



The role of humus substances in soil enzymatic processes

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Humus substances plays in soil the role as sorbents and possible substrates for hydrolytic enzymes, particularly, proteases with various specificity. Due to the high amount of carboxylic, carbonyl, hydroxyl and amide groups in HS structure, they can be served as supports for immobilized enzyme. On the other hand, various mineral and organo-mineral surfaces may have an inhibitory influence on the activity of enzymes.

In present work, we had attempted to elucidate of mechanisms the complex formation of humic acids (HA) of various origin: oxidized brown coals, soil, peat (Kyrgyz deposits), products of anaerobic fermentation of organic raw material with proteases (α -chymotrypsin, trypsin, subtilisin, respectively) and metal ions. The stability of forming humic-enzyme complexes and the types of influence of HA on the activity of enzymes had been determined using kinetical methods. It was shown that humic acids are non-specific inhibitors of proteases. Data have indicated that metal ions inhibited proteases activity.

Organometallic complexes composed of HA and metal ions (M) show enhanced inhibition of enzymes activity as compared with either HA or metal ions alone. It can be caused by the formation of the enzyme-inhibitory complexes at the expense of formation of mixed ligand coordination centers with the participation of the functional groups both of HA and protein molecule on metal localization, and also at the expense of displaying electrostatic and hydrophobic interactions on the other parts of the macromolecular chains. The stability of enzyme-inhibitory complexes depends on the metal nature and on the amount of coordination centres on the polymeric chains of humic macroligands.

The kinetical scheme of inhibition by metalcomplexes of HS was proposed. Defined in the study the constants of the inhibition of the proteases activity by the humic acids at the existence of the metal ions can be used to forecast fermentation processes *in vivo*.

Water-soluble and insoluble complexes studied by high exclusion chromatography are formed upon addition of divalent copper ions to the solution. The contacts between molecules of HA and protein are achieved via chelate unit formation in which the metal ions are located at the center. In the mixture of humic substances-metal ions-enzyme (HA-M-E), insoluble complexes are formed at $\text{pH}=\text{pI}$ starting with very low concentrations of metal ions. On the other hand, these polycomplexes remain soluble at $\text{pH} > \text{pI}$.

The formation of the polycomplexes in the mixture HA-M-E were also studied using sedimentation and spectrophotometric methods. The solubility, composition, and stability of these polycomplexes depend on the metal/HA and HA/E ratio. Insoluble polycomplexes are formed when concentration of metal ions reaches the critical ratio $M/n\text{HA}$ is 0,25. At this concentration of metal ions, phase separation takes place, starting with very low concentration of enzymes in the system. Over the critical ratio of the protein/HA, the mixture again exhibits water-soluble character.

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