

Biological and Microbiological Effects of MRET Activated Water.

The examinations of influence of MRET activated water on biological systems have been conducted within 3 months (June- August 2004) at Institute of Microbiology and Institute of Plants Genetics of Ukraine Academy of Sciences under supervision of Prof. V. Vysotskii, Kiev National University.

Introduction:

The investigation was conducted regarding the effect of MRET water on the growth of mutated cells of botanical origin (Kaluss tissue), on metabolic activity and growth of pathogenic bacteria E-coli, on growth activity of E-coli in the presence of various types of antibiotics, on the process of plants growth cycle.

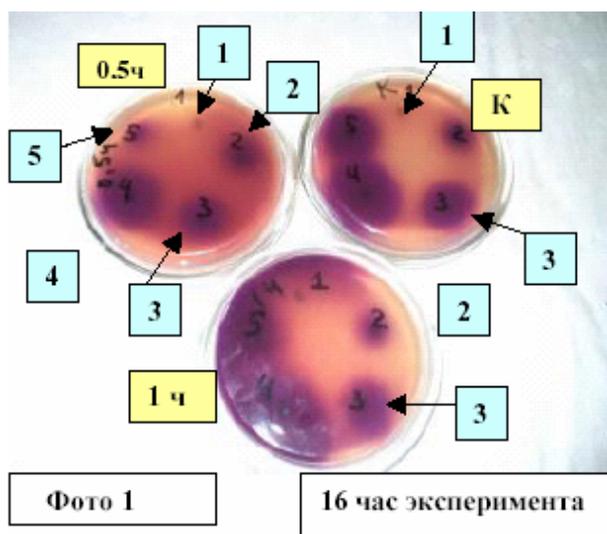
In order to imitate the environmental conditions similar to the conditions in the human's and animal's digestive systems the test on metabolic activity of microbial associations was conducted in anaerobic environment. Taking into consideration that a small population of pathogenic bacteria, such as E-coli, is usually presented in microbial association in the human body intestine system, the test on the metabolic activity of E-coli in anaerobic environment was also conducted.

The different kinds of organisms were used in the experiments to investigate effects of MRET activated water. These organisms can be divided into four hierarchical levels of architecture of living substance:

- the *first level* - microbial culture *Escherichia coli K 12*
- the *Second level* - microbial association including the highest possible variety of species and physiological groups of microorganisms.
- the *Third level* - higher plants grown in a nutritious medium,
- the *Fourth level* - higher plants grown in a soil.

Totally about 30 different types of examinations were carried out. It was discovered in the majority of them that MRET activated water essentially influences to biological objects. We inform you about the most interesting results. These results are also partially presented in form of photographs.

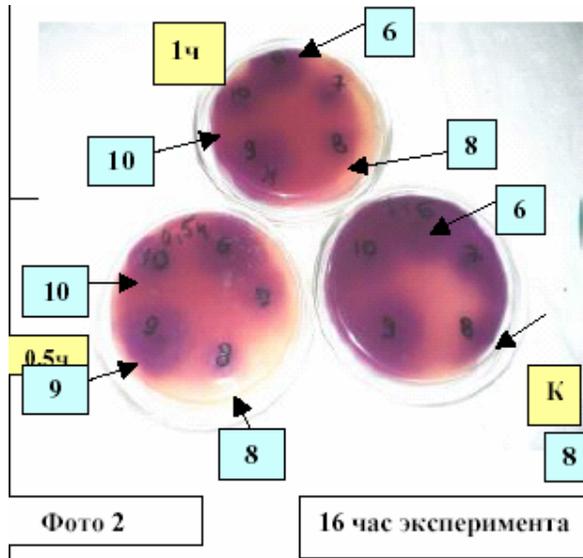
1. The examination of action of 10 different antibiotics (including *clindamycin*, *kanamycin*, *chloramphenicol*, *ampicillin*, *tetracycline*) on culture *Escherichia coli* in MRET activated water has been carried out. The following antibiotics were used in this experiment: (1) Clindamycin, (2) Kanamycin, (3) Cephalexin, (4) Cephtriaxon, (5) Levomiticin, (6) Ciphran, (7) Ampicillin, (8) Tetracycline, (9) Cephaclor, (10) Karbenicyline. The resistance to the effect of antibiotics (RA) is determined by the size (diameter) of the area of inhibition of bacteria growth. It correlates with the size of the purple color area (no reductant activity of bacteria as a result of suppression of bacteria growth). (K) –control sample, (0.5) – 30 minutes activated sample, (1) –60 minutes activated sample.



