# POSSIBLE ROLE OF WATER RESTRUCTURING IN LOW ELECTRIC FIELDS DURING ELECTRIC ADAPTATION OF MICROORGANISMS TO STRESSES

V.S. Andreev, "ElectroEcoTechnologies, LLc",



International Congress "Low and superlow fields and radiations in biology and medicine"

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## Surface charge formation of microbial cells (main types of the ionic balance)

for ionogenic groups of the cell surface:

$$-S - COOH \stackrel{K-}{\leftrightarrow} -S - COO^{-} + H^{+}, \qquad (1)$$

$$-\mathbf{S} - \mathbf{N}\mathbf{H}_2 \stackrel{K+}{\longleftrightarrow} -\mathbf{S} - \mathbf{N}\mathbf{H}_3 + \mathbf{O}\mathbf{H}^-, \tag{2}$$

$$-S - PO_4H_2 \stackrel{K^-}{\leftrightarrow} -S - PO_2H^- + H^+, \qquad (3)$$

$$-S - PO_4H^{-} \stackrel{K2}{\leftrightarrow} -S - PO_4^{2-} + H^{-}, \tag{4}$$

for hydrated parts of the cell surface:

$$-SH_{2}^{+} \stackrel{K^{+}}{\longleftrightarrow} -SH - H^{+}, \tag{5}$$

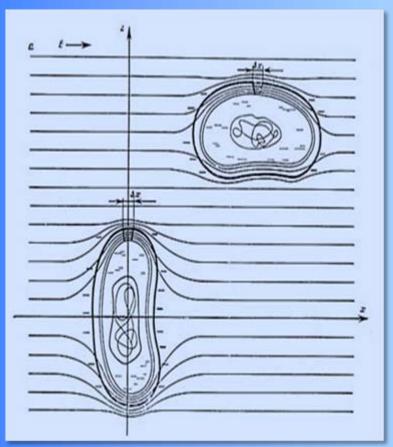
$$-SH \stackrel{K^{-}}{\longleftrightarrow} -S^{-} + H^{+}, \tag{6}$$

for the adsorption mechanism of the cell surface:

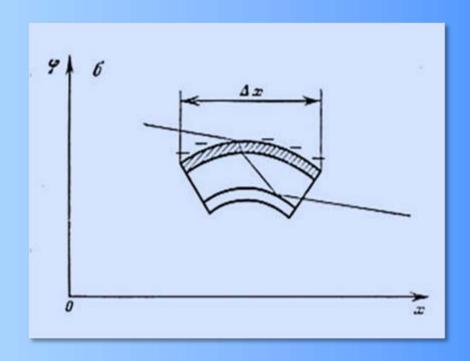
$$-SH + OH^{-} \stackrel{K1-}{\longleftrightarrow} -S + H_2O, \tag{7}$$

