III. RADIATION ECOLOGY

APPLICATION OF THE PLASMA FOCUS DISCHARGE IN THE RADIOBIOLOGY STUDIES

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Abstract. The purpose of this short review article is to present the results of the influence of the ionizing X-ray pulses of plasma focus on living organisms, by performing experiments *in vivo* and analyzing changes in some vital characteristics of the objects, such as survival ability, efficiency of photosynthesis of unicellular organisms at moderate doses (up to 65 mSv); efficiency of enzyme production, change in protein content and change in fungi mass in the radiation dose range 7 mSv ÷ 45 Sv; changes in mammalian blood characteristics and appearance of signs of anemia due to the significant dose load.

Keywords: Plasma focus, X-ray radiation, radiobiology studies

INTRODUCTION

Since the discovery of the Plasma Focus phenomenon in 1960 it was revealed that this discharge is a source of various emissions – high energy particle beams (ions and electrons), fusion neutrons in the case when the filling gas is deuterium or DT mixture, plasma streams and short wave EM radiation. The last consist of continuous soft and medium range X-ray radiation and characteristic hard X-ray lines. All of these emissions are used in different plasma focus applications. Although the plasma focus X-ray radiation has some advantages over the conventional ionization sources, the number of such papers in the literature is limited. In several works [1÷3] medium and hard X-rays of the Plasma Focus (PF) discharge have been used for the needs of radiation enzymology. Different enzymes have been treated *in vitro*, changing the conditions e.g. the X-ray spectral range, doses, dose power, etc. The results obtained show huge difference in the enzyme activation/deactivation after irradiation of cell cultures by pulse X-rays burst from PF and by conventional continuous radio isotope source (γ -

source ^{137}Cs) or X-ray tube. In particular, the above effect is seen at doses five to six orders of magnitude lower for this radiation source than in the case of prolonged action of hard radiation having low intensity. The authors presume that the most adequate characteristic of the action is not dose but the product of the dose and the dose power.

Investigations presented here are done with various types of live organisms, exposing the objects on the PF ionizing EM radiation and examining the most important characteristics of the species (and their strains) *in vivo*.

EXPERIMENTAL PART

A. Experimental setup

A short description of the plasma focus will be given here. Usually the words “plasma focus” or “dense plasma focus” are used to denote both the phenomenon of focusing the plasma in a small volume as well as the device performing it in the literature it is accepted to use the term Plasma Focus (PF) as a name of the device creating the effect.

Fig. 1. The three phases of the discharge development are shown above

There are three different constructions of the PF discharge chamber. Two of them are named after their creators – Fillipov type (first operation – in 1954) and Mather type device (1961). The last one (Sarov type) is named after the town, where it was created. The figure below shows the PF principle of operation.

There are no substantial differences in the operation of all three types of PF geometries – Fillipov, Mader and Sarov: in the pinch phase Rayleigh-Taylor instabilities appear. As a result, the so-called “break of the current” is observed. For a very short time the discharge current is switched off and replaced with streams of fast electrons and ions. These streams deposit their energy in the pinch plasma, giving start to various emissions known for the dense plasma focus phenomenon.

Here we will present the results of the experiments with living microorganism, irradiated by hard and soft X-ray generated by the PF device. The data is obtained with the 3 kJ plasma focus machine of the Faculty of Physics at the University of Sofia. We have Mather type PF device, with aspect ratio, the ratio between the cathode diameter to its length, $A < 1$. The

condenser bank has capacitance of 20 μF , with the maximal charging voltage of 40 kV. The insulator is a quartz tube (2.6 cm in diameter, 3 cm length in the chamber) the main switch is a vacuum spark gap. The anode is a hollow copper tube with 2 cm diameter and 14,5 cm length, the cathode consists of 6 copper rods (0,8 cm diameter, 16 cm length) mounted in the massive cathode bottom on a circle with a radius 3,5 cm. The experiments are conducted in D, Ar, or air, in the pressure range $1.0 \div 2$ mBar. The voltage operating range is chosen to be $15 \div 18$ kV, therefore the energy level of the PF standard operation is not higher than 3 kJ. We are monitoring the discharge current, the current derivative, the hard X-r ay emission by scintillation detector and the soft X-ray by PIN diodes. For this aim 4 PIN BPX 65 photo diodes are mounted. The glass cover of the diodes is removed and in front of each photodiode a metal filter with different thickness is placed (10, 20 and 30 μm aluminum and 0.5 mm lead respectively). The filters transmit the X-ray quant with energy above certain values.

Fig. 2. PF current (curve 1); soft X-ray detectors signals (curves 2 and 3) and hard X-ray signal (curve 4), respectively.

The Fig. 2 shows the discharge current and the X-ray emissions.

