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ANTIOXIDANT PROPERTIES OF BROWN COAL HUMIC SUBSTANCES

Humic substances (HS) belong to a fairly common class of organic compounds in nature, which are a component of soils, peat, lignite and sapropels. Some properties of humates (salts of humic acids) make it possible to use them in various fields – crop production, animal production, well drilling and soil structuring. The biological and chemical activity of humic substances is well known. It is caused by the presence of such functional groups as carboxyl, hydroxyl, carbonyl, nitrogen- and sulfur-containing in the composition of GR macromolecules. This fact also serves as a basis for a more extensive study of other properties of GR, namely antioxidant activity.

Purpose. Deepening and expanding the understanding of the biological and antioxidant activity of lignite humic (HA) and hylatomelanic (HmA) acids, determining its nature and evaluating the prospects of its use in little-studied processes of oxidation of organic and consumer substances.

Methodology. The paper used a photocolometric method for determining the antiradical activity (ARA) of two components of humic substances of domestic lignite – humic and hylatomelanic acids. The antioxidant activity (AOA) of humic and hylatomelanic acids from lignite was determined using the gas volumetric method, evaluating the rate of reaction inhibition using the example of the initiated liquid-phase oxidation of cumene with oxygen.

Findings. It was established that humic acids exhibit more pronounced antioxidant properties in the processes of radical-chain oxidation of hydrocarbons compared to hylatomelanic acids. The total AOA of HA is 1.68 relative units, which is almost three times higher than this figure for HmA (0.50). On the other hand, it has been experimentally proven that hylatomelanic acids have more pronounced antiradical properties than humic acids. ARA HmA is 0.90 relative units, which is approximately three times more than the similar value for HA (0.57).

Originality. Despite the fact that a number of methods for determining the antiradical and antioxidant activity of low-molecular antioxidants and their mixtures have been developed, a method for determining these indicators for high-molecular native compounds has not yet been created. For the first time, a method for determining the antiradical effect of humic substances using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was proposed and experimentally tested. A method for determining the antioxidant activity of humic and hylatomelanic acids in hydrocarbon oxidation processes (on the example of cumulus) has been developed. The convenience and satisfactory reproducibility of the developed methods are shown.

Practical value. The green-chemistry related development and application of drugs based on native and modified humic substances as polymers with adjustable redox properties (redox polymers) is a relevant direction of sustainable development of society. These substances are the basis for obtaining new medicines, plant protection products, new sorbents, preparations for the reclamation of territories contaminated with radionuclides, heavy metals, organic ecotoxicants, oil products, etc.

Keywords: *antiradical activity, antioxidant activity, inhibitors, hylatomelanic acids, humic acids*

Introduction. Humic substances are a fairly common class of organic compounds in nature, which are part of soils, peat, lignite and sapropels. The reactivity of humic substances is due to the presence of a large number of functional groups, primarily carboxyl, hydroxyl, carbonyl, nitrogen- and sulfur-containing, in the composition of their macromolecules, in addition to polyarene clusters of various degrees of condensation. Due to this, humic substances are capable of complex formation, oxidation and reduction reactions. Their biological activity is due to participation in ion transport and influence on the action of enzymes [1, 2].

Literature review. High-molecular compounds of organic nature with condensed aromatic nuclei – humic and hylatomelanic acids are the main components of humic substances. Moreover, the fraction that can be dissolved in alkalis and insoluble in acids is humic acids (HA), and the alcohol-soluble fraction is hylatomelanic acids (HmA). All of them have side chains of varying degrees of branching. Humic acids are the main part of humic substances, which are extracted from lignite with weak alkali solutions. The alcohol-soluble fraction is hylatomelanic acids. High molecular natural polymers belonging to aromatic acids and including systems of several aromatic heterocyclic cores containing sulfur, nitrogen, and oxygen [1, 3]. Shown in Fig. 1, a probable molecular fragment of the peripheral part of humic acids [4] contains all the most important structural components of HA. The redox balance is

