Electron beam agrobionanotechnologies for agriculture and food industry enabled by electron accelerators

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Abstract. Electron beam (EB) radiation technologies have been employed to increase efficiency of biologically active nanochips developed for agricultural plants seed pre-treatment with purpose of enhancing crop yield and productivity. Iron-containing nanoparticles (NPs), synthesized in reverse micelles following known radiation-chemical technique, have served as a multifunctional biologically active and phytosanitary substance of the chips. Porous chip carriers activation has been performed by EB ionization (dose 20kGy) of active carbons (AC) prepared from agricultural waste and by-products: Jerusalem artichoke (Helianthus tuberosus) straw, rape (Brassica napus L. ssp. oleifera Metzg) straw, camelina (Camelina sativa (L.) Crantz) straw, wheat (Triticum aestivum) straw. Three methods, UV-VIS spectrophotometry, Electron Paramagnetic Resonance (EPR) spectroscopy, cyclic voltammetry (CV) have been used for process control and characterization of radiation-activated and NPs-modified ACs. The results show a notable effect of ACs activation by electron beam radiation, evidenced by FeNPs-adsorption capacity increase. Studies of the impact of Fe NPs-containing nanochip technology on enhancement of seeds germination rate and seedlings vigour suggest that reported electron beam radiation treatment techniques of the ACs from selected agricultural residues may be advantageous for industrial application.

1. Introduction
Development of enhanced agricultural bionanotechnologies is becoming vital to sustainable crop production [1, 2]. Focused integration of research approaches is unavoidable for successful introduction of new pre-sowing seed treatment procedures involving multifunctional and multicomponent (nano)systems, deriving, preferably, from renewable resources, including
agricultural residuals[3-5]. Biologically active nanochips (BANchips), when used to treat seeds of agricultural plants, allow application of desired ultra small amounts of active substances for the purpose of concurrent achievement of phytosanitary plant protection, nutrition or microelements feeding, seed germination enhancement, and crop yield increase under adverse conditions.

The BANchip usually consists of porous carrier matter and nanoparticles of biologically active and phytosanitary substances, and nutrients. Multiple nano pores of the carrier accommodate and hold nanoparticles of the impregnated substances selected for further seeds treatment. The carrier is usually mixed with mineral, polymer, clay, etc in a variety of proportions. BANs are then applied to the seed covers.

Electron beam (EB) radiation technologies[6-10] can offer a variety of solutions capable of increasing BANchip efficiency. They provide unique tools and methods for fundamental research of both homogeneous and heterogeneous systems, precise modification techniques, wide range of possibilities for varying parameters of radiation-chemical production of nanostructured composites with predetermined properties.

In earlier papers [12-14] suggested methods for activation of carbonaceous sorbent from natural materials and pre-sowing seed treatment using developed BANchips with AgNPs content, and related crop yield increase issues were discussed [15-18]. Current studies are focused on MeNPs introduction to BANchips, metal precursors being chosen from the microelements of vital importance for plant growth, preferably, not included within noble metals range. Iron-containing NPs, synthesized in “micoreactors” of reverse micelles pools following known radiation-chemical technique [7, 19-21] could be offered as a multifunctional biologically active and phytosanitary substance for the chips [22-25]. Porous chip carrier’s treatment is being presently investigated and tried using EB ionization (doze 20kGy) in order to further activate the carbons (ACs), derived from agricultural waste and byproducts.

2. Radiation-chemical metal NPs synthesis (RadChem)

Radiation-chemical reduction of metal ions and stable NP formation in reverse micelles systems (RMS) [7, 19-21,34] – such as Fe^{2+}/H_{2}O/0.15M AOT/isooctane – can be induced and implemented by EB radiation straight after solubilization of the components and solution’s saturation with inert gas. Metal ions reduction then occurs due interaction with short-lived reductant products of water radiolysis: solvated electrons e^{-}_{\text{aq}} , hydrogen H-atom and the radicals R_{(0)}. Radiolysis reaction can be viewed below.

