activity and growth of conditionally pathogenic microbiological culture Escherichia coli K-12 (E.coli) and on metabolic activity of complex microbial associations (similar to microbial associations in the intestine). The microbiological investigation described in this article was conducted at Kiev Institute of Microbiology and Virology of Ukrainian Academy of Science. After 25 hours of experiment in aerobic *environment it revealed significant inhibition of growth of E.coli: about 30 times in nutrient medium MRET activated for 30 minutes and about 300 times in nutrient medium MRET activated for 60 minutes. The metabolic (reductant) activity of E.coli reduced up to 3 times in 30 minutes activated water and up to 1.6 times in 60 minutes activated water during the first 6 hours of experiment in* aerobic *environment. In* anaerobic *environment the metabolic activity of E.coli practically did not change. In order to simulate the environmental conditions similar to the conditions in the intestine the test in* anaerobic *environment was conducted on metabolic activity of complex microbial associations. It revealed that MRET water substantially increased reductant activity of microbial associations during the first several hours of experiment.*

Keywords: *MRET water,* E.coli bacteria, *microbiological, pathogenic bacteria.*

Introduction

MRET Activated Water is produced with the help of patented in the USA Molecular Resonance Effect Technology (MRET). MRET water activator is the stationary source of subtle, low-frequency, resonant electromagnetic field with composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity of nano-rings formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when polymeric body is exposed to the external electromagnetic fields of specific frequency and wavelength.[1]

This particular article relates to a detailed observation of the effect of MRET activated water with the modified molecular structure, physical and electrodynamic characteristics on metabolic activity and growth of conditionally pathogenic microbiological culture *Escherichia coli* K-12 *(E.coli)* and on metabolic activity of microbial associations (similar to microbial associations in the intestine). The research was conducted under supervision of Prof. Vladimir Vysotskii (Kiev State University, Ukraine), Alexander Tashyrev, Ph.D., Anna Tashireva, Ph.D. (Kiev Institute of Microbiology and Virology of Ukrainian Academy of Science), and Alla Kornilova, Ph.D. (Moscow State University, Russia).[2]

The research regarding the physical parameters of water conducted earlier at Moscow State University, Russia confirmed that MRET treatment of distilled water leads to substantial modification of basic physical-molecular properties of distilled water. The level of modification of properties of MRET water depends on the duration of the process of activation. The results also confirmed the ability of MRET activated water to keep its anomalous characteristics for several hours or days at room temperature and especially at low temperature (known in physics as the "long-term water memory" phenomenon).[1]

[1] Vysotskii, V.I., Smirnov, I.V. and Kornilova, A.A., Introduction to the Biophysics of Activated Water. Universal Publishers, 2005 [2] Vysotskii, V.I., The Biophysical Model and Experimental Observation of Strong Inhibition Activity of Water Activated with the help of MRET Process. *Program and Abstract Book, International Congress on Medical Physics and Biomedical Engineering, Seoul, Korea,* 2006

The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirmed the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process.[3] The prior researches confirmed the ability of MRET water to enhance morphology of blood cells, immune response and to inhibit the growth of mutated cells.[4, 5, 6]

Significant positive effect of MRET activated water for preventive treatment and enhanced tumor resistance in oncology in animal model was observed in the investigation conducted on 500 mice (laboratory models of Ehrlich's ascites tumor and Sarcoma ascites form). The best results were observed in the groups of mice on MRET water activated for **30 minutes (optimal regime of activation)**. The substantial anti-tumor efficacy was confirmed by very high level of reduction of Total Number of Viable Tumor Cells by **76%** for animals in "preventive treatment" group. The application of MRET water in "therapeutic treatment" regime was less effective. The reduction of Total Number of Viable Tumor Cells by **55%** was observed for the optimal 30 minutes activated water. The survival of the investigated animals was daily monitored in order to study the effect of different activated water fractions on dynamic and survival indices of tumor-bearing mice. Water activated for 30 minutes produced the most significant effect on survival of mice with transplanted tumors. The life span of mice which received optimal activated water in "preventive treatment" regime increased by 61%. The increase of life span by 43% was observed in "therapeutic treatment" regime for the optimal 30 minutes activated water.[8]