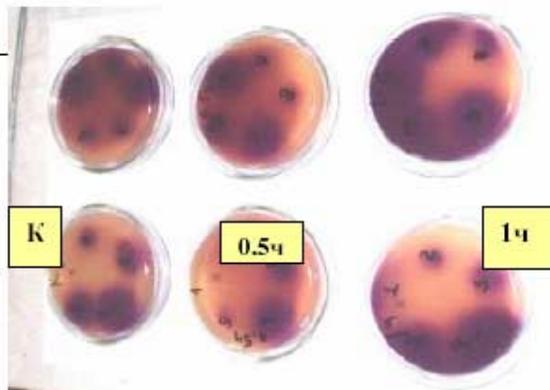
The 16th hour of experiment. Antibiotics #1-5.

The maximal resistance to the effect of antibiotics was observed for 30 minutes activated samples.

The minimal resistance to the effect of antibiotics was observed for 60 minutes activated samples.



The 16th hour of experiment.
Antibiotics # 6-10.
The maximal resistance to the effect of antibiotics was observed for control and 30 minutes activated samples.
The minimal resistance to the effect of antibiotics was observed for 60 minutes activated samples.



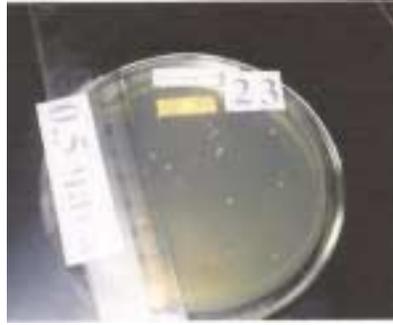
The 24th hour of experiment. The areas of the inhibition of bacteria growth significantly decreased by the end of this experiment.
The maximal resistance to the effect of all antibiotics (the smallest diameter of area) was observed for 30 minutes activated samples.
The minimal resistance to the effect of all antibiotics was observed for 60 minutes activated samples

It was shown that both amplification of efficiency of activity of some antibiotics in 2-3 times on growth of microbiological culture and impairment of this activity in 2-10 times takes place. This effect depends on the time of water activation. The directedness of change of activity of antibiotics and value of this change is different for different antibiotics.

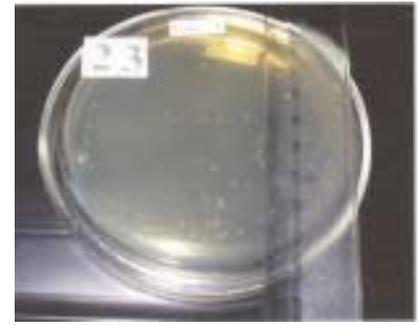
2. The examination of influence of MRET activated water on growth of culture *Escherichia coli* in beef-extract agar in the aerobic environment was conducted within 25 hours of experiment.



Control sample.

 $N = 1.7 \times 10^8 \text{ cell/ml}$


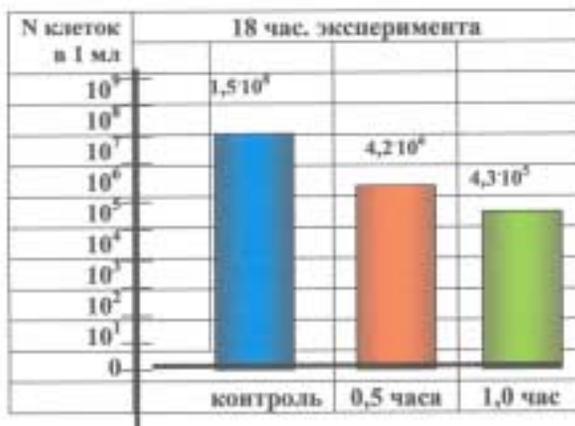
30 minutes of activation.