\[
\begin{align*}
\text{H}_{2}\text{O} & \rightarrow e^{-}_{\text{aq}} \ (2.7), \text{H}^{+}_{\text{aq}}, \text{OH}^{-}_{\text{aq}}, \text{H}^{-} \ (0.6), \text{OH}^{-} \ (2.7), \text{O}, \text{H}_{2}, \text{H}_{2}\text{O}_{2} \\
\text{e}^{-}_{\text{aq}} & \text{ is the unique reductant (E_{d.e} = -2.9 \text{ V})} \\
\text{e}^{-}_{\text{aq}} + \text{Fe}^{2+} & \rightarrow \text{Fe}^{+} \\
\text{e}^{-}_{\text{aq}} + \text{Fe}^{+} & \rightarrow \text{Fe}^{0} \\
\text{Fe}^{2+} + \text{Fe}^{+} & \rightarrow \text{Fe}^{2+}
\end{align*}
\]
During NPs synthesis risks of possible oxidation of Fe\(^+\) and other transients by radicals OH\(^-\) must be considered:

\[
\text{OH} + \text{Fe}^+ \rightarrow \text{FeOH}^+ \quad (5)
\]

Unwanted oxidation can be avoided by OH\(^-\) transformation into radical products with reducing properties by introduction of the acceptors, such as alcohol, or carbonates ions (reaction 6, 7):

\[
\begin{align*}
\text{OH} + (\text{CH}_3)_2\text{CHOH} & \rightarrow (\text{CH}_3)_2\text{C}^\cdot\text{OH} + \text{H}_2\text{O} \quad (k=1.6\times10^{-9} \text{M}^{-1}\text{s}^{-1}) \\
\text{OH} + \text{HCOO} & \rightarrow \text{CO}_2^\cdot + \text{H}_2\text{O} \quad (k=3.1\times10^8 \text{M}^{1}\text{s}^{-1})
\end{align*}
\]

(6)  
(7)

Thus formed reducing agents contribute to reduction reactions responsible for consequent nanoparticles yield. Transient metal species are unstable and consequently form various aggregates Fe\(^{n+m}\) (n>m).

Conditions of the RadChem process, primarily, the radiation dose (usually chosen in 10÷50 kGy range), lab temperature, component ratios and concentrations can influence resulting NPs structure, size, shape and functionality.

3. Experimental

3.1. Chemicals
All chemical were in analytical grade, were purchased from Aldrich (USA) and used as received, unless otherwise stated. AOT (bis(2-ethylhexyl) sulfosuccinate sodium salt) purity specification was 98%. Solvents were obtained from Component Reactive (Russia) or Aldrich.

3.2. Active Carbons (ACs).
ACs from carbonized straw have been produced by pyrolytic treatment [26-27] by Dr. Mukhin, JSC ENPO "Neorganika" (Elektrostal, Russia). Table 1 shows typical specifications set for received ACs [23-25]: AC origin, surface. Received samples have been manually powered and used as is, i.e. powdered active carbons (PAC). Residues (straw) of the following cultivated plants were selected for processing into ACs: Jerusalem artichoke (Helianthus tuberosus), rape (Brassica napus L. ssp. oleifera Metzg), camelina (Camelina sativa (L.) Crantz), wheat (Triticum aestivum).

**Table 1.** Typical specifications of experimental ACs samples

| Sample Assigned No. | AC origin       | \(V^3\) \(\gamma\) \(\text{cm}^3\) /g | Humidity, \(\%\) | \(\Delta\) \(g/dm\) | Adsorption of iodine, \(\%\) |
|------------------|----------------|----------------------------------------|----------------|----------------|-------------------------------|
| AC1              | Jerusalem artich. | 3,00                                    | 1,0             | 95,3           | 34                            |
| AC2              | Camelina         | 2,43                                    | 5,7             | 140            | 43                            |
| AC3              | Wheat            | 3,61                                    | 1,9             | 66,5           | 64                            |
| AC4              | Rape             | 4,14                                    | 2,4             | 135            | 39                            |

3.3. Spectroscopic Measurements
UV-VIS spectroscopy was performed with a Hitachi U-3310 spectrophotometer (Japan) using a 1-mm quartz cuvette, and 0.15M AOT/ isooctane solution as reference.