a key factor in all processes occurring in both normal and pathological cells, as reactive oxygen species can significantly disrupt normal metabolic pathways. Disturbance of the redox balance, caused either by excessive production of reactive oxygen species, or insufficient activity of the antioxidant defense system, inevitably leads to oxidative stress [4, 5]. There are some natural molecules, mainly nutrients, that have antioxidant activity. They include carotenoids, vitamins E and K, ascorbic acid (AA), flavonoids and phenolic acids, zinc, selenium and many other chemicals. Humic acids (HA) can also be considered effective antioxidants; many research groups reported their high potential for reducing active free radicals [6, 7]. According to the generally accepted opinion, HA are natural high- and supramolecular polyfunctional colloidal bioorganic molecules that have both amphoteric and amphipathic properties. The antioxidant activity of HA can be explained by a large number of phenolic hydroxyl, quinoid and other chemical groups that have a highly delocalized molecular orbital. As a result, HA can donate protons, trap free radicals and chelate reactive ions under normal physiological conditions.

In addition, being powerful antioxidants and substances that trap free radicals, HAs do not show specific toxic activity in relation to cells, tissues or organisms [6, 9]. Thanks to this, humic substances are a promising natural source of new raw materials for the food and pharmaceutical industry [10, 11]. Despite the fact that HA is a very promising potential new drug, the chemical structure of HA has not yet been identified, and the quantitative structure-activity relationship remains

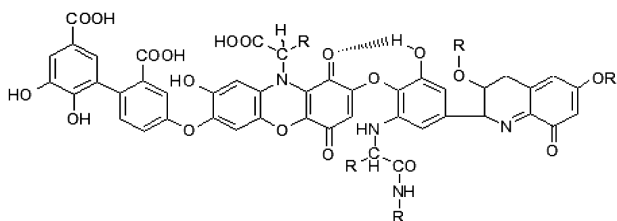


Fig. 1. Example of the structure of the humic acid macromolecule peripheral part

unmodeled. The main reason for this is the increased chemical complexity of HA [1, 12]. Another important factor is that HA samples obtained from different sources have quite significant variations in their physical and chemical properties and are accompanied by nonlinear dependencies between these parameters and biological activity.

HA are used in oriental medicine for the treatment of skin diseases, cold stress, rheumatic pains, diabetes, urolithiasis, heart diseases, leprosy, and diseases of the immune system [6, 13]. Peat preparations are mainly used as external means, but they can also be used as means of internal use. For example, HA from various sources was used externally for the treatment of hematomas, phlebitis, desmoresis, myogelosis, arthrosis, polyarthritus, osteoarthritis, and osteochondrosis. As for internal use, GCs have proven to be particularly useful in the treatment of diarrhea, gastritis, stomach ulcers, dysentery, colitis, and diabetes [6, 14]. In a number of studies, it was reported that HA exhibit anti-inflammatory and immunomodulatory properties [5, 15].

As was mentioned above about the anti-radical and antioxidant activity of some natural humic substances [4, 14], but there is no information about specific HAs isolated from lignite. Since lignite can be an important source of HAs for the pharmaceutical industry, the characterization and standardization of their physicochemical parameters is necessary, especially since no clear relationships have been reported between the pharmacological activity of lignite-derived HA and their humification, molecular size distribution or content of stable free radicals [11]. Lignite, unlike, for example, peat, is subject to relatively weak oxygen saturation, which leads to a low intensification of oxidation processes during the formation of humic substances and changes in their chemical and biological characteristics. The process of humification can, in some cases, lead to changes in the pharmacological properties of HA, such as increased anticarcinogenic and antiradical activity.

From the point of view of the elemental representation of the composition of humic substances, they mainly contain carbon, oxygen, hydrogen, nitrogen and sulfur. From the point of view of the structure of the macromolecule, humic substances are composed of parts of structurally heterogeneous carbon chains (of which approximately 40–50 % are aliphatic, the rest – alicyclic, and aromatic chains make up a total of 35–60 %). By functional groups, carboxylates, phenolic and non-aromatic hydroxyl groups, oxo- and quinone groups, amino- and imino groups, as well as esters, amides (cyclic and non-cyclic), acetals and ketals, ether and peroxy groups prevail. According to the classes of structural components, the molecules of humic substances contain fragments of oligo- and polysaccharides, sugar acids, oligo- and polypeptides, remnants of fatty acid chains, fragments of lignin, condensed aromatic substances, which quite often contain heterocyclic nuclei [4, 16].