 $N = 6.4 \times 10^6 \text{ cell/ml}$


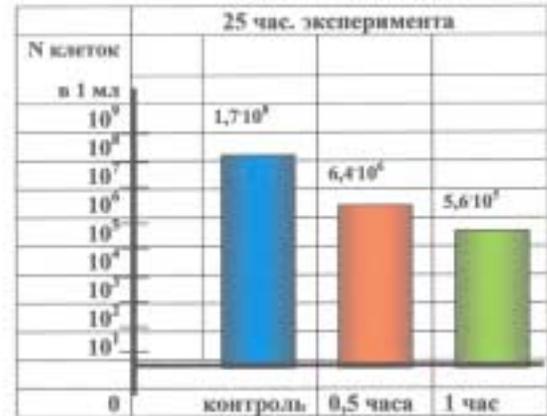
60 minutes of activation.

 $N = 5.2 \times 10^5 \text{ cell/ml}$

Two samples of water based agar medium were treated by MRET device for 30 minutes and 60 minutes respectively. After that activated medium was kept for 24 hours in sterile environment. Then *E. coli* bacteria were introduced to not activate sample and two activated samples of medium. The growth of *E. coli* bacteria began on the 17th hour of experiment. The photographs show test results on the 23d hour of experiment. There is significant inhibition of growth of *E. coli* in activated samples. MRET treatment has strong sterilization effect.



18 hours of experiment

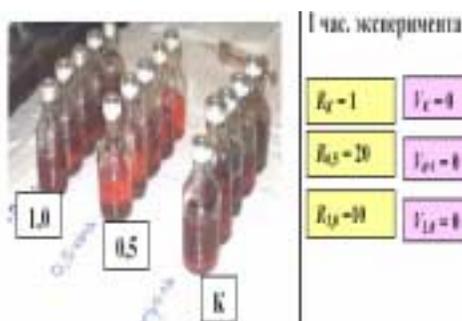


25 hours of experiment

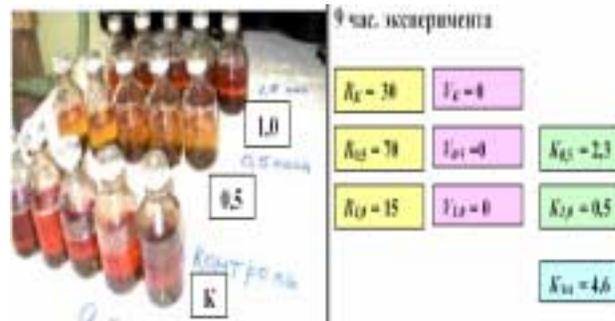
Blue color – control sample; Red color – 30 minutes of activation; Green color – 60 minutes of activation. The activation of water based media has suppressed growth of *E. coli* bacteria in 30 to 300 times. The inhibition of *E. coli* growth is more effective when activation time is increased. This test shows that MRET water has very strong germicidal activity. At the increasing of the time of water activation inhibition of culture growth is more effective. In this case activated water has very strong germicidal activity. In other words, in activated water at an optimum regime of

activation the growth of culture *Escherichia coli* are completely suppressed in the **aerobic** environment.

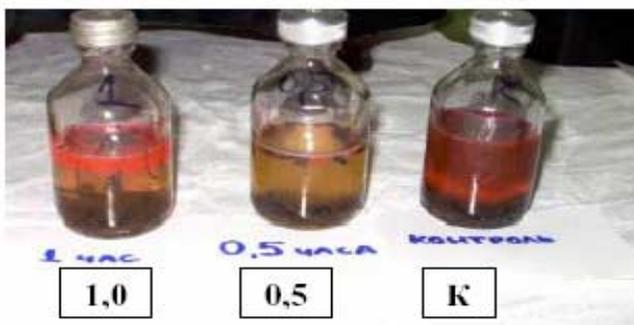
3. The study of influence of MRET activated water on reductibility and enzyme activity of microbial *associations* has been conducted in the **anaerobic** environment. It was discovered that the water activated for 0.5 hours provides the effect of very strong increase of reductant and enzyme activity of microbial *associations* during several next hours after activation takes place.



