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Surface-Active Properties and Biological Activity of Mechanically Activated Humic Acids Extracted from Peat

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Abstract

The features of structural transformations of peat humic acids in the course of mechanical activation with iron oxyhydroxides and the changes in their surface-active and adsorption properties are revealed. It is shown that the maximum biological activity of the aqueous solutions of sodium humates is manifested when the concentration of the active substance of humic acids is below the critical concentration of micelle formation.

Keywords: peat, humic acids, mechanical activation, iron oxyhydroxides, surface-active properties, adsorption, biological activity

INTRODUCTION

Humic acids (HA) of peat, coal and soil exhibit sorption and detoxifying properties with respect to oil, oil products and heavy metals [1, 2]. In addition to the protective function in ecosystems, HA are natural stimulators which enhance the general nonspecific resistance of plants and intensify the growth of the root system [3]. Sorption properties and biological activity of HA are connected with the features of the structure of macromolecule, represented by the hydrophobic part in the form of aromatic, alkyl fragments, and the hydrophilic part with functional groups including micro- and microelements [4–6]. The authors of [7] demonstrated the possibility to modify the structure of humic acids enriched with oxygen-containing functional groups for the detoxication of the medium, and with iron for the correction of iron-deficient chlorosis.

One of the efficient, less labour-consuming and ecologically safe methods to enhance the sorption and complex-forming ability of HA is mechanochemical activation of caustobiooliths [8, 9]. Mechanical action provides the mobility of solid caustobiooliths, promotes the formation of new pores and opening of inaccessible ones, an increase in the external and internal surface. Changes of the physicochemical properties and an increase in the reactivity of HA are due not only to the indicated phenomena but also to possible rupture of chemical bonds, the formation of active radicals, and the generation of excited states.

The modifying substances that are widely used in the mechanical activation (MA) of solid caustobiooliths are alkaline and acid reagents [10], the substances stimulating oxidation and reduction reactions [11, 12], etc. As a result of these operations, the yield of water-soluble substances

increases, the molecular mass decreases, and the fragment composition of HA changes.

According to [7], humic substances are prone to self-association with the formation of supra-molecular structures with a complicated arrangement, stabilized through hydrophobic interactions and hydrogen bonds. Depending on the concentration, these associates in the aqueous media form micelle structures changing the physico-chemical properties of HA. The authors of [13] demonstrated the effect of HA concentration on the biodecomposition of MTsPA herbicide (4-chloro-2-methylphenoxyacetic acid) by the microbiosynthesis of active sludge. In addition, the macrostructural properties of HA and biological activity are interrelated values. In [14], the supply of HA into the intracellular space of plants is linked with their adsorption and transformation of the root surface.

The goal of the work was to study the effect of MA conditions and HA concentration on the surface-active, adsorption properties and biological activity of HA.

EXPERIMENTAL

Mechanical activation of peat was carried out in the AGO-2S activator mill of planetary type in the mode: the frequency of cylinder rotation 1820 r.p.m., centrifugal acceleration 600 m/s². Milling bodies were steel balls 8–10 mm in diameter. The mass of balls loaded into one cylinder was 0.2–0.5 kg, the weighted portion of the sample was 15–20 g, the time of MA of caustobiooliths was 2 min.

The samples of humic acids HA1 and HA2 were extracted from initial and mechanically activated peat, respectively, with the solution of 0.1 M NaOH at a temperature of 20 °C and mixing for 1 h. Alkaline extraction was carried out three times. The obtained HA (HA1 and HA2) in the alkaline solution were precipitated by adding 10 % solution of HCl to pH 2. The brown amorphous precipitate of HA was separated by centrifuging, then washed with distilled water to pH 7 and dried in Petri dish in the vacuum box till the constant mass [15].