The bacteriostatic activity of MRET water (the inhibition of growth and reproduction of pathogens) was confirmed by testing conducted at C.A.I. Environmental Laboratory, Carlsbad, USA. It revealed the significant reduction of the amount of total coliforms following the process of MRET activation. The reduction of coliform population by **86%** was observed in the rainwater activated for 30 minutes compare to non-activated rainwater.[7] The significant bacteriostatic effect of **70 – 100%** (depending on initial concentrations of pathogens) was observed in MRET-activated nutrient medium with *Staphylococcal aureus* cultures in the recent investigation. This research in animal model also revealed the fact that the consumption of MRET water stimulated the phagocytic activity and immune response. There was no case of animal death in all investigated groups within the first 24 hours after intraperitoneal inoculation of *Staphylococcus* culture which is a pretty standard result. During the next 8 days **30%** of animals died in control group which is an expected result for such experimental procedure. There was no death case in both groups of mice that ingested MRET activated water and it is a very unusual result. Thus, the consumption of MRET water reduced the death rate from 30% (control group) to 0% (MRET groups) during the first 9 days of experiment.

Taking in consideration the high bacteriostatic activity of MRET water already confirmed by previous researches the goal of this investigation was to study the effect of MRET water activated for different periods of time (30 and 60 minutes respectively) on metabolic activity and growth of conditionally pathogenic microbial culture *Escherichia coli* K-12 in aerobic and anaerobic environment and on microbial associations in anaerobic environment. The study revealed the significant inhibition of growth and the inhibition of metabolic activity of *E.coli* bacteria in **aerobic** environment. It confirms that the process of MRET activation and the sterilization effect of MRET water can be applied in food industry and for water purification.

Taking in consideration that a small population of pathogenic bacteria, such as *E.coli*, is usually presented in complex microbial associations in the intestine, the test on metabolic activity of *E.coli* bacteria in anaerobic environment was conducted. The investigation showed that the metabolic activity of *E.coli* bacteria in **anaerobic** environment practically did not change. In order to simulate the environmental conditions similar to the conditions in the intestine of humans and animals the test on metabolic activity of complex microbial associations was conducted in **anaerobic** environment. It was found that MRET activated water substantially increased metabolic activity of microbial associations during the first several hours of experiment. Thus, this investigation shows that the ingestion of MRET water is beneficial for the process of digestion and can enhance metabolism of the body.

Materials and Methods

MRET water effect on the growth of microbiological culture *Escherichia coli* K-12 in nutrient medium (meat broth with 1.5% of agar) was studied at the first stage of investigation. The goal of the investigation was to find out the effect of MRET-activated water based nutrient medium on the physiology of bacteria culture, especially on the inhibition of growth of colonies (bacteriostatic activity), their size and the mechanism of cell division.

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[3] Smirnov, I.V., The Anomalous Low Viscosity and Polarized-Oriented Multilayer Molecular Structure of MRET Activated Water. *Explore,* 2007, Vol.16, No.4, 37-39 [4] Smirnov, I.V., The Physiological Effect of MRET Activated Water on Patients Suffering from AIDS. *Explore,* 2006, Vol.15, No.2, 37-40 [5] Smirnov, I.V. and Peerayot, T., The Physiological Effect of MRET Activated Water. *Explore,* 2006, Vol.15, No.1, 38-44 [6] Smirnov, I.V., Mechanism of Activated Water's Biological Effect on Viruses. *Explore,* 2003, Vol.12, No.4, 34-36 [7] Smirnov, I.V., Activated Water. *Explore.* 2002, Vol.11, No.2, 49-53 [8] Smirnov, I.V., MRET Activated Water and its Successful Application for Preventive Treatment and Enhanced Tumor Resistance in Oncology. *European Journal for Scientific Research,* 2007, Vol.16, No.4, 575-583, ***http://www.eurojournals.com/Vol%2016%20No%204.htm***

Two groups of samples of water based nutrient medium were treated by MRET device for 30 minutes and 60 minutes respectively. After that both non-activated (control) and activated medium samples were kept for 24 hours in sterile environment at 5°C temperature. *E.coli* bacteria culture was inoculated on the surfaces of non-activated samples and of two groups of activated samples. Cultivation of colonies was produced at 20°C in ***aerobic*** environment. The growth of *E.coli* bacteria began on the 17th hour of experiment and was studied during the following 12 hours (up to 29 hours of experiment).

