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Intensification of Biomass Cultivation Process of *Lemna minor* at Physical Exposure

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ABSTRACT

The study suggest methods for biomass of *Lemna minor* increase by means of plant treatment by various physical impacts (ultraviolet, infrared, low intensity electromagnetic radiation, magnetic fields). Enhancement of plant reproduction rate and biomass production are caused by increase of energy absorbed by the plant, perimembrane water molecule cluster structure changing, changing of potential difference at the cell membrane, its permeability and enlargement of nutrient transport from the environment to the cell.

INTRODUCTION

Lemna minor is a monocotyledon metasperm plant of the genus *Lemna*. *Lemna minor*, due to its fast vegetatively reproduction, which duplicates its biomass for 36 hours and high protein content, is very attractive as a potential biofactory for production of biogas, livestock feed and pectin.

Lemna plants could be grown in aseptic containers with controlled cultivation conditions. The plants need only water, inorganic nutrients and CO_2 for growth. Thus, artificial and fully closed cultivation environment combined with rapid growth, high protein content in biomass and mainly vegetative reproduction make this system unique and easily controllable. Consequently, *Lemna*-based system has high potential and a lot of advantages over existing systems as it is a highly effective plant system for biogas production.

In the past decades, numerous evidence was discovered which state for high sensibility of biological system to external physical exposures (EPE): constant magnetic field (CMF), electromagnetic (EM) and electric fields, ultraviolet (UV), infrared (IR) and laser radiation, which create additional currents in biological objects. These exposures vary the membrane potential and can manage the organisms growth and evolution process having both stimulative and inhibitory impacts. This impact depends on the exposure characteristics: wavelength (λ), oscillation frequency (f) of the electromagnetic radiation (EMR), CMF strength and exposure time. A number of hypotheses for possibility of resonance interaction of EMR with biological systems have been formulated, relation between biological efficiency and exposure frequency and intensity, "frequency" and "amplitude" frames were determined by Devyatkov et al. (1991). Positive impact of EMR on bio-objects was highlighted and its various physiological effects were studied: growth enhancement, biomass increasing, photosynthesis processes stimulation, accompanied by increase in oxygen release and photosynthesizing pigments in cells, increase of excretion of organic compounds to the environment, changes in exometabolite reactivity and ion transport, etc. (Tambiev et al. 1986, Kazarinov 1990). Earlier studies by Gapochka et al. (2003) have shown an increase in average life span of organisms and their survivability at electromagnetic field exposure.

Numerous investigations have shown that electromagnetic field (EMF) and electromagnetic radiation may have a favourable effect under specific conditions. A cell under stress is characterized by metabolic processes reconstruction, which should lead the plant to adaptation of the changed conditions (Ali-Zade 2009).

Immunity of plants to various exposures of outside environment are characterized not only by specific responses of cells (which depend on exposure characteristics), but nonspecific responses of cells, arised at various impacts. Nowadays, it becomes increasingly evident that nonspecific responses essence is to a great extent limited to the changes found in membrane cell formations. Furthermore, a relation between plant resistivity to various impacts and its membrane component conditions were found. Membranes, as natural barriers, are the first ones to be affected by stress factors. Being dynamic structures, membranes are able to be quickly responsive to living conditions variation. However, the changes in membranes cause a cascade of shifts in metabolic process of cell as a whole. Membrane permeability increases, depolarization of plasmalemma membrane potential takes place, cytoplasm pH becomes acidic and hydrogen ions H^+ activity becomes more. Shifts in membrane functional activity are accompanied by reconstruction of its structure, which in the initial stage express their stability increase until stressor activity will come to exertion. In phospholipid molecules the most important elements are strictly qualitative and quantitative determined (Plonsi & Barr 1991, Antonov 1997, Chernavskaya 1989).

It is known that, for example, at ultra high frequency electromagnetic radiation (UHF EMR), impact on plant cells ion current through cation-regulated membrane systems is increased (H^+, K^+, Ca^{+2}, Na^+), which is explained by resonance impact of EMR on weak hydrogen bonds of dipole water molecules, solution convection strengthening and proton transport acceleration (Kataev 1993). Tambiev (2003, 2007) showed that the effects arising from UHF radiation lead to growth acceleration, biomass increasing, photosynthesis processes stimulation, accompanied by increase in oxygen release and photosynthesizing pigments in cells, as well as to changes in reactivity and ion transport, etc. However, various EMR and EMF impact mechanisms are not yet thoroughly studied.