$$-SH + H^{+} \stackrel{K1+}{\longleftrightarrow} -SH_{2}^{+}. \tag{8}$$

## Simplified model of a gram-negative microorganism' cell in homogeneous electric field



lines of current in a culture liquid

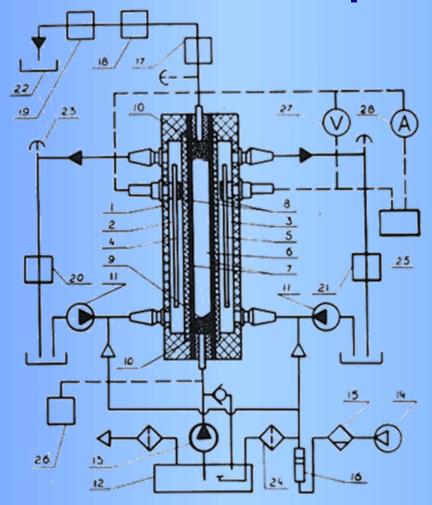


reduction of potential in the cell membrane area

## This research has been tasked to find out the following key issues:

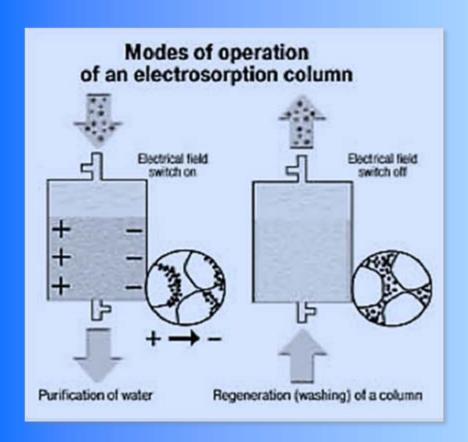
- 1. Is it possible, considering the inevitable role of mutual electrical effects of biological cells throughout their evolution, to "trick" the microbial population (for cognitive purposes) and cause a complex, vital transformation of microorganisms by an external ("false") electric stimulation.
- 2. Is it possible, considering the inevitable action of the external field on the extracellular environment's water as a mediator, to understand what will be a direct effector in the planned experiment: the effects of electric current on the cell, electrically induced chemical changes of medium, the changes of the redox potential of the extracellular water or all of this together.
- 3. What are the possible perspectives and specific ways of practical use of the discovered effects in biotechnology

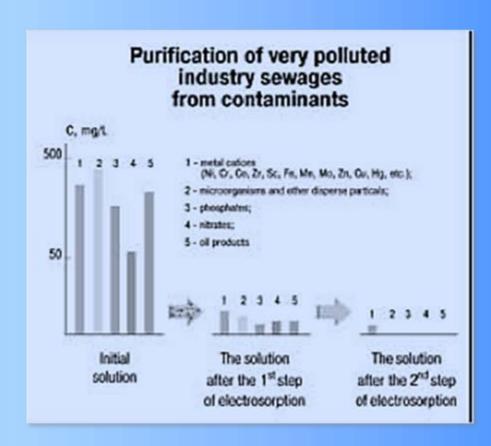
#### The scheme of the main experimental setup



1-case, 2.3-electrode chamber, 4.5-electrodes, 6-the working chamber, 7-semipermeable membrane, 8-porous pads, 9,10-sorbent pellets and filters (in the case of a flow-through culturing of microbial cells), 11-pump for pumping buffer solution through the electrode chambers, 12–nutrient medium tank, 13-pump for pumping the culture medium through the working chamber, 14-compressor for culture medium aeration, 17,18-optical detectors, 19.20,21- conductometric detectors, etc., 22, a vessel for the spent medium collection

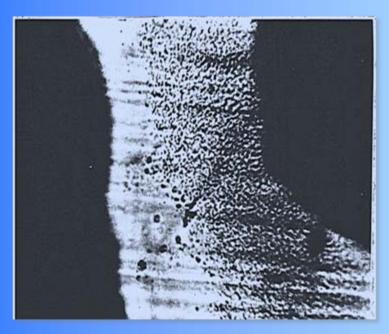
### FOR UNDERSTANDING OF THE MAIN IDEAS OF WATER ELECTROCONDITION (1)





#### FOR UNDERSTANDING OF THE MAIN IDEAS OF WATER ELECTROCONDITION (2)

Electrosorption of microparticles on the granulare beds, as the theoretical analysis shows, essentially differ in cases of highly and slightly polarizable materials



Photomicrography (enlargement x 500, the intensity of electrical field is 3000 V/m, a field acts from left to right) illustetes the considerable effect of electrosorption of E.coli cells on the granules of ferroceramics T-10 000 (plant «Coulomb», Russia).



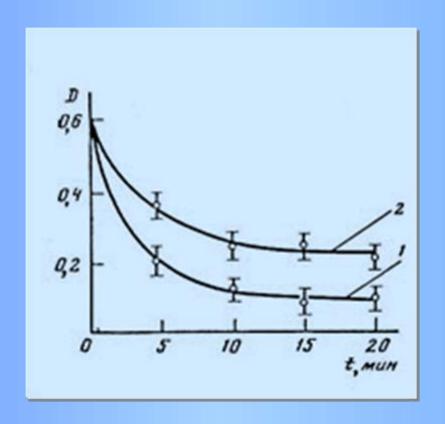
Photomicrography (the same conditions) illustrates the substantially less effect of dipolephoretic focusing of cells due to superposition of external electrical field on the granules of the porous glass, trademark "Bio-Glass-500" (firm "Pharmacia", Sweden)

## Experiments for following ascertainment: "Can we talk about something like an action mechanisms of electrical and thermal treatment factors (thermal shock proteins)"

Table 1

	Survival rate of cells(%) obtained after		
The type of effect	thermal shock	dehydration(drying in a thin layer, 40°)	
Electrotreatment	3,3±0,3	3,6±0,4	
Resuspending in EF-factor containing supernatant	2,4±0,2	4,5±0,6	
Resuspending in supernatant of native culture subjected to thermal shock (50°)	1,2±0,1	1,3±0,1	
Resuspending in supernatant of native culture heated to 60°	1,4±0,1	2,0±0,2	
Resuspending in supernatant of native culture heated to 80°	1,6±0,2	2,5±0,3	