1st hour of experiment



9th hour of experiment

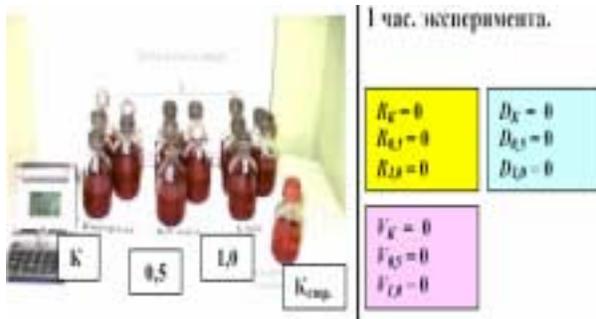
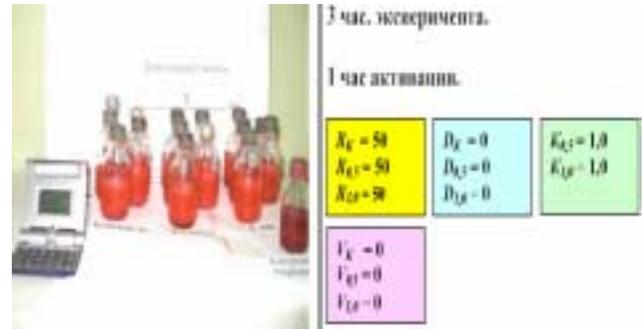
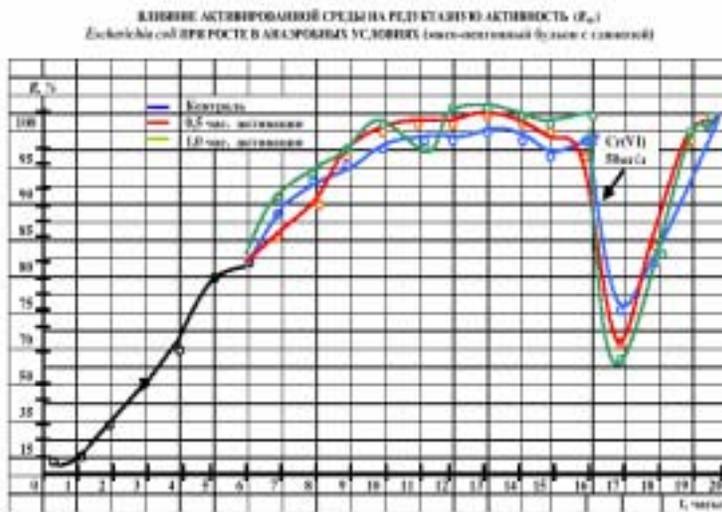


Final results of experiment

R – mean value of reductant activity; **V** – mean value of gas volume in bottles; **K** – coefficient of reductant activity.

- ✦ 30 minutes of MRET activation leads to maximum reductant and enzyme activity – color transparent 100% ;
- ✦ 60 minutes of MRET activation leads to significant reductant and enzyme activity – 80% transparent and 20% coloration;
- ✦ Control sample – no reductant and enzyme activity – 100% coloration

4. The reductant activity of E-coli bacteria in the **anaerobic** environment practically did not change.

1st hour of experiment3rd hour of experiment

Reductant activity of *E. coli* in the anaerobic environment

MRET water activation does not have any significant effect on reductant and enzymatic activity of *E. coli* microorganisms in the anaerobic environment. Reductant activity is an integral characteristic of metabolic activity of microorganisms. It is measured with the help of Sodium Resazurine color indicator in the percentage degree of decolorization (purple = 0%, red = 50%, and transparent = 100%).