3.4. Electrochemical characterization
ACs electrochemical properties were studied by alternating current polarography cyclic voltammetry (CV) methods using an Intelligent Potentiostatic Control – ProMF (Russia) with IPC2000 SW in a three-electrode set-up with disposable planar working electrodes, Pt auxiliary and silver chloride reference electrodes in aqueous 0.5 M sulfuric acid electrolyte. All of the measurements were carried out at room temperature (25°C).

3.5. EPR measurements
EPR spectra were recorded using EPR spectrometer PS 100.X (Russia). AC samples were placed into glass vials made of "Luch" glass, allowing irradiation of samples and further registration of
paramagnetic AC particles signals without impact of irradiated glass EPR signal. Typical parameters were: middle of range – 3310 (G), scan range – 400, 2000, 4000 (G), modulation amplitude – 300 (mG) – 500, attenuation – 0 (dB), Gain: value – 3 to 9, order – 3, Phase (0/90) – 90 (deg). Measurements were carried without carbons deaeration, at room temperature.

a. FeNPs synthesis

Aqueous 0.3M Mohr's salt ((NH₄)₂Fe(SO₄)₂·6H₂O) solution was injected into 0.15M AOT/isooctane RMS in volume as determined by chosen solubilization coefficient value ω=3. RMS was, saturated by He, sealed and EB-irradiated using UELV-10-10-C-70 accelerator (10 MeV). Five samples received different absorbed radiation doses in 10-25 kGy range. UV-VIS spectroscopy records indicate, that optimal absorbed doze was achieved at 20kGy, and its further doze increase did not contribute to RadChem FeNPs formation in RMS with listed set of initial parameters, component quality and concentrations (see figure 3). FeNPs obtained in RMS with 20kGy adsorbed dose were used for ACs modification series. Results of FeNPs particle size and distribution characterisation performed by DSL (Zetasizer, Malvern, UK) method and corresponding AFM (BerMad 2000, Nanotec Electronica, Spain) data [28] indicated m.p.d. of ~3.5 nm of FeNPs in RSM with ω=1-3, and ~4.8 nm for FeNPs, obtained in RMS with ω=4-5.

b. ACs activation

Powdered AC1, AC2, AC3, AC4 were EB-irradiated using UELV-10-10-C-70 accelerator (10 MeV); absorbed radiation dose was 20 kGy for all samples: AC1-20kGy, AC2-20kGy, AC3-20kGy, AC4-20kGy. Second-step activation was performed via ACs surface modification with FeNPs. Original AC1-AC4 samples and radiated AC1-20kGy – AC4-20kGy samples were placed into in FeNPs RMS (ω=3, RadChem, D=20kGy), volume ratio of RMS and AC powder in all samples was ~ 1: 1. UV-VIS spectral control of NPs absorption process in RMS continued for 14 days.

4. Results and discussion

EPR spectroscopy is the standard tool for the detection of paramagnetic species and has been traditionally widely used to elucidate the nature of the carbons [29-32], it has proved existence of different types of ACs paramagnetic reaction centers, constituted by trapped electrons, and other ones - free radicals in the conjugated aromatic systems. EPR signals of these centers overlap, but have different values of the g-factor. AC radiation-induced activation appears to lead to structural changes, detected through the change of the narrow and broad signals correlation and g-factors shifts.

Obtained EPR spectra of original (xybtreated) AC1-AC4 samples have indicated the difference of the properties of detected centers dependent on the AC origin (plant). As it can be seen on figures 4 and 6, the properties of AC1 sample are characterized by three EPR signals varying in type and width: narrow signals noticed within the range of 5 ÷ 10 Oe, and two broad signals, first one ΔH is ~ up to 250 Oe, and second one has got ΔH ~ up to 600 Oe. Intensity and shape of AC4 EPR- recorded signals are notably different: the signal can be clearly seen be composed of two features: a narrow signal line, ΔH ~ 45 Oe, and second broad signal of low intensity.
Figure 4. EPR spectrum of untreated AC1, derived by pyrolysis from Jerusalem artichoke straw

Figure 5. EPR spectrum of untreated AC4, derived by pyrolysis from Rape straw

After EB-radiation treatment only subtle EPR signals changes were detected for in all four radiated samples AC1-20kGy (see figure 6) – AC4-20kGy (see figure 8). No EPR-sensitive effects of ACs irradiation, or center decomposition got revealed during reported experiment. Significant changes of paramagnetic properties of all samples got recorded after completion of the FeNPs adsorption by irradiated ACs.