After the cultivation the morphological and tinctorial properties of cultures were observed and the numbers of colonies grown on MPA were counted. The bacteriostatic activity of MRET activated nutrient medium (MPA) was measured as an Index of Bacteriostatic Activity (IBA). An Index of Bacteriostatic Activity is defined as a coefficient of the inhibition of growth and reproduction of pathogens in bacteriostatic medium, particularly in MRET activated nutrient medium. It is calculated as reduction of the number of colonies (CFU – Colony Forming Units) in MRET activated medium related to the control samples not exposed to the activation:

$$IBA = (N_{control} - N_{act})/N_{control}$$

where N – number of bacteria colonies (CFU) in Control (non-activated) and MRET activated nutrient medium respectively.

At the second stage of the investigation the metabolic activity of *E.coli* bacteria was studied in aerobic and anaerobic environment based on measurements of reductant activity of microorganisms. Reductant activity is an integral characteristic of metabolic activity of microorganisms and it is measured with the help of Sodium Resasurine color indicator in the percentage degree of discoloration (purple = 0%, red = 50%, transparent = 100%).

The symbols on the pictures and on the charts of experiments are as follows:

K – control samples (blue color on the charts);
0.5 – 30 minutes of MRET activation (red color on the charts);
1.0 – 60 minutes of MRET activation (green color on the charts);
K_{CTEP} – reference to sterility;
R – mean values of reductant activity (R_C or R_K – control, $R_{0.5}$ – 30 minutes activation, $R_{1.0}$ – 60 minutes activation);
V – mean values of gas volume in the bottles;
D – mean values of optical density;
K – relative reductant activity (coefficient of alteration of reductant activity $K_{0.5}=R_{0.5}/R_C$, $K_{1.0}=R_{1.0}/R_C$).

Results and Discussion

The Inhibition of *E.coli* Bacteria Growth in Aerobic Environment: The investigation[2] revealed the significant effect of MRET-activated nutrient medium in ***aerobic*** environment on the process of growth and reproduction of *E.coli* microorganisms, their division, the size of colonies and the modification of forms of culture cells. It was observed that at low initial concentration of cells of investigated culture *Escherichia coli* K-12 MRET nutrient medium activated during **30 minutes** and **60 minutes** inhibited the growth of culture 27 and **303 times** respectively during the 25 hours of experiment.

Initial view of Petri dishes with different fractions of nutrient medium at the beginning of experiment is presented on *Fig. 1*. Petri dishes with grown colonies and statistical parameters of the colonies after 23 hours of experiments are presented on *Fig. 2*: (a) non-activated nutrient medium (control); (b) nutrient medium activated for 30 minutes; (c) nutrient medium activated for 60 minutes. Petri dishes after 29 hours of experiment are shown on *Fig. 3*. The significant inhibition of growth of *E.coli* in activated samples was revealed and it confirmed the strong bacteriostatic effect of MRET-activated medium.

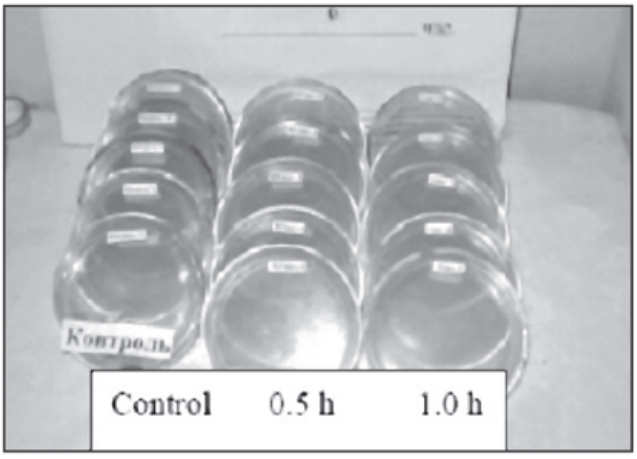
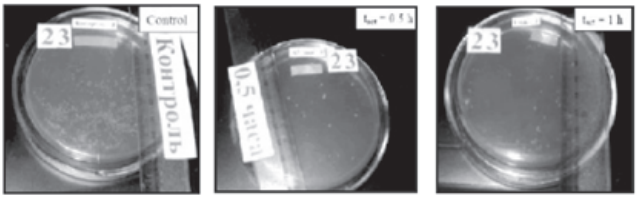