$K_{\text{ср.}}$ – reference for sterility; $K_{0.5}$ – coefficient of inhibition of reductant activity (30 minutes of activation); $K_{1.0}$ – coefficient of inhibition of reductant activity (60 minutes of activation);

R_k , $R_{0.5}$, $R_{1.0}$ – mean values of reductant activity in percentage.

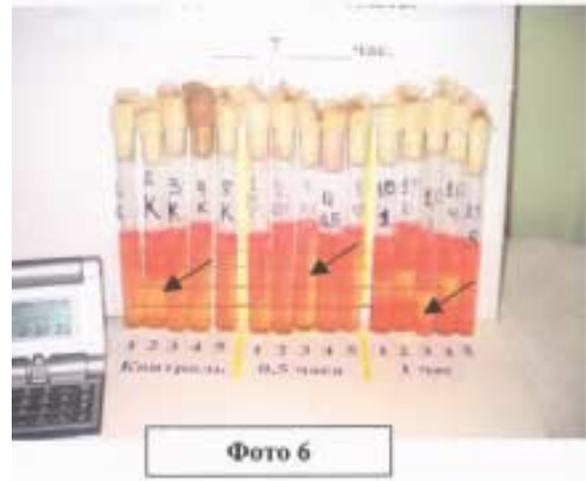
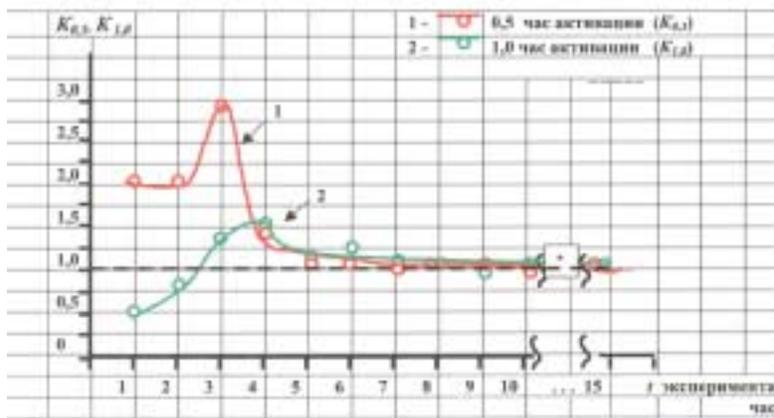
D_k , $D_{0.5}$, $D_{1.0}$ – mean values of optical density;

V_k , $V_{0.5}$, $V_{1.0}$ – mean values of gas volume in containers

5. On the contrary, in the aerobic environment reductant activity of one line culture *Escherichia coli* in this period of time decreases greatly. Based on the increasing or decreasing of the time of water activation in relation to optimal duration this effect becomes weak or generally incidental.



Beginning of experiment

7th hour of experiment

R – reductant activity (R_c – control, R_{0.5} - 30 minutes of activation, R_{1.0} - 60 minutes of activation. Reductant activity is an integral characteristic of metabolic activity of microorganisms. It is measured with the help of Sodium Resazurine color indicator in the percentage degree of decolorization (purple = red = 50%, and transparent = 100%). K – shows the inhibition of reductant activity in relation to control sample ($K_{0.5} = R_c/R_{0.5}$ and $K_{1.0} = R_c/R_{1.0}$). MRET activated water shows strong inhibition effect on E. coli bacteria. The reductant activity of E. coli in activated water is reduced up to 3 times during the first 6 hours of experiment. It leads to suppression of E. coli growth.

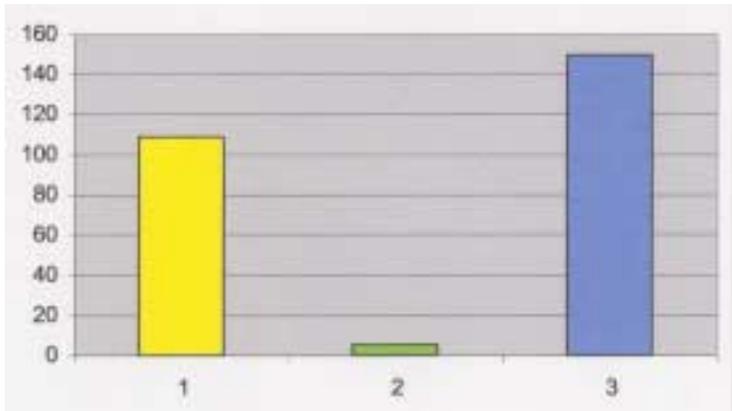
6. The very strong influence of MRET activated water on the process of growth of Kaluss tissue cells (like psoriasis) of botanical (vegetative) origin grown in the nutritious medium has been discovered.