At a discharge in air in the pinch phase more than one contraction can happen as it is on the figure.

It is seen that high intensity bursts of both soft and hard X-rays appear for time intervals less than 100 ns. Therefore, the dose power of the plasma focus X-ray emission is very high.

Thermo-Luminescent Dosimeters (TLD) are used to measure the full X-ray dose in the stainless steel chamber of the PF machine and to control the radiation outside the device. The data of all of the

detectors mentioned above are recorded by two 4 channel oscilloscopes (TDS 3034C, TDS 2004B). By TLD detectors we determine the dose of the radiation inside and outside the chamber. It turns out that average dose inside is about 0.3 Sv /shot, while outside the dose is on the level of the background radiation. The stainless steel walls of the chamber absorb the X-rays. However, if the living cells or microorganism are inside the chamber during the operation they will die. So they should be in the

close vicinity of the chamber being in the same time at atmospheric pressure. For the purpose an X-ray window with thin metal foil is mounted in the

upper lid. The samples are placed upon this window. Fig. 3 shows schematically the PF chamber with the X-ray port on the upper lid.

Fig. 3A) Schematic representation of the PF chamber with the port on the upper lid

Fig. 3B) The last version of the vessel for biology samples, allowing fast change and reliable work with thin foil.

B. Measurements procedures and results

1. A study of cell viability *Saccharomyces cerevisiae* – (yeast) after irradiation.

The sample with the cells was irradiated by 14 shots of the PF through 100 μm Al foil. The total dose of the X-ray radiation determined by the TLD detectors placed upon the metal foil near the sample was 65 mSv. No difference of the viability between the control probe and the sample was observed after irradiation. Similar results have been obtained before that with another type of yeast – *Kluyveromyces marxianus*, where no change of the survival activity was found after irradiation through a thick foil.

2. Monitoring of the photosynthetic activity of *Chlamydomonas reinhardtii* – (duckweeds) [5]:

C. reinhardtii cells were grown photomixotrophically in a Tris-Acetate-Phosphate (TAP) medium with pH of 7.0, at a temperature of 25°C. The cultures were first grown in 25 ml glass flasks, and were illuminated by luminescent light with a photon flux density (PFD) of 80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ 24 h/d at continuous shaking. The microalgal suspension was cultivated during 7 days to achieve late exponential phase with cell density of about $4\text{--}6 \times 10^6$ cells/ml.

We checked the impact of X-ray irradiation on the fast processes occurring in the photosynthetic apparatus in unicellular alga. The Chlorophyll fluorescence was measured using mPEA (multi-functional Plant Efficiency Analyzer, Hansatech Ltd., Kings Lynn, UK) as reported before [4].

The first irradiation of samples was done through 20 μm Al foil and 10 μm plastic foil – 4 shots; the dose was ~ 11 mSv. In the second experiment the samples were irradiated through 100 μm Al foil and 10 μm plastic foil – 14 shots; the dose load was ~ 65 mSv.

The method, used for analysis of photosynthetic activity in living system is proposed by Strasser et al [4]. According to the procedure given by these authors, the probe treated by ionizing radiation (soft X-rays in this case) is illuminated afterwards with certain visible light pulses ($627 \pm 10\text{nm}$) and then the prompt and the delayed fluorescence emission ($735 \pm 15\text{nm}$) of the sample is recorded *in vivo*. The changes of fluorescence intensity during dark to light transition, so called “induction curves” are derived and analyzed by the JIP-test [4]. Fig. 3A shows the induction curves. Suspension of *C. reinhardtii* was adapted to dark during 1h and chlorophyll fluorescence transients was measured at actinic light intensity $5000 \mu\text{mol.m}^{-2}.\text{s}^{-1}$

Testing of photosynthetic activity of algal cultures shows that X-ray irradiation has strong effect on photosynthetic apparatus. The shape of the induction curves was modified (Fig. 4A). The characteristic increasing of both J and I phases of fluorescence transients suggests a slowdown of electron transfer in acceptor sides of Photosystem II and I (PS II and PS I), respectively. For visualization of the X-ray effect the data is presented as differential curves (see Fig. 4B). Most pronounced effect is at about 2 ms (phase

J) when the stationary state of electron transfer reactions toward primary quione acceptor Q_A and from Q_A^- to Q_B is established. It means that during Test 1 the reoxidation reaction in PSII acceptor side was suppressed.

The effect of X-ray treatment during Test 2 can be explained as a weak stress. From these curves Quantum efficiency at different points of the electron transport machinery can be deduced.