Mechanical activation of the HA1 sample was carried out in the activator mill AGO-2S of planetary type in the presence of nano-dispersed powders of iron oxyhydroxides FeOOH (23.0 ± 6.2) nm and FeOOH · Fe₂O₃ (28.0 ± 9.5) nm, which resulted in obtaining HA samples modified with iron -

HA3 and HA4, respectively. The process was carried out with the cylinder rotation frequency 1820 r.p.m., centrifugal acceleration 600 m/s² for 2 min. Milling bodies were steel balls 8–10 mm in diameter. The obtained samples were dissolved in 0.1 M NaOH and precipitated by adding a 10 % solution of HCl to pH 2. The precipitate was washed with distilled water to pH 7.

The surface tension (σ) of the aqueous solutions of sodium humates was determined by means of tensiometry with the help of tensiometer KRUSS EasyDyne K100 (Germany) at room temperature 22 °C and pH 11.7. The ionic strength was maintained by adding 1 M NaCl solution. The tensiometer allows measuring the surface tension at the interface with the help of an optimally wetted platinum-iridium ring combined with the weight system. The accuracy of the measurement of surface tension for solutions was 0.08 %. Measurements of surface tension were carried out after 24 h from the preparation of the solution. The critical concentration of micelle formation (CCM), the change of the standard Gibbs energy of micelle formation (ΔG_M^0) were determined from the dependence of the surface tension of solutions on HA concentration [16–18]. The concentration of functional groups in the CCM point (C_{CCM} , mol/L) was calculated from the content of acid ionogenic groups (C , mol/g) in the composition of HA determined by means of potentiometric titration.

To analyse the sorption ability, HA powder samples with particle size 100–150 nm were used. The adsorbate was a solution of cation-adsorption indicator [C₁₆H₁₈ClN₃S]H⁺ – methylene blue (MB), with the surface area of the flat molecule being 0.69 nm [19–21]. The optical density of MB solutions was measured with the help of 2800 UV/VIS Spectrophotometer UNICO (Germany) at the wavelength of 670 nm. The molecular absorption coefficient was equal to 2.7 · 10⁵ g/(mol · cm). The MB solution and HA powder were mixed for 2 h. The heterogeneous equilibrium in the system HA – MB was established for 24 h in the thermostat at a temperature of 20 °C. Then HA particles were separated by centrifuging, and the optical density of the MB solution was measured. Adsorption value (A , mmol/g) was calculated using the equation

$$A = \frac{(C_0 - C_p)V}{m} \cdot 1000$$

where C_0 and C_p are initial and final MB concentrations in aqueous solutions, mol/L; V is the solution volume, L; m is the mass of adsorbent, g;

1000 is the transition factor used to express A in mmol/g.

The structural-group composition of HA samples was determined by means of ^{13}C NMR spectroscopy with the help of Bruker 300 radiospectrometer (Germany) at the working frequency of 100 MHz using the Fourier transform technique with accumulation. The sweep width was about 26 000 Hz, the time of recording the free induction decay signal was 0.6 s, time between pulses was 8 s with the pulse width 90° , the time of spectrum accumulation was 24 h. The weighted portion of the sample 50–70 mg was dissolved in 0.7 cm^3 of the 0.3 M NaOD solution.

Iron content in the samples was determined using the energy-dispersive X-ray fluorescence method with the BRA-18 spectrometer (Russia). The operation principle of the analyzer is based on the excitation and subsequent registration of the X-ray fluorescence spectrum of the sample under investigation. The energies of the characteristic lines of the spectrum correspond to chemical elements, while their intensities are proportional to the concentrations of the corresponding elements.

The biological activity of HA was evaluated from sprouting the seeds of tomatoes. For this purpose, the seeds were placed in a Petri dish, wetted with the aqueous solutions of sodium humate (pH 7) with the concentration of 0.01, 0.075, 0.05, 0.1 and 0.5 g/L, and kept in the climatic box at a temperature of $20\text{ }^\circ\text{C}$ for 10 days. The reference samples were treated with distilled water.