60 minutes

30 minutes

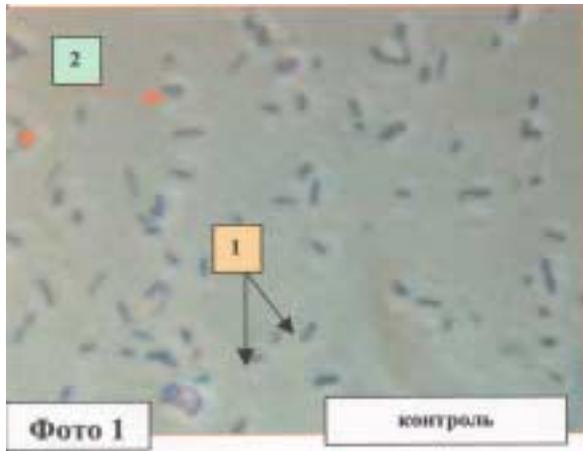
control



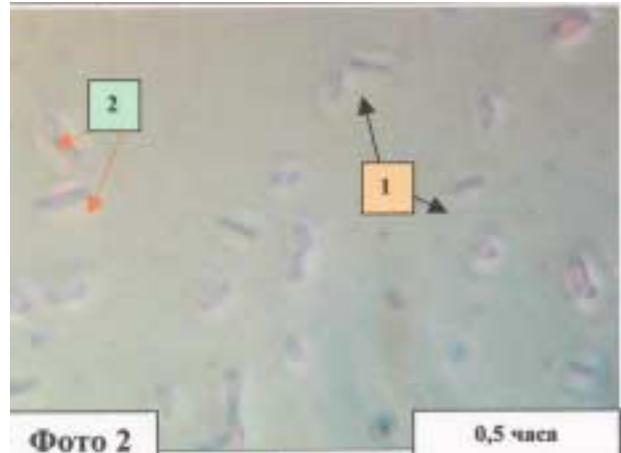
- ◆ 1 – average increase of single segment weight of tissue (mg) in activated medium (60 minutes, dilution ratio 1:2.5);
- ◆ 2 – average increase of single segment weight of tissue (mg) in activated medium (30 minutes, dilution ratio 1:2.5);
- ◆ 3 – average increase of single segment weight of tissue (mg) in control sample (distill water);

The strong inhibition of the growth of “kallus cells” (*Solanum Rickii*) was observed in activated medium. Kallus cells are mutated cells of botanical origin with ability to produce uncontrolled, unlimited growth in the nutrient medium. The similar mutated cells of animal origin are known as psoriasis cells. The 30 minutes activated water produces inhibition of Kallus cells growth in 15 times, the 60 minutes activated water produces inhibition of Kallus cells growth in 1.4 times to compare with control sample. It was shown that at a particular mode of water activation (30 minutes) the very strong suppression of this process takes place. It means that with a high probability such MRET activated water may be an effective agent for treatment of psoriasis and other diseases.

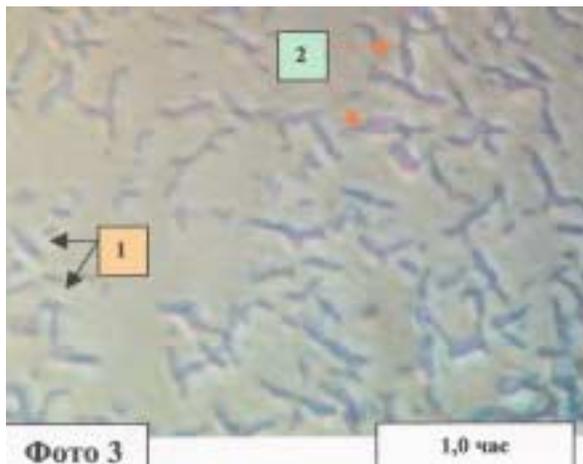
7. The strong influence of MRET activated water on the process of cloning of microorganisms, their division, the size of microorganisms' colony and the form of cell-like division has been discovered. In a number of cases forms of cell division unknown to the science were discovered.