Fig 1: Petri dishes at the beginning of experiment. Identical very small amount of *Escherichia coli* K-12 cells was introduced on a surface of non-activated nutrient medium of **control** dishes and on a surface of dishes with nutrient medium MRET-activated for **30 and 60 minutes(t_{act}=0.5h and t_{act}=1.0h) in aerobic environment.** There are no colonies of microorganisms in Petri dishes in the beginning of experiment.



(a) Control: Number of colonies **N_C = 1.7x10⁸ CFU/ml.** Average diameter of grown colonies d = 1.1 mm.

(b) MRET-activated, tact = 30 minutes: Number of colonies **$N_{0.5}$ = 6.4x10⁶ CFU/ml.** Average diameter of grown colonies d = 1.8 mm.

(c) MRET-activated, tact = 60 minutes: Number of colonies **$N_{1.0}$ = 5.2x10⁵ CFU/ml.** Average diameter of grown colonies is d = 1.5 mm

Fig 2: Petri dishes after **23 hours** of experiment.

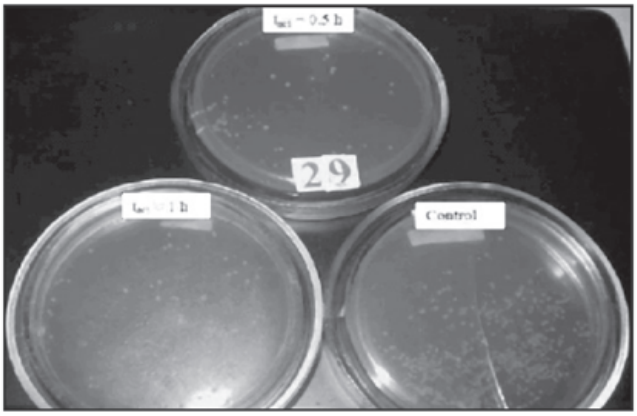


Fig 3: Selected Petri dishes with grown colonies of E.coli K-12 after 29 hours of experiment.

This experiment shows that MRET-activation process has very strong bacteriostatic effect on conditionally pathogenic *E.coli* microorganisms and that the inhibition of *E.coli* growth is more effective when activation time is increased. It was observed that at low initial concentration of cells of *E.coli* in nutrient medium MRET-activation during **30 minutes** and **60 minutes** period of time inhibited the culture growth $N_C/N_{0.5} = 27$ and $N_C/N_{1.0} = 303$ times respectively after 25 hours of experiment *(Fig. 4 – please see enclosed color plate)*. Consequently, the level of bacteriostatic activity was **96%** in 30 minutes activated nutrient medium and **99.7%** in 60 minutes activated medium. Thus, the direct correlation between bacteriostatic activity of MRET-activated nutrient medium and the time of activation was confirmed.

This experiment also revealed the strong effect of MRET-activated water on the process of division of *E.coli* microorganisms, the modification of forms of culture cells and the size of colonies. It was observed that one of the reasons of abnormally low growth of *E.coli* bacteria was related to the modification of the process of cell division in MRET-activated nutrient medium. In the process of growth and reproduction a large number of cells did not separate from each other and the linear line-ups consisting of 2-3 sequentially paired cells were formed. The culture cells grown in non-activated and MRET activated medium are shown on *Fig. 5* and *Fig. 6* respectively.