RESULTS AND DISCUSSION

The isotherms of surface tension of the aqueous solutions of sodium humates depending on the concentration of HA (C_{HA}) are shown in Fig. 1. The surface tension at the initial region of the concentration curve decreases monotonously with an increase in C_{HA} . At a definite critical concentration, micelles are formed in the system from HA molecules. These micelles do not possess surface activity. Because of this, a further decrease in the surface tension of the solution does not occur. One can see in Fig. 1, *a* that similar behaviour of the changes of surface tension depending on concentration is observed for the HA1 and HA2 samples, with a more intense decrease of σ in the case of the solution of mechanically activated sample HA2. For HA1 and HA2 samples, CCM is the same and equal to 0.8 g/L. Analysing the effect of the addition of iron oxyhydroxides during MA of HA samples, it should be stressed that the surface tension isotherms for HA3 and HA4 samples are identical, and the formation of micelles occurs at lower HA concentration: CCM = 0.6 g/L (see Fig. 1, *b*).

To calculate the concentration of functional groups of HA in the point of CCM (C_{CCM}), one should know the molar concentration of the substance. However, it appears impossible to determine the molar concentrations of micelle formation for HA due to the non-stoichiometric composition, irregular structure, heterogeneity of structural elements and polydispersity. Because of this, the contributions from all functional groups ionized

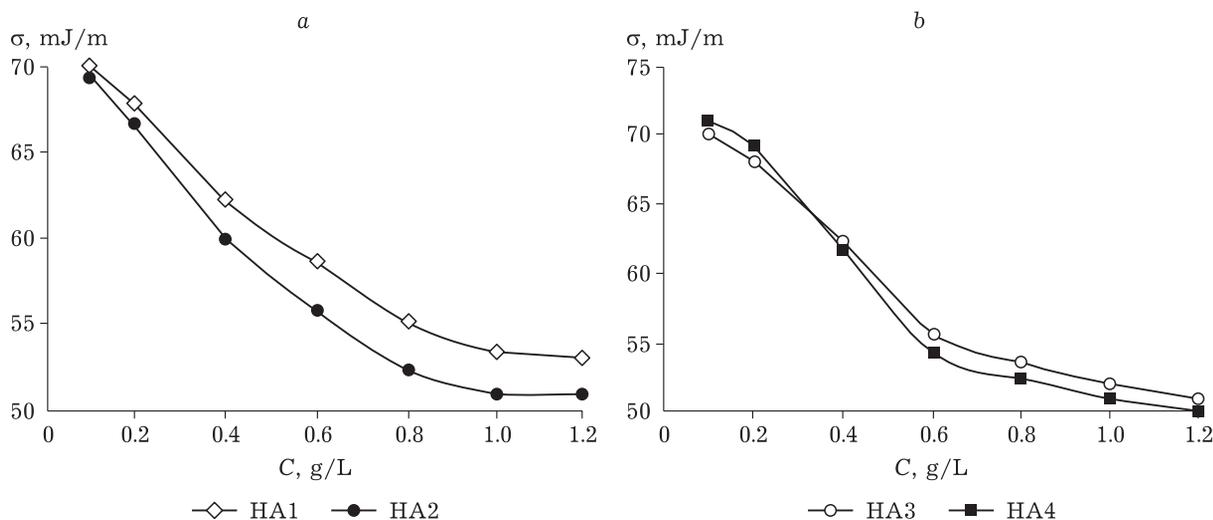


Fig. 1. Dependence of surface tension of the aqueous solutions of sodium humates on concentration: HA1 and HA2 (*a*); HA3 and HA4 (*b*).