Fig. 4A. Induced fluorescence curves

Fig. 4B. Induced fluorescence curves in differential form

Fig. 5. Various photosynthetic characteristics of the *Cl. Reinhardtii*, calculated from the data, shown on Fig. 4

The results obtained so far [5] show that in the irradiated sample through 20 µm Al foil with a dose of 11 mSv affects the photosynthetic electron transport chain at all investigated sites – in PS II, in PS I and between both photo systems. The Performance Index (it is an index quantified vitality of photosynthetic machinery and reflects both structure and functional characteristics of the total photosynthetic activity) decreases more than twice in comparison with the same value of the reference sample (Fig. 3). In the same time a 20 percent increase of the efficiency of the electron transport increases is observed for the sample irradiated through 100 µm Al foil with a dose of 65 mSv. It is clear that these results should be accompanied by the data about spectral distribution of the soft X-rays but unfortunately our lab has no such equipment.

3. Influence of hard X-ray upon vital characteristics of special class of fungi *Trichoderma reesei* M7 [6, 7]

When hard or medium-energy X-radiation is aimed, certain solid metal caps are put on the top of the anode of the device. We use tungsten caps. In this case characteristic radiation arises due to the classical mechanisms of interaction between the beam of relativistic electrons and the solid metal. Exactly this effect was used for irradiation of the fungi species.

Series of experiments were conducted with the well known cellulase producer *Trichoderma reesei* M7, which spores were exposed to certain doses of hard X-radiation (from the K α 1 and K α 2 lines of tungsten), with a very high dose rate. Doses were within the interval from 7 up to 45 000 mSv. This significant dose load was applied on two types of samples:

A) 10 days-old culture of the spores upon PDA agar.

B) A spore suspension of 10 days-old culture grown in agar and transformed to a solution in saline and processed at the same conditions.

Detailed description of the preparation of the samples and processing of them after the treatment with X-ray burst is given in papers [6, 7].

General observation: The analysis of the endoglucanase activity and the measurement of the residual biomass and proteins quantities have shown that for moderate doses absorbed (200÷1200mSv) the effect of the micromycete producer is clearly expressible [6].

Trichoderma reesei M7 shows exceptionally high resistibility in respect to high doses of absorbed X-radiation, with the vitality of spores practically unaltered – experiments were conducted with doses as high as 45 Sv.

The pulsed mode of irradiation fundamentally differs from the continuous one when considering its effect on biological objects – they can rapidly absorb great amounts of radiation in a glimpse, leading to a qualitatively different interaction with their matter. In the experiments done the viability was determined, as well as the vegetative biomass, four types of cellulase activity and quantity of total protein after the treatment of the spores suspension ($2 \cdot 10^5$ CFU/ml) of *Trichoderma reesei* M7 with this pulsed X-radiation. The absorbed doses were in the range of 7 to 45000 mSv paper [7].

It was noticed that the viability of the strain, if compared with the control samples drops, reaching 57% of the initial value (of the untreated spores) for the sample with 45010 mSv of absorbed dose.

The data obtained for the four experimentally determined cellulase activities (endoglucanase, Filter Paper Activity - FPA, β -glucosidase, endoxylanase), as well as for the total extracellular protein show that there is no clear (explicit) dependence between the dose absorbed and the changes that have occurred biosynthetic capacity of the micromycete (Table 1).

Table 1) Hydrolase activities and total soluble protein after irradiation of *T.reesei* M7 with a 3 kJ DPF device: A) Endoglucanase, B) FPA, C) β -glucosidase, D) Xylanase.

Absorbed dose [mSv]	Endoglucanase activity [IU/ml]	FPA* [FPU/ml]	β -glucosidase activity [IU/ml]	Endoxylanase activity [IU/ml]	Total soluble protein [mg/ml]
control	30,4	3,79	1,51	5,62	2,11
7	29,2	3,81	1,54	5,98	2,15
28	28,6	3,78	1,39	5,89	2,09
37	29,2	3,51	1,54	5,91	2,13
89	28,4	3,73	1,47	5,77	2,01
134	28,3	3,81	1,61	6,18	1,97
178	29,9	3,49	1,58	5,83	2,21
206	30,8	3,81	1,47	5,57	2,08

232	32,1	3,89	1,61	5,98	2,20
475	33,8	3,77	1,47	6,19	2,19
515	35,2	4,35	1,75	6,43	2,29
541	35,8	4,21	1,89	7,08	2,31
618	31,3	3,91	1,66	7,14	2,25
669	30,6	3,82	1,54	6,32	2,17
1174	28,1	3,50	1,31	5,87	1,97
2360	28,7	3,63	1,49	5,69	2,06
4216	29,4	2,49	1,38	5,46	1,84
5601	26,8	3,41	1,57	5,11	1,69
6265	29,9	4,13	1,61	4,92	1,87
7318	28,3	3,66	1,35	5,87	1,98
7491	31,5	3,41	1,62	6,12	1,86
9542	30,2	3,66	1,34	5,68	1,94
10346	28,3	3,18	1,80	6,42	2,08
11754	35,3	3,83	2,18	5,93	2,24
12662	25,4	3,45	1,41	4,76	2,01
19439	37,2	3,86	1,36	6,79	2,17
31893	30,6	3,77	1,62	5,74	1,99
42413	29,8	3,94	1,43	5,67	2,09
42917	42,9	4,94	2,91	7,65	2,18
45010	23,7	2,81	1,23	4,41	1,74