Based on the analysis of the question, such properties as inhibition of oxidation processes, i. e., the ability to stop or delay unwanted reactions involving active forms of oxygen, have not been sufficiently studied. Namely, the presence of hydroxyl, quinoid, carboxyl, amino groups in the composition of macromolecules of humic substances is the basis for predicting the capacity for antioxidant action [8, 17].

It is known that antioxidant (AOA) and antiradical (ARA) activities are pharmacological tests for biologically active substances. Preparations of humic substances, both native and modified, are already used in agriculture, veterinary medicine, medicine and some technical areas. Determining the prospects of GR in a new range of possible uses, such as when obtaining preparations with specified antioxidant properties based on humic substances, is an urgent task.

Several methods for determining ARA and AOA of low-molecular antioxidants and their mixtures are described in the literature, but clear methods for evaluating these indicators for high-molecular compounds have not been developed [4, 6]. In general, it is known that it is quite problematic to determine the total antioxidant efficiency of a substance. This is due to such features as the transition from one test system to another. The studied substance can change the characteristics studied when using different compounds, so the effect of the studied substance as an antioxidant will also change. Thus, the question of creating a universal test for antioxidant activity remains open. Understanding the detailed mechanism of the oxidation reaction opens ways to overcome this negative result, especially for complex natural compounds, both in the case of food products and biological objects. It is known [16] that a large number of biochemical reactions in the living organism take place with the participation of free radicals, which have exceptionally high chemical activity. During pathological processes, the balance of formation and decay of free radicals is disturbed, which leads to a sharp increase in their level in the body. At the same time, cellular structures may be affected, vital processes, etc., may be disturbed. In the process of interaction of free radicals (forms of active oxygen, etc.) with biologically active substances that have antioxidant properties, the chain reaction with the participation of free radicals, which are formed in various pathological processes in the cells of living organisms, is partially or completely stopped had [17, 18].

Works [16, 17] claim that quinoid fragments are responsible for electron transfer reactions involving macromolecules of humic acids, which form free radicals (semiquinones) during one-electron reduction. In addition to quinoid fragments, phenolic hydroxyls, which are oxidized to phenoxyl radicals, can contribute to the redox capacity of humic acids. This assumption was put forward in [17], based on the similarity of the redox capacity dependences on pH for phenols and humic acids. Radicals of humic substances of semiquinone or phenoxyl types can neutralize radical centers of reactively unstable compounds, thereby reducing the likelihood of developing pathology.

Purpose. Deepening and expanding the understanding of the biological and antioxidant effect of lignite humic and hmatomelanic acids, determining its nature and evaluating the prospects of their use in little-studied oxidation processes of simple organic compounds and consumer substances constituted the main goal of our development.

Methods. HmA and HA for the experiment were obtained similarly [4] with a yield of approximately 19 % for HA and 1–2 % HmA and a molecular weight of ~20,000 a.o.m., which makes it possible to classify them as natural high-molecular compounds.

Humic substances are extracted from soil, low-metamorphic coal or other natural raw materials with alkali solutions. Any acid up to pH 1–2 is added to the resulting alkaline extract, humic and hmatomelanic acids precipitate, and fulvic acids (FA) remain in solution. The precipitates of humic and hmatomelanic acids are filtered, dried and obtained in a dark brown color. Obtaining the mentioned humic substances from natural raw materials is shown in Fig. 2.

The antiradical activity (ARA) of humic substances was determined by analogy with plant extracts, and AOA – by studying the inhibitory properties of HA and HmA in hydrocarbon oxidation processes.

To determine the optimal concentration of humic substances, their content in the system varied in the range of 0–10.0 g/L.