E.coli control (no introduction to MRET water)



E.coli after introduction to MRET water (30)



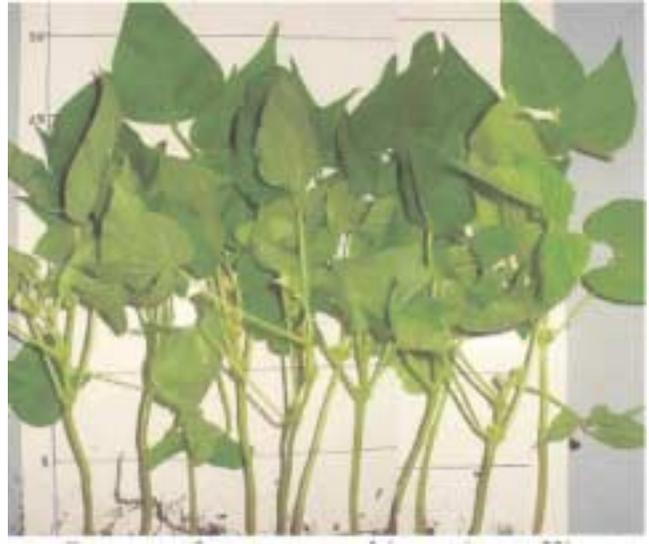
E.coli after introduction to MRET water (60)

Test was conducted at laboratory of Institute of Microbiology, Kiev, Ukraine. Experiment was conducted on *E. coli* bacteria in the **aerobic** environment. Picture #1 – control sample after 24 hours of incubation. Picture #2 – activated sample (30 minutes activation) after 24 hours of incubation. Picture #3 – activated sample (60 minutes activation) after 24 hours of incubation. *I*₉ – index of abnormal cellular division. Control sample *I*₉ = 4%; 30 minutes activation sample *I*₉ = 280%; 60 minutes activation sample *I*₉ = 220%. Activated (MRET) water produces significant changes of *E. coli* morphology: different shape, color and size of cells, disturbance of process of cellular division. . *Adaptation mutation* of *E. coli* cells. /1/ - normal cellular division; /2/ - abnormal cellular division.

8. MRET activated water with an optimum mode of activation accelerates the process of seed germination of some plants (in particular of cabbage, pumpkin, string bean, garden radish and peas) and favors significant increase of their amount of fiber.



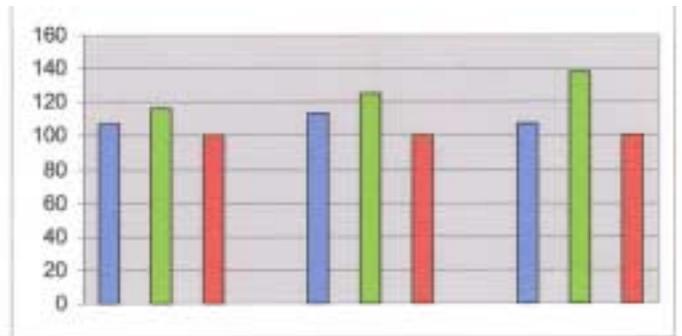
MRET water, 30 minutes of activation



MRET water, 60 minutes of activation



Control, regular water



Average height Weight of stems Area of leaves

Red – control; Green- 30 minutes activation; Blue – 60 minutes activation

Conclusions:

Interpretation of test results and possible applications of MRET Activated water (based on test results).

1. Benefits for humans health and well being;
2. Agricultural industry – benefits for crops irrigation and feeding of animal stock;
3. Sterilization of food products;
4. Pharmaceutical industry

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