Plant cell is an electrochemically active biomembrane. To get into the cell, every substance should pass through the cell wall, which comprises proteins, pectins, phospholipids, cellulose microfibrilles and others containing fixed charged groups (primarily carboxyl ones) (Linnik & Nabivanets 1986). They govern the cation-exchange capacity and accumulation of various substances in the cell. Transport of ions and large polar molecules is mainly provided by specific integral proteins.

Biological effects of combined exposure of electromagnetic fields and chemical substances is now under attention with a view perspective of their application for bio-objects behaviour modification (Tambiev et al. 1997, Sinitsin et al. 1998).

All the aforesaid proves the possibility of application of *Lemna minor* (which rapidly gains weight after external physical exposure) for obtaining biogas.

The aim of this work was to study impact of external physical fields on *Lemna minor* productivity growth and to determine rational conditions for increase of its biomass which have the capability for biogas formation.

RESULTS AND DISCUSSION

Phytosorbent *Lemna minor* was under study in this work. Aseptic plant material presence is the necessary condition for the experimental performance. Even at the beginning of our work we faced the difficulty of plant material disinfection, as lemna plants from Saratov region natural environment were heavily polluted. When placing these plants into the sterile Hoagland nutrient solution we saw growth of bacterial and fungal infections. For aseptic treatment of *Lemna minor* we used 4% sodium hypochlorite for 4 minutes and 70% ethanol for 30 seconds (Gaidykova 2008). This preparation method yielded in 65-70% of aseptic plants.

This work presents an analysis of the impact of ultra high frequency EMR exposure of various strengths and time on the growth and evolution processes of natural biosorbent *Lemna minor* (Tables 1-3, Fig. 1).

High-frequency signal generator G4-142 of 65 GHz frequency and 120 μW/cm^2 energy flux density at plant location was used to obtain UHF EMR. The irradiation duration was 5, 10, 15 and 30 minutes. For IR- irradiation (1, 5 and 24 hours) we used infrared light source (blue "Minin" lamp with wavelength λ=780-1400 nm). For UV irradiation (0.5, 1 and 5 hours) we used germicidal lamp SBPe 3×30 W with constant UV-C light of λ=257 nm. Laser irradiation for 1, 3, 5 and 10 minutes was made by He-Ne laser of 2 mW power, operating in red visible range (λ=632.8 nm).

Lemna minor plants of equal growth periods in the amount of 20 g without (control) and after irradiation were placed into Petri dish with 2 days settled water (3 concurrent experiments). The number of fronds (UV, IR, laser irradiation) and plant mass (UHF EMR) were controlled daily within 20 days.

The obtained data analysis shows that the enhanced growth and reproduction of *Lemna minor* (the maximum test response of organisms in our experiments) was detected at 15 min irradiation time. This confirms the mechanism of water electric effect and structurization of thin water containing layer (Il'ina et al. 1979, Sinitsin et al. 2003). According to this mechanism, the initial target at UHF EMR exposure of

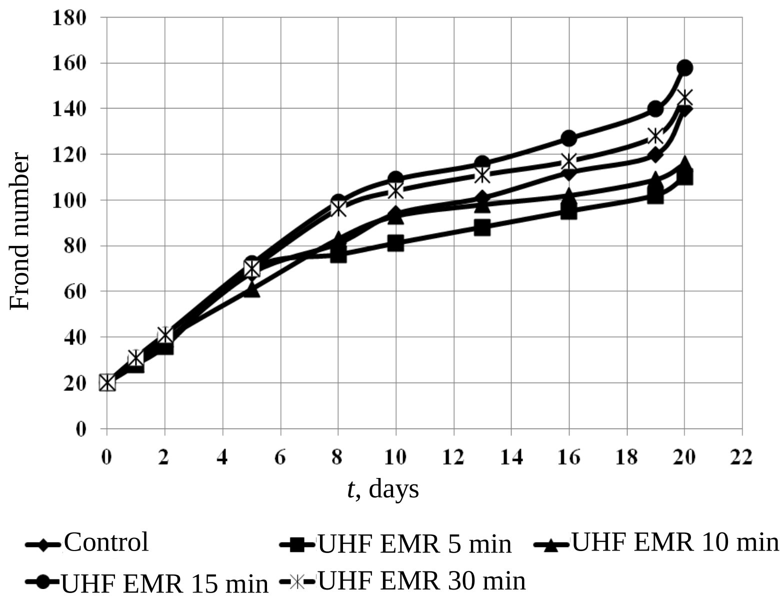

Fig. 1: Mass change (g) of *Lemna minor* with and without 65 GHz UHF EMR exposure for various irradiation times.