Figure 6. EPR spectrum of AC1-20kGy (radiated AC1, derived from Jerusalem artichoke straw).

Figure 7. EPR spectrum of FeNPs-impregnated AC1-20kGy (derived from Jerusalem artichoke straw).

According to preliminary analysis, EPR spectra indicate that all EPR signals – narrow and broad ones – disappear from EPR spectrum of NP-modified and irradiated AC1 (see figure 7), while EPR spectrum of NP-modified and irradiated AC4 (see figure 9) clearly demonstrate intensity increase of the broad signal, going along with the weakening of the narrow one.

Figure 8. EPR spectrum of AC4-20kGy (radiated AC4, derived from Rape straw).

Figure 9. EPR spectrum of FeNPs-impregnated AC4-20kGy (derived from Rape straw).

Experimental EPR data suggest, that ACs paramagnetic centers must be involved in complex FeNP adsorption processes during ACs modification in RMS media.

Characterization achieved by CV methods consistently found that the nanomaterials based on preliminarily radiation-activated AC exhibited higher electro-catalytic activity (see figures 10-12). The shown voltammogram demonstrate that in case of FeNPs-impregnated AC1-20kGy (AC1, derived from Jerusalem artichoke straw, and FeNP-modified after being irradiated), the currents in the cathode area -750 mV at potential increase from -18 to -30 μA, while in the anode at a potential +750 mV they double in value, when compared to CV of NP-modified sample, not treated with radiation.
For FeNP-modification of ACs same FeNPs RMS (ω=3, RadChem, D=20kGy) was used. UV-VIS optical absorption spectra of this FeNPs RMS demonstrate characteristic peak with $\lambda_{\text{max}} \sim 215$ nm, and weakly expressed bands at 264–270 nm, and at 340 nm.

UV-VIS spectra (see figures 13, 14) recorded during modification process of AC1-20kGy illustrate multiple-stage complicated FeNPs adsorption process characteristic for all studied AC samples.

Spectrophotometric monitoring of the optical density (D) of FeNP RM modifying solutions helped providing persuasive records of initial D increase at the first stage of AC NP-modification, as well as the difference in kinetics of the absorption processes of NPs, which are accounted to ACs pre-treatment and carbon’s origin.

As it has been supposed [33], this phenomena can be explained by AC-induction of further NPs formation in the RMS, being the result of the reaction between remaining metal ions, - still present in the RMC along with NPs, - and active centers found on the surface of the adsorbents. In due time,
each RMSs with FeNPs, used for ACs modification, reached “FeNPs-saturation” state, followed by custom D decline. It agrees with NPs concentration decrease in liquid media due to NP adsorption within AC pores and on its surface.

A histogram, providing consolidated input on the time rate and intensity of FeNPs absorption, correlating to RMS optical density change at $\lambda=264\text{nm}$ observed with time for all studied samples, can be seen on figure 15.

![Figure 15. Optical density change recorded at $\lambda=264\text{nm}$ for modifying FeNP RMSs with following samples: AC1–1, AC2–2, AC4–3, AC1-20kGy–4, AC2-20kGy–5, AC4-20kGy–6. Spectra Registration Time, days after adsorption start, legend: Initial 2 days 4 days 7 days 9 days 14 days](image)

UV-VIS optical absorption spectra provide evidence that EB-radiation treatment of ACs make notable impact on absorbance rate and FeNP-capacity of the tested ACs (see Table 2).

