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Effect of Humic Acid on Growth of Zymogenous Consortium of Microorganisms Used in Bioremediation Process

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[Introduction]

Environmental pollution with toxic metals, petroleum-type contaminants and new classes of Persistent Organic Pollutants (POPs), such as perfluoroalkyl and polyfluoroalkyl substances (PFASs) is always a problem. Microorganisms as "biological agents" are always actual in remediation, especially in the historical pollution, protection and preservation of the environment. Biochemical pathways and mechanisms of degradation and inactivation of pollutants are challenges for researchers and for those who implement those researches in practice. Because of their ability to remove metabolic hydrogen peroxide, special attention is focused on humic substances, an important ingredient of soil organic substances and end products of degradation of organic pollutants and organic substances on the substrate by the consortium of microorganisms during simultaneous bioremediation and humification. Humic substances bind metals (such as manganese, copper, iron, etc.) which are redox-active and react with $\text{H}_2\text{O}_2$ in the Fenton reaction giving hydroxyl radical ($\text{OH}^\cdot$) which is bonded by humic acid.

[Methods]

To monitor the changes during biodegradation that indicate the free radical mechanisms and their possible stimulation we have used the electron paramagnetic resonance (EPR) spectroscopy. Varian E104-A EPR spectrometer, X-band (9.51 GHz). Modulation amplitude 2G, frequency 100 kHz, 10 mW, scan time 4 minuta. We used EW software (Scientific Software Inc, IL, USA). Experiment was conducted in Haber-Weiss (HWS) and in Fenton's systems (FS) with addition of standard and test humic acids.
[Results and discussion]

Figure 1. EPR spectrum
A) HWS; B) HWS + standard of humic acids, * - unknown organic radical; C) HWS + tested humic acids

Figure 2. EPR spectrum:
A) Fenton’s system (FS); B) FS + standard of humic acids; C) FS + tested humic acids

Results show direct reaction of humic acids with reactive oxygen species (ROS) formed in HWS and their removal.

[Conclusion]

The results have showed that the test humic acids directly affect the binding of hydroxyl radicals by binding of Fe$^{3+}$. Because of its reactive nature, iron is highly toxic to the body if its intracellular concentration is not adequately regulated. Toxicity of Fe$^{3+}$ ions is based on its ability to react with superoxide thereby forming a Fe$^{2+}$.

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[References]