biological systems are the processes of perimembrane water molecule cluster structure changing, changing of potential difference at cell membrane, its permeability and, consequently, physiological cell parameters alteration. This leads to enlargement of nutrients transport from outside environment to the cell (Alekseev & Ziskin 1995) and to increase in growth rate and evolution of plants. Frond mass has increased for more than 5-12 % relatively to control. This could be explained by the reason that at long-term irradiation by UHF EMR, the amount of energy absorbed by the object under investigation increases. Electromagnetic energy reception is performed by molecules of free and bound water which is a part of biological membranes. The observed phenomenon is in agreement with the well-known statements about rise of biological effects under UHF EMR exposure (Shlyahtin 2007). It was found that mechanisms of radiation interaction with both standalone life cell and with multicellular organism influence their fundamental vital functions: behaviour, survivability (physiological state), reproduction rate, etc. This is in good agreement with literature data (Sinitsin et al. 1998) and states for maximum response of aquatic organisms at 65 GHz frequency, 120 μW/cm^2 energy flux density and irradiation duration of 15 min. The inhibitory effect, which is observed after 5 and 10 minutes irradiation is caused by insufficient plant cells “energy pumping” obtained at irradiation and required for plant growth. At this, failure of vital functions takes place, which adversely affects plant evolution and reproduction processes. Duckweed mass turn out to be less than that of control.

The obtained data prove the aforesaid assumptions stating that UHF EMR is a stimulating factor, and under its exposure at resonance frequencies disorder of hydrogen bonds system takes place. This leads to its cluster structure imperfection and change in biological activity of the cell (cell reproduction and photosynthetic activity) by means of variation of transport activity of Ca-AT phase and, consequently, its energetic potential. Presence of rigid cell envelope at plant cell leads to a slower involving of electromagnetic waves through water molecules into its major vital processes.

For investigation of UV-IR and laser irradiation influence on growth and evolution processes of plants, duckweed with equal frond dimensions and growth periods were studied. Twenty plants were grown in Petri dishes containing not less than 48 hours settled water. These plants were exposed by various duration physical irradiations. Source of exposure was located at 1 m distance. The plants were incubated in water and the number of fronds was counted daily.

It is established that the effect of the magnetic field can manifest either as a stimulant or as a retarder for the development of cells and the root system of plants (Savostin 1937 & 1928, Kholodov 1971). Investigation of magnetic field impact on living organisms achieved considerable success over the past decades. Savostin (1928 &1937) showed that magnetic field has an effect on living organisms of every organization degree: from unicellular to higher plants. This phenomenon is characterized by various effects: from

Table 1: Mass change (g) of *Lemna minor* with and without 65 GHz UHF EMR exposure for various irradiation times.

Incubation period, days	0	1	2	5	8	10	13	16	19	20
Control m, g	20	29	38	68	81	94	101	112	120	140
EMR irradiation, 5 min, m, g	20	28	36	70	76	81	88	95	102	110
EMR irradiation, 10 min, m, g	20	28	39	61	83	93	98	102	109	116
EMR irradiation, 15 min, m, g	20	31	41	72	99	109	116	127	140	158
EMR irradiation, 30 min, m, g	20	31	41	70	96	104	111	117	128	145

Table 2: Dynamics of *Lemna minor* ponds reproduction with and without CMF exposure of various field strengths (E, kA/m).

Time (24 hours)	Frond number				
	Without CMF	0.5	1.0	2.0	4.0
0	20	20	20	20	20
1	25	20	20	20	20
3	29	21	22	26	22
5	35	21	22	35	23
10	36	22	24	53	26
15	60	24	27	62	30
18	67	29	29	70	35
20	72	32	36	75	44

Table 3: Dynamics of *Lemna minor* ponds reproduction with and without UV, IR and laser irradiation.

Time days	Without EPE	IR, h			Laser, min			UV, h		
		24	5	1	10	5	3	5	1	0.5
		Frond number								
1	20	21	21	21	20	20	20	21	21	21
3	25	26	21	23	25	21	22	21	22	24
5	29	31	22	24	26	24	24	22	28	26
10	36	35	24	27	28	26	23	23	35	28
15	60	56	34	33	47	44	41	27	43	36
18	67	67	45	39	65	69	59	31	57	49
20	72	75	53	43	76	73	68	39	64	56

changes at the molecular level to responses of organism as a whole.