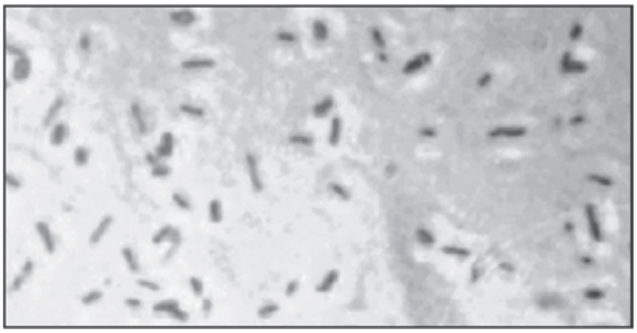


Fig 5: Cells of *Escherichia coli* K-12 grown in non-activated medium.

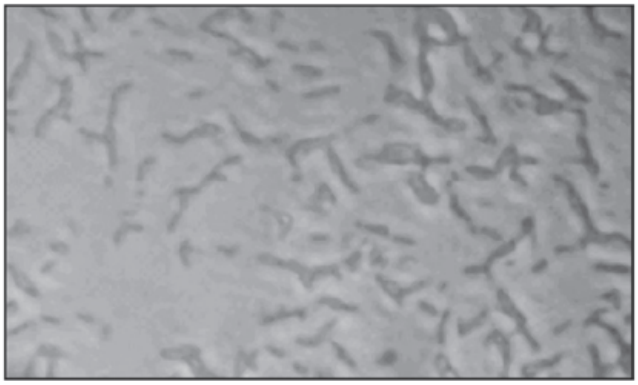
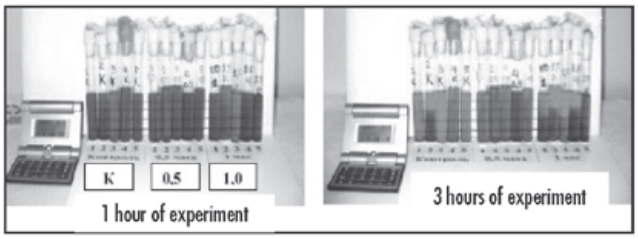


Fig 6: Cells of *Escherichia coli* K-12 grown in MRET -activated medium (t_{act} =1 hour).

Metabolic Activity of *E.coli* Bacteria in Aerobic and Anaerobic Environment: The reductant activity of *E.coli* bacteria reduced **up to 3 times** in **30 minutes** MRET-activated water and up to 1.6 times in 60 minutes activated water during the first 6 hours of experiment in aerobic environment *(Fig. 7 and 8 – please see enclosed color plate)*.



K – Control; 0.5 – 0.5 hour MRET-activated water; 1.0 – 1 hour MRET-activated water

$R_C = 0.2, R_{0.5} = 0.1, R_{1.0} = 0.4$	$R_C = 25, R_{0.5} = 9, R_{1.0} = 19$
$K_{0.5} = 0.5, K_{1.0} = 2.0$	$K_{0.5} = 0.36, K_{1.0} = 0.76$
$D_C = D_{0.5} = D_{1.0}$	$D_C = D_{0.5} = D_{1.0}$

Fig 7: Comparative test on metabolic (reductant) activity of E.coli (control samples, 30 and 60 minutes MRET- activated water) in **aerobic** environment: R – reductant activity (in Control, 0.5 hour and 1.0 hour MRET-activated water), K – relative reductant activity, D – optical density.

This experiment showed that there was no direct correlation between the inhibition of metabolic (reductant) activity and the inhibition of growth of *E.coli* (bacteriostatic activity) in MRET activated water. The bacteriostatic effect is substantially higher in 60 minutes activated water and the inhibition of reductant activity during the first 3 hours is higher in 30 minutes activated water. Thus, this experiment revealed that **the optimum time** of activation for the maximum inhibition of metabolic activity of *E.coli* bacteria in **aerobic** environment is **30 minutes**. The same optimum time of activation was found in the process of another investigation regarding the application of MRET activated water for preventive treatment and enhancement of tumor resistance *in vivo* on 500 mice for two types of cancer conducted at Kiev Institute of Experimental Pathology, Oncology and Radiobiology of Ukrainian Academy of Science.[8]