#### Curves of E. Coli - M17 cells lysis



Effect of DDS on cells before (1) and after (2) electric field treatment

## The influence of various intensity electric fields at different exposure time (t) on average $\zeta$ -potential ( $\overline{\zeta}$ , mV) of E. coli M-17 cells

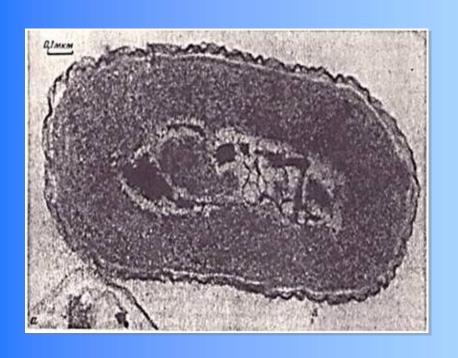
t, min	Reference (E=0)	$E=10^3 \text{ V/m}$	$E=3\cdot10^3\mathrm{V/m}$	$E=5\cdot10^3\mathrm{V/m}$
10	27,4±0,8	33,2±0,8	37,8±0,9	46,2±0,9
20	27,4±0,8	39,1±0,9	44,7±0,9	49,1±1,0
30	27,4±0,8	40,1±0,9	35,0±0,8	30,2±0,8

Table 3

### The influence of various intensity electric fields on average $\zeta$ -potential $(\overline{\zeta}, mV)$ of microbial cells

Microorganism species	Reference (E=0)	E=10 <sup>3</sup> V/m	$E = 3.10^3 \text{ V/m}$	$E = 5.10^3 \text{ V/m}$			
Exposure time 10 min							
E. coli M-17	22,3±0,7	23,8±0,7	25,0±0,8	24,0±0,7			
S. marcescens	21,8±0,7	23,1±0,8	24,3±0,8	23,3±0,8			
Exposure time 20 min							
E. coli M-17	22,3±0,7	25,7±0,8	26,9±0,8	25,8±0,7			
S. marcescens	21,8±0,7	24,0±0,8	26,0±0,8	24,5±0,7			

## Electron micrographs of ultra thin slices of E.coli M-17 cells (x 84 000)



before the action of the electric field



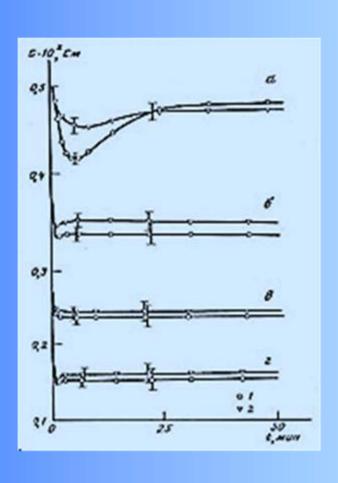
after the action of the electric field

## Survival rate of bacterial cells (stationary phase) during the thermal drying in a thin layer (40°C) after different types of field treatment

	The relative amount of viable cells in the dried specimen, %						
	Native	Resuspending	Culture,	Resuspending of	Resuspending of		
The species of bacteria	culture,	of cells in	exposed in	cells in	cells in		
	E=0	supernatant of	electric field	supernatant of	supernatant,		
	(reference)	native culture	E=1,5·10	culture, exposed	exposed in		
			V/cm	in electric field	electric field		
				E=1,5· 10 V/cm	E=1,5· 10 V/cm		
E. coli K-12	6,0 ±0,5	6,4±0,6	11,3±0,9	31,6±2,9	6,2±0,6		
S.marcescens B10M-1	4,1 ±0,4	4,4±0,4	9,1±0,9	24,7±0,2	5,1±0,5		

**Table** 

## Conductograms for survival of hyperosmotic shock (1M solution of L-arabinose) by microbial cells of different taxonomic groups before and after the electric treatment



- a E.coli
- б S.marcescens
- в Micrococcus Lysodeikticus
- г Arthrobacter sp.
- 1 Experimental points before the electric treatment of cells
- 2 Experimental points after the electric treatment of cells

#### EXPERIMENTS WITH ELECTROCONDITION OF AQUEOUS MEDIUMS

As against filters of other types, electroconditioners will manage with physiologically important problems of water quality which are not being solved at usual technologies purification, namely:

- will allow to correct composition of potable water bearing in mind the majority of the normalized chemical parameters, which may be checked objectively, and the characteristics which are usually being perceived subjectively and not measured by analytical instruments;
- will provide stability of a complex of quality characteristics of water at a level of recommended sanitary norms under various seasonal conditions, sudden emergency deterioration of a condition of water sources or pipelines;
- will improve structural characteristics of water (due to the local electric influences), having provided higher availability of water molecules to use by cells of the most various biological tissues, i.e. the best biocompatibility of water with various organic structures; will decrease redox- potential of water and as a result its bioenergetic, metabolic and immune properties will be improved as well as condition of human internal, integument, mucous membranes, joints and hair. Development of normal microflora of a human body will be stimulated and negative consequences of a disbacteriosis will be reduced



#### How does electroconditioners of water "Cascade"® look?



NEW APPROACH TO WATER TREATMENT
7 significant technological processes in 1 device



System of water-preparation on the basis of electroconditioners "Cascade"® AVE-32/300 at the pharmaceutical enterprise "POLYSAN" in Saint-Petersburg (output 20,000 l/h)

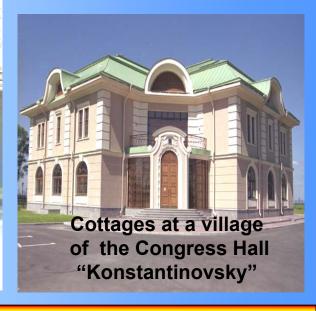


NEW APPROACH TO WATER TREATMENT
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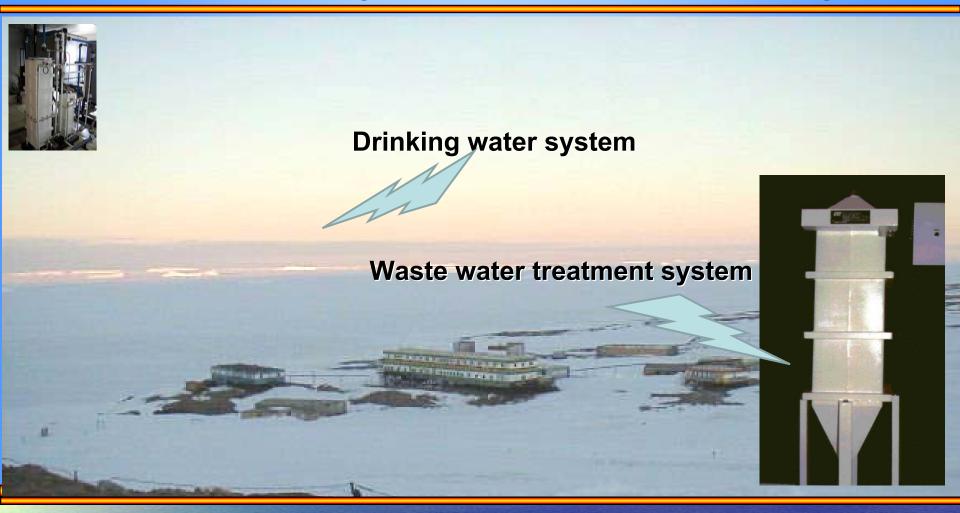
#### Where already drink and use "Revitalized water"? (only a few examples from two hundreds objects)

- Cottage settlement « Russian Switzerland » companies " HONKA ".
   (Leningrad region)
- The newest pharmaceutical enterprise "Polysan" (Saint-Petersburg)
- Hotels in Saint-Petersburg and Nyagan ("Smolninskaya", "Ambassador", etc.)
- Mounting skiing resort "The Gold valley" about settlement Korobitsyno (Leningrad region)
- Motel and autocamping at lakes Vandliz (Germany)
- St.Petersburg office of the Federal Trust "Spetsstroy"
- Summer residence of the firm "Eagle Group" (Hamina, Finland)
- Factory of carpet coverings "Neva Taft" (Saint-Petesburg)
- Apartment house and a business centre "Mont Blanc" (development company "Stroymontage", St. Petersburg)
- Apartment house "Dominant" (development company "Elis",
   St. Petersburg)
- High-rise apartment building "Finansist" ("Gerasimov's Architectural workshop" and the Public Company "ProjectService", St.Petersburg)
- Apartment house "Van Vitelli" (the development company "Business Link Estate, St.Petersburg)





Systems "CASCADE"® for drinking water (from lake Stepped) and sewage treatment in severe conditions of Antarctica (a housing space "Lena", station " Progress ")



NEW APPROACH TO WATER TREATMENT
7 significant technological processes in 1 device