TABLE 1

Content of functional groups and the constants of adsorption equilibrium at the liquid – gas interface

Sample	$C \cdot 10^3$, mol/g	CCM, g/L	$C_{ccm} \cdot 10^3$, mol/L	$-\Delta G_M^0$, kJ/mol	K_a , L/g
HA1	13.9 ± 0.1	0.8	11.8	10.8	2.8
HA2	14.4 ± 0.2	0.8	11.5	10.9	3.0
HA3	13.3 ± 0.3	0.6	8.0	11.7	4.9
HA4	14.9 ± 0.2	0.6	8.9	11.5	4.2

within the pH range under investigation (at pH 11.5) were taken into account (Table 1).

The C_{CCM} value depends on a number of factors: HA nature, the size of the hydrophobic radical, salt background, acidity of the medium, the presence of proton donating groups in the hydrophilic part of the molecule. The highest C_{CCM} and ΔG_M^0 are detected for HA1 and HA2 samples. The HA3 and HA4 samples are characterized by a trend to a decrease in the energy of micelle formation.

Calculation of the constants of adsorption equilibrium K_a at the liquid – gas interface (see Table 1) was carried out by means of graphical differentiation over the experimental curve $\sigma = f(C)$. Analysis of the values was carried out in agreement with the data on the content of functional groups and the structural group composition determined by means of NMR ^{13}C spectroscopy (Table 2). The high fraction of oxygen-containing alkyl ($C_{alk}O$) and alkyl (C_{alk}) fragments in all HA samples should be stressed. Under the effect of MA, the concentrations of carboxyl groups (COOH), aromatic ($C_{ar}C,H$) and alkyl fragments increased.

One of the major factors affecting CCM is the structure of HA, namely the structure of the hydrocarbon radical and the nature of the polar group. Aggregation processes depend on the structure of hydrophilic parts, which affects the electrostatic repulsion between the polar heads of micelles, changes the hydrophilic-hydrophobic balance and the degree of micelle binding with counter-ions in the HA structure. On the basis of the structural group composition, the hydrophilic-hydrophobic balance of the molecular assembly of HA may be presented [22]. According to [23], hydrophilic fragments include the following components: $COOH + C_{OHPhen} + C_{alk}O + CH_3O$. The content of carbon atoms connected with phenol groups (C_{OHPhen}) was determined from the difference of integral intensities within the ranges of $C_{ar}O$ and CH_3O fragments in the ^{13}C NMR spectra of HA. Hydrophobic fragments are represented by the sum of aromatic and alkyl carbon $C_{ar}C,H + C_{alk}$. It follows from the data shown in Table 2 that hydrophilic fragments including carbohydrates, peptides, phenol and carboxyl groups dominate in the peat HA samples, which determines the possibility of the formation of bonds

TABLE 2

Iron content and the structural group composition of humic acids (HA)

Content	HA1	HA2	HA3	HA4
Iron, mass %	0.15	0.23	2.42	1.21
Carbon in structural fragments, rel. %:				
C=O	1.1	2.1	1.0	2.0
COOH	9.2	10.1	11.5	12.2
$C_{ar}O$	9.3	9.7	7.6	8.1
$C_{ar}C,H$	12.2	11.5	16.3	13.0
$C_{alk}O$	37.3	34.2	30.0	30.7
CH_3O^-	2.9	3.7	4.2	4.4
C_{alk}	28.0	28.7	29.4	29.5
Hydrophilic carbon, rel. %:				
$\Sigma C_{hydrophil} = COOH + C_{OHPhen} + C_{alk}O + CH_3O$	55.8	54.0	49.1	51.0
Hydrophobic carbon, rel. %:				
$\Sigma C_{hydrophob} = C_{ar}C,H + C_{alk}$	40.2	40.2	45.7	43.5
$\Sigma C_{hydrophob} / \Sigma C_{hydrophil}$	0.72	0.74	0.93	0.85

with adsorption centres. The amount of molecules adsorbed at the interface, determined by the constant of adsorption equilibrium K_a , is higher for HA3 and HA4.