Our investigations have shown (Tables 2 and 3) that various physical exposure have an impact on *Lemna minor* behaviour.

It was found that the maximum growth and reproduction of *Lemna minor* occur without CMF and with 2.0 kA/m CMF exposure. Magnetic fields of 0.5, 1.0 and 4.0 kA/m strength inhibit the duckweed. This approves the well-known data about existence of "frequency-amplitude (bioeffective) windows". Within these frames we observe the bio-object response (in our case it is CMF of E=2.0 kA/m), and we do not see it in other cases. This leads to enhancement of substance flux rate to the cell from the solution and, consequently, to plant reproduction rate and biomass production enhancement as compared with processes running without magnetic field.

Savostin (1928 & 1937) was the first one to point out that constant magnetic field of 1600 Oe strength has a lower impact on wheat husk growth than that one of 60 Oe (Sinitsin 2003, Alekseev et al. 1995, Shlyahtin 2007). It is worth noticing that short-time (up to 30 min) exposure of 20 Oe constant magnetic field, co-directed with the earth geomagnetic field (GMF), stimulated root growth, acting as a stimulating agent. However, the same field, directed against GMF, did not influence the root growth, thus allowing to consider CMF as not just a mechanical factor, but as a physiological stimulating agent.

Investigations of UV exposure time impact on growth and reproduction of *Lemna minor* show its inhibitory effect, especially at long-term exposure. This proves probability of plant stress state generation at long-term exposure of UV-C, which possesses high energy and can damage biomolecules by changing or destruction of its chemical bonds. Proteins stop functioning, and nucleic acids are subjected to muta-

Table 4: Relation between T and pH and time of IR exposure of water with Lemna minor.

Time, h	T, temperature °C			pH		
	IR-1	IR-2	IR-3	IR-1	IR-2	IR-3
1	21.8	21.9	21.8	6.94	6.94	6.94
5	22.0	24.5	24.3	6.85	6.83	6.83
12	22.3	25.0	24.7	6.93	6.73	6.97
24	22.4	22.4	25.0	6.88	6.98	6.8
72	20.7	20.6	24.8	7.11	7.09	6.96
120	20.2	20.1	23.8	7.14	7.32	6.98
168	21.6	22.0	24.2	7.07	7.30	7.05

tions, so the cell destroys (Kovac & Keresztes 2002, Frederick & Lubin 1988, Rozentcvet et al. 2000). Investigation of irradiation period has shown that optimum UV irradiation time is 1 hour.

It is known that infrared radiation penetrates into tissues to a depth of 20 to 50 nm, therefore, surface layers are heated to a greater extent. The plant cell contains a large amount of water. The water in the cell is heated evenly, if necessary, the cell evaporates water, protecting plants from overheating by infrared rays (Kyklev 2001).

According to the obtained data, IR layers penetration and plant heating are more intensive during the first days of exposure. At this, photosynthesis processes and chlorophyll creation are accelerated. Bright green color of the leaves of the *Lemna minor* is the evidence of it.

Temperature and pH solution measurements at IR irradiation have shown that under IR exposure a tiny increase of pH value took place (Table 4). This is more likely caused by absorbance of hydrogen ions H^+ and other nutrients by plant cell.

It is known from literature (Kaplan 1999), that low intensity laser irradiation stimulates metabolic activity of the cell. These processes are based on photophysical and photochemical responses, which arise at the organism under laser irradiation. Photophysical responses are predominantly caused by object heating (from 0.1 to 0.3 °C) and heat transmission at biotissues. Temperature difference is more pronounced at biological membranes. This leads to Na^+ and K^+ ions outflow, expansion of protein channels, biopotential change, plant cell and cell membrane permeability increase and, consequently, molecules and ions transport into the cell. Photochemical reactions are caused by exiting of electrons in atoms of the substance which absorbs light. At molecular level it is expressed by photoionization of the substance, its photoreduction, photooxidation, molecules photodissotiation, or by photoisomerization of molecules (Kyklev 2001).

At this, water absorbs visible light and red light. This affects membranes by changing of its water layer structural organization and function of its thermolabile channels.

Besides this, biological structures of organism possess the intrinsic electromagnetic fields and free charge, which reorganize under exposure of radiation photons. This leads to "energy pumping" of the irradiated organism. As a result, mechanisms of EMR and EMF exposure on tissues are based on processes occurring at cell and molecular levels (Lotova 2007, Bragina & Mironov 2002).

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