#### Mounting of "Cascade"® units in field conditions (auto-camping near the lake Lipnitzsee Germany, 2001)

#### Second generation of models







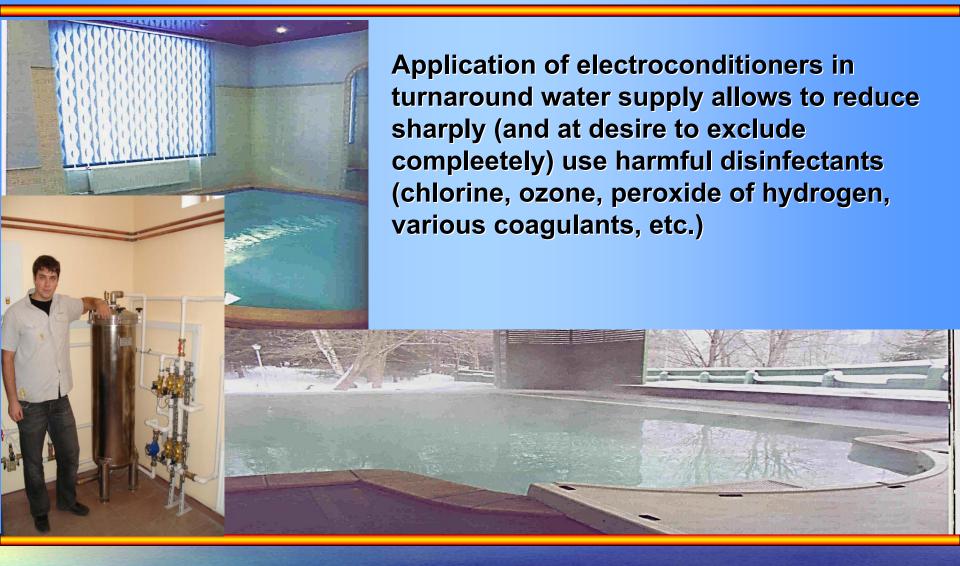
#### Installation "CASCADE"® for purification of waste water from laundry (Hotel "Ambassador", Saint-Petersburg)



NEW APPROACH TO WATER TREATMENT
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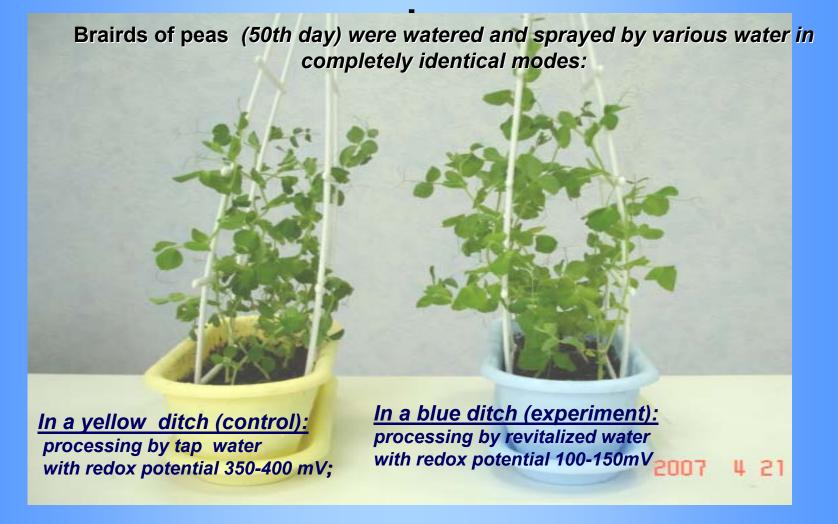
#### What's news in swimming pool technologies?



NEW APPROACH TO WATER TREATMENT
7 significant technological processes in 1 device



#### Illustration №1 of life-giving power of revitalized





#### Illustration №2 of life-giving power of revitalized water

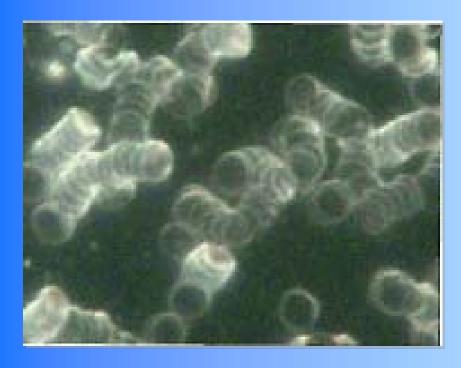
Brairds of viola red (56th day) were watered and sprayed by various water in completely identical modes:



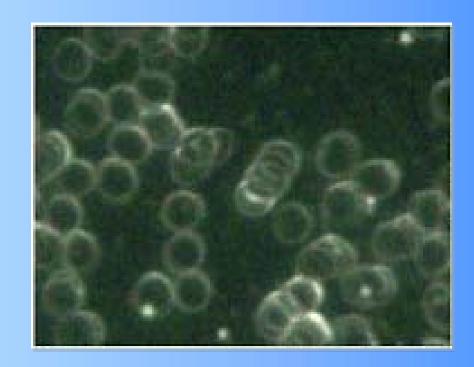


#### By the way

## Photomicrographs of red blood cells, obtained by "Dark field"



Aggregation of the patient's red blood cells, causing many diseases associated with metabolic disbalance (coin columns)



Aggregation of the patient's red blood cells is reduced by regular use of water with lowered redox potential

### Experiments on electric treatment of aqaeous mediums

Table 5

#### The relation between water redox potential and the exposure time in the electric field

The linear flow velocity, cm/min	0 (Reference)	6	24	60
The voltage on the electrodes, V	0	12	12	12
Current, A	0	0,9	0,9	0,9
The value of redox potential, mV	420	-10	0	5

Table 6

### The relation between water redox potential and the electrical conductivity of culture medium

Conductivity of water, µS·cm-1	11(Reference)	11	62	202	255	353	502
The voltage on the electrodes,V	0	12	12	12	12	12	12
Current, A	0	0,1	0,6	2,0	2,5	3,5	5,0
The value of redox potential,mV	380	-13	-10	54	61	369	378

## Survival rate of bacterial cells (stationary phase) during the thermal drying in a thin layer (40°C) after different types of field treatment in lowered conductivity solution

	The relative amount of viable cells in the dried specimen, %						
	Resuspending	Resuspending	Resuspending	Resuspending	Resuspending		
	of cells in	of cells in	of cells in	of cells in	of cells in		
The species	supernatant of	0,15M	0,07M	supernatant	0,15M		
of bacteria	native culture	L-arabinose	L-arabinose	exposed, in	L-arabinose		
or bacteria		solution	solution,	electric field	solution,		
			exposed in	E=1,5·10	exposed in		
			electric field	V/cm	electric field		
			E=1,5·10V/cm		E=1,5·10V/cm		
E. coli K-12	6,4±0,6	5,8±0,5	39,1±2,9	6,2±0,6	46,2±3,1		
S.marcescens B10M-1	4,4±0,4	3,9±0,4	31,4±0,3	5,1±0,5	35,1±0,4		

#### Resume of the series of studies

- 1. There are all reasons to talk about the universal phenomenon of electroadaptation of microorganisms to stress factors as a quite certain fundamental process of regulation of their physiological activity. One can with certainly assume that the mechanism of electroadaptation has originated and perfected during the evolution by natural selection of microbial populations in the changing environmental conditions, various natural disasters.
- 2. The external electrical signals as well as changes in transmembrane potentials, induced by different stressors, are launching biophysical and biochemical processes which lead to the excretion of regulatory factor (EF-factor) into the extracellular medium by microbial cells. This factor, absorbed by the cells (as producers of EF-factor, as native), moves them into the hypobiotic state, contributing to a better surviving of microorganisms during the stress and enhance their viability in the future.

### Resume of the series of studies: (continuation)

- 3. Redox potential reduction of water in the extracellular medium under the influence of the electric field contributes to the achievement of effects, specified in claims 1, 2. That indicates the possible involvement of this process in the electroadaptation of microorganisms to stress. The occurrence of water shortages due to dehydration of the cells or increasing of microbial populations is a basic, in evolutionary terms, probably is a primary process during development of electro-biochemical mechanism of electroadaptation.
- 4. The prospects of the practical implementation of the results are obvious: it is the widespread use of electric treatment of microbial cultures in all the diverse biotechnological processes in which it is important to maintain cell viability, as in the preliminary stages of theprocess, as in the final stages of preparation of ready-made forms of microbiological drugs, for the food, medical or agricultural destination.

The main part of research has been done on the basis of FSUE "State Res. Inst. Extra Pure Preparations" FMBA and the Scientific-Production Company "ElectroEcoTechnologies"

The author appreciates the assistance of the following specialists:



Специалист подобен флюсу: полнота его одностороння his fullness is opecialist is like a flux.



Козына Прутков (Koz'ma Prutkoff)

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