From the data presented above it can be concluded that three of the samples with high absorbed doses, namely with 11754, 19439 and 42917 mSv, demonstrated increases of their cellulase activities. In the case of 42917 mSv the increment of the endoglucanase activity, compared to the control, reaches 41,1%, FPA rises with 30,3%, and the β -

glucosidase activity nearly doubles with an increase of 92,7%.

Using the quantity of the extracellular protein and the corresponding hydrolase activity, the specific enzyme activities after the 120-th hour of the fermentation process are calculated and shown on Fig. 6. A, B, C, D below.

Fig. 6. Specific hydrolase activities after irradiation of *T.reesei* M7 with a 3 kJ DPF device:
A) Endoglucanase, B) FPA, C) β-glucosidase, D) Xylanase.

Results of irradiation of animals (rats) with the X-rays burst of a plasma focus

In this section are presented data obtained by G.

M. El-Aragi, N. M. El-Abiad, and E. A. Marzook, in Plasma Physics and Nuclear Fusion Department, Nuclear Research Center, Egypt, Egypt, 2009.

Fig. 7. The experimental arrangements with the PF chamber and the cage with the animals.

In this study Mather type PF device is used with parameters: $C = 1 \mu\text{F}$; $U = 15 \text{ kV}$, $E = 0.1 \text{ J}$; Distance between the rat cage and the focal zone $\sim 20 \text{ cm}$; irradiation is conducted through 10 mm plastic insulator. The animals have been divided to four groups: control group, and three other groups

subjected respectively to 10, 15 and 20 shots per day, 3 times in a week, for 3 weeks. After that blood samples have been taken from the animals in order to determine some biochemical and hematological parameters. The results are shown on the figures below.

Fig. 8. Thyroid hormones (hormones created by the thyroid gland) as a function of dose load

The results show that a radiation induced anemia is observed in the treated animals indicated by decreasing of the Hemoglobin concentration and the Erythrocyte number. The increased number of Thrombocyte also is a specific feature of hemolytic

anemia. The increased Reticulocyte number as well as the increased activity of the thyroid gland is the typical reaction of resistance of the organism subjected to such treatment. The same effect is observed in the enhanced activity of the thyroid gland.

Fig. 9. Blood biochemical and hematological parameters as a function of degree of dose load

CONCLUSIONS

The plasma focus device proves to be quite promising tool for radiobiology studies. However, in order to reveal its full capacity in this field much more studies should be carried out.

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REFERENCES

1. Gribkov V.A., M.A. Orlova, "On various possibilities in pulsed radiation biochemistry and chemistry", Radiation Environmental Biophysics, 43 (2004), 303-309
2. V. Dubrovsky, I. G. Gazaryan, V. A. Gribkov, Yu. P. Ivanov, O. A. Kost, M. A. Orlova and N. N. Troshina, "On the possible mechanism of activation changes of enzymes under pulsed irradiation" Journal of Russian Laser Research, 24, № 4, (2003) 289-300
3. V. A. Gribkov et al., "PF 6 – An effective plasma focus as a source of ionizing radiation and plasma streams" Nucleonica, 51 (2006) 55-66

4. Reto J. Strasser; Merope Tsimilli-Michael; Sheng Qiang, Vasilij Goltsev (2010) “Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820 nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*”. *Biochimica et Biophysica Acta* 1797 (2010), 1313-26

5. Zapryanov S., V. Gol'tsev, B. Galutsov, M. Gelev, A. Blagoev, “Influence of the soft X-ray Plasma Focus radiation on living microorganisms”, *European Journal of Applied Physics*, 58 (2012), 11201

6. Gemishev O., S. Zapryanov, A Blagoev, M Markova, V Savov “Effect of multiple highly energetic X-ray pulses on the synthesis of endoglucanase by mutant strain of *Trichoderma reesei*”, *Biotechnology and Biotechnology Equipment*, 2014

7. O. Gemishev, S. Zapryanov, D.Nikolova, Y. Evstatieva, A. Blagoev “Influence of multiple short highly energetic X-ray pulses on the biosynthetic and physiological activity of the strain *Trichoderma reesei* M7”, *Journal of Physics: Conference Series* 1598 (2020), 012003

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