### Table 2. Typical specifications of studied ACs samples

| Sample | $D_{264}$ a.u. $\Delta T=0$ | $D_{264}$ a.u. $\Delta T=2$, days | $D_{264}$ a.u. $\Delta T=4$, days | $D_{264}$ a.u. $\Delta T=7$, days | $D_{264}$ a.u. $\Delta T=9$, days | $D_{264}$ a.u. $\Delta T=14$, days | $\Delta D$, % |
|--------|-------------------------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------|---------------------------------|----------------|
| AC1    | 0.763                         | 1.013                            | 1.039                            | 1.052                            | 1.039                           | 0.940                           | 11             |
| AC2    | 0.763                         | 1.072                            | 1.056                            | 1.034                            | 1.026                           | 0.957                           | 12             |
| AC4    | 0.763                         | 0.983                            | 0.982                            | 0.969                            | 0.966                           | 0.922                           | 7              |
| AC1-20kGy 4 | 0.763                     | 1.008                            | 0.993                            | 0.952                            | 0.917                           | 0.845                           | 19             |
| AC2-20kGy 4 | 0.763                     | 0.975                            | 0.951                            | 0.892                            | 0.836                           | 0.826                           | 18             |
| AC4-20kGy 4 | 0.763                     | 1.006                            | 0.977                            | 0.912                            | 0.851                           | 0.822                           | 22             |

FeNP-modification of AC4-20kGy (irradiated AC4 derived from Rape straw) showed this AC sample to be most active adsorbent, presumably due to inherited plant properties and EB-radiation activation, and in comparison with the untreated original AC (AC1).

FeNP-impregnated AC4-20kGy was selected for incorporation into BANchip, and biological testing of the applicability of FeNP-modified AC as the carrier for BA substance within the chips. The
crop germination tests were run at FSBSI ARRI of Rapeseed, FASO Russia, Lipetsk, Russia, within local bionanotectnological R&D program “Nanochip for the Seeds”.

Bittercress (*Barbarea vulgaris*) seeds were treated with BANchips, containing varying amounts of FeNPs-impregnated AC4-20kGy carbon carrier, and tested vs untreated seeds in line with GOST 12038 – 84 test procedures.

Recorded test data [23-25] included seedling dimensions, fresh and dry weights, moisture content, relative humidity, germination total and rate values, etc. The laboratory fourfold 10-day germination tests were carried out on the filter tissues in Petri dishes at 25 °C.

Determined germination energy and seed germination exhibited process activation due to BANchip-treatment of the seeds:
- the number of "germinated" seeds on the first day of germination, depending on the variance in their chip’s composition, was 19.4–34.9% (compared to 10.1% in the control), raising rapidly to 80.9 - 93.3% next (second) day;
- germination vigour (germinated seeds number/ total seeds ratio 3 days after seeding (DAS3), and laboratory sprouting of a seedling from a seed DAS5 have reached 100% in a number tests;
- seedlings length, their fresh and dried mass weight gain were also dependent on actual BFNchip content.

Pre-sowing treatment of plant seeds with “FeNP-impregnated AC4-20kGy” - experimental ACs applied via BANchip technology - was recognized to be efficient practice.

5. Conclusion

The absorption capacity of a studied range of carbonized materials from natural straw increase in test cases, when EB pre-treatment of the sorbents was done. According to UV-VIS spectra records, ACs EB-ionizing radiation with absorbed dose of 10-20 kGy can double the rate of FeNPs absorption from micellar solution at initial “slow” modification stage, which is complicated and contradicted in modifying micellar media, as it has been observed, by an alternative NP-formation process.

CV express registration of electrocatalytic and magnetic properties of nanomaterials, obtained as a result of absorption of FeNPs on the surface of EB radiation-treated ACs, show changes of their catalytic and magnetic activity, and suggest continuing EPR studies.

Reported experiments provide evidence, that radiation-activated and FeNPs-modified ACs from agricultural byproducts and wastes have good potential of becoming the materials of choice for further development of NPs-containing nanochip technology.

They confirm, that not only Ag and other noble metal NPs, but FeNPs, and other metal microelements should be considered for phytosanitary and safe plant seeds BAN treatment done for crop yield and productivity increase, especially under adverse conditions, which include plant diseases, insect attacks, soil and climate extremes.

Employed EB-radiation scientific tools and techniques – radiation-induced adsorbent activation, radiation-chemical metal NPs synthesis in liquid phase, and further adsorbent modifications, – are becoming an integral part of modern agrobiomaterials, and should eventually considerably contribute to their development, suggesting ways of effectively using materials found among renewable resources and agricultural residues. Obtained results clarify direction for further interdisciplinary studies and the path for optimization of EB accelerator-aided methods, required to be in place to meet varying needs imposed by a wide range of tasks in agriculture and food industry worldwide.

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