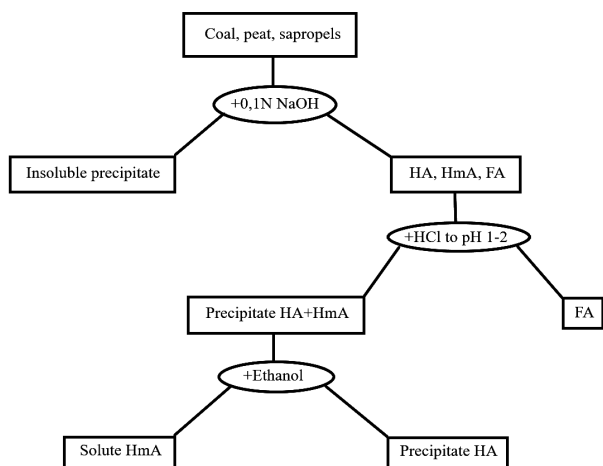
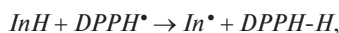


Fig. 2. Production of humic, humatomelanolic and fulvic acids from natural raw materials

The experiment was conducted at a temperature of 348 K and a partial pressure of oxygen of 760 mm mercury Art. The amount of absorbed oxygen was recorded using a gas volumetric device [20]. ARA HA and HmA were studied using the interaction of cumene with molecular oxygen as a model reaction. Dimethylsulfoxide (DMSO) was chosen as the solvent, and azodiisobutyronitrile (AIBN) as the initiator.

Based on the goal and task of researching the anti-radical properties of humic substances, we used the method for determining ARA of plant extracts, which is based on the ability of natural inhibitors to interact with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). We used such solvents as ethanol and dimethyl sulfoxide (DMSO).

Using the UV spectrophotometric method, the change in the concentration of the 2,2-diphenyl-1-picrylhydrazyl radical during its reaction with the radical centers of the studied systems was determined according to the equation



where InH is an inhibitor molecule (HA, HmA, AA).

The study of changes in the optical density of solutions during this reaction was carried out on a Specord UV VIS spectrophotometer in cuvettes $l = 1.0$ cm at $T = 298$ K. To determine the optimal concentration of humic substances, their content in the system varied in the range of 0 to 10.0 g/L.

DMSO, cumene, and azodiisobutyronitrile were used in the work. All substances were prepared for the experiment according to the methods described in [20]. Cumene concentration in the reaction mixture is 3.59 mol/L, AIBN is 2.00×10^{-2} mol/L. To determine the optimal concentration of humic substances, their content in the system varied in the range of 0–10.0 g/L.

The stable radical 2,2-diphenyl-1-picrylhydrazyl from Aldrich had a purity of 95 %, and the used ascorbic acid (AA) had a specific optical rotation of $+20.9 \pm 0.4$.

Results.

1. Determination of ARA of humic acids. Studying the proposed reaction mixture of HmA with DPPH in ethanol, kinetic dependences of DPPH consumption were obtained, where the value of HmA concentration varied in the range of 0.1–0.4 g/L. The figure (Fig. 3) shows the change in the optical density (D) of HmA solutions of different concentrations with time (t). As can be seen, the presented dependencies are described with high accuracy by an exponential equation of the first order.

From the results of Fig. 3 it can also be seen that the maximum rate of radical death is observed in the first 10 minutes of the process. During this time, when the concentration of HmA

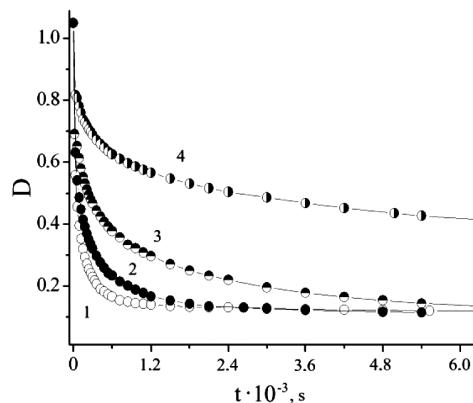


Fig. 3. Change in the optical density (D) of HmA solutions of different concentrations with time (t):

1 – 0.36 g/L; 2 – 0.27 g/L; 3 – 0.18 g/L; 4 – 0.09 g/L

increases by 4 times, the number of “neutralized” radicals decreases by 6 times. In general, the process of death of DPPH radicals stabilizes in 40–60 minutes, depending on the concentration of humatomelanolic acid.

The obtained results give reason to believe that the optimal reaction time is 10–20 minutes, and the optimal (to achieve 90 % of the result) HmA concentration will be about 0.3 g/L.

Similar dependences were obtained for the interaction of DPPH with HmA and HA in DMSO medium. They have the same character. The optimal reaction time, which leads to the death of 90 % of radicals, is 