The surface properties of HA in the dispersing medium containing charged probe particles of MB with known geometric parameters were studied similarly to the sorption of humic materials in [19]. MB adsorption isotherms are shown in Fig. 2. Mechanical activation causes an increase in the sorption capacity of HA with respect to MB within the range of concentrations of the latter $(4.0\text{--}23.0) \cdot 10^{-6}$ mol/L (HA2 sample). The HA3 and HA4 samples are characterized by close sorption capacity within the whole concentration range. The adsorption of MB by the samples modified with iron is higher than its adsorption by HA1 and HA2 samples. This difference increases with an increase in MB concentration.

To establish the mechanism of MB adsorption, pH of aqueous solutions was controlled during the experiments. It was shown that before and after adsorption pH varied in the studied MB solutions within the range 7.1–7.6, which is the evidence of the absence of chemical reactions between MB and HA with the participation of ions. An increase in the hydrophobicity of molecules in HA3 and HA4 samples (see Table 2) and an increase in MB sorption are the evidence of the hydrophobic binding of the organic cation with the carbon matrix of HA.

Results of the investigation of the biological activity of HA samples depending on C_{HA} are shown in Table 3. All the studied samples exhibited rather high biological activity with respect to the test object (tomatoes). The treatment of seeds with the aqueous solution of sodium humates HA2 affects the height of seedlings. Samples HA3 and HA4 are characterized by a more pronounced effect at the stage of the development of the root system of the seedlings. According to the data of X-ray fluorescence studies, iron content in HA3 and HA4 samples increased in comparison with

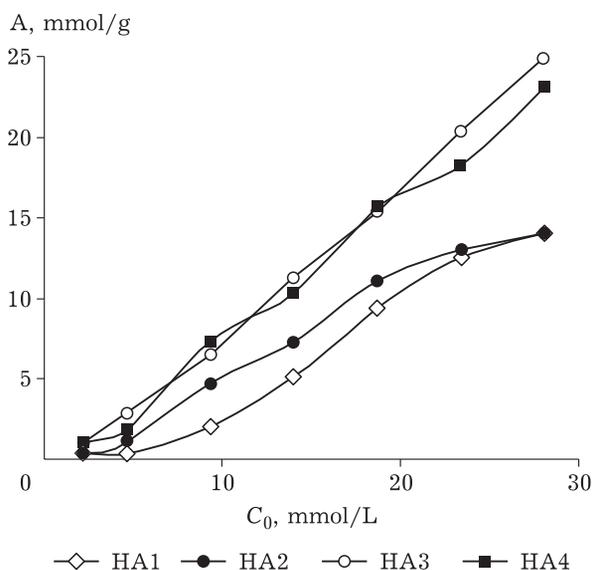


Fig. 2. Adsorption isotherms for MB dispersed in water with HA.

initial and mechanically activated HA (see Table 2). Iron participates in oxidation-reduction processes and is a component of the respiratory enzymes, accelerates the formation of chlorophyll, causes an increase in general productivity and plant stability against diseases. The concentrations of HA are not less important in the investigation of the biological activity of HA. One can see in the data presented in Table 3 that the maximal biological activity of the aqueous solutions of sodium humates manifests itself at HA concentration below the CCM value.

CONCLUSION

The surface-active properties of HA are determined by the structural features of macromolecules and the conditions of the formation of associates and micelles in solution. The HA samples obtained after MA with iron oxyhydroxides are characterized by a decrease in CCM, ΔG_m^0 and an increase in K_a . An increase in the hydrophobic

TABLE 3

Effect of HA concentration on stalk height and root length in the seedlings

Concentration, g/L	Stalk/root, % to the reference			
	HA1	HA2	HA3	HA4
0.5	110/105	119/75	105/155	131/153
0.1	112/107	139/80	124/185	155/173
0.075	135/110	145/119	158/201	178/192
0.05	145/110	158/120	164/202	180/179
0.01	144/109	156/122	168/204	184/183

component in the molecular assembly of iron-modified HA promotes an increase in the sorption properties with respect to MB.

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