[8] Smirnov, I.V., MRET Activated Water and its Successful Application for Preventive Treatment and Enhanced Tumor Resistance in Oncology. *European Journal for Scientific Research*, 2007, Vol.16, No.4, 575-583, *http://www.eurojournals.com/Vol%2016%20No%204.htm*

Taking in consideration that a small population of pathogenic bacteria, such as *E.coli*, is usually presented in complex microbial associations in the intestine of the body, the test on metabolic activity of *E.coli* bacteria in anaerobic environment was conducted. Anaerobic environment simulates the environmental conditions similar to the conditions in the intestine of humans and animals. The investigation showed that the reductant activity of *E.coli* bacteria in **anaerobic** environment practically did not change *(Fig .9 and 10 – please see enclosed color plate)*.

This experiment revealed that the process of MRET activation did not have any significant effect on reductant activity of *E.coli* bacteria in **anaerobic** environment.

Metabolic Activity of Complex Microbial Associations in Anaerobic Environment: In order to simulate the environmental conditions similar to the conditions in the intestine of humans and animals the test on metabolic activity of microbial associations was conducted in **anaerobic** environment. It was found that MRET-activated water substantially increased reductant activity of complex microbial associations during the first several hours of experiment *(Fig. 11 – please see enclosed color plate)*. This experiment revealed that **the optimum time** of activation for the maximum increase of metabolic activity of microbial associations in **anaerobic** environment was **30 minutes**. The same optimum time of activation was found in the process of inhibition of metabolic activity of *E.coli* in aerobic environment and in another investigation regarding the application of MRET activated water for preventive treatment and enhancement of tumor resistance *in vivo*.[8]

Conclusions

This investigation revealed that at low initial concentration of cells of conditionally pathogenic microbiological culture *Escherichia coli* K-12 in water based nutrient medium activated for **30 minutes** and **60 minutes** the growth of culture was inhibited 27 and **303 times** respectively after the 25 hours of experiment in **aerobic** environment. This experiment also revealed the strong effect of MRET activated water on the process of division of *E.coli* microorganisms, the modification of forms of culture cells and the size of colonies. It was observed that one of the reasons of abnormally low growth of *E.coli* population was related to the modification of the process of cell division in MRET-activated nutrient medium. These results allow admitting that the process of MRET activation and the sterilization effect of MRET water can be applied in food industry and for water purification.

The second stage of investigation revealed that the metabolic (reductant) activity of *E.coli* bacteria reduced **up to 3 times** in 30 minutes activated water and **up to 1.6 times** in 60 minutes activated water during the first 6 hours of experiment in **aerobic** environment. Another experiment showed that the process of MRET-activation did not affect the reductant activity of *E.coli* bacteria in **anaerobic** environment and, consequently, should not affect a small population of conditionally pathogenic bacteria, such as *E.coli*, usually presented in microbial associations in the intestine of the body.

In order to simulate the environmental conditions similar to the conditions in the intestine of humans and animals the test on metabolic activity of complex microbial associations was conducted in **anaerobic** environment. It was discovered that MRET activated process substantially increased reductant activity of complex microbial associations during the first hours of experiment. The same **30 minutes optimum time of activation** was observed in the process of inhibition of metabolic activity of conditionally pathogenic *E.coli* bacteria in **aerobic** environment and for the maximum increase of metabolic activity of complex microbial associations in **anaerobic** environment (presented in the intestine). The previous investigation regarding the application of MRET activated water for preventive treatment and enhancement of tumor resistance in oncology *in vivo* on 500 mice also showed the best results on 30 minutes MRET-activated water. Thus, this investigation shows that the ingestion of MRET water is beneficial for the process of digestion and can enhance metabolism of the body.

[8] Smirnov, I.V., MRET Activated Water and its Successful Application for Preventive Treatment and Enhanced Tumor Resistance in Oncology. *European Journal for Scientific Research*, 2007, Vol.16, No.4, 575-583, *http://www.eurojournals.com/Vol%2016%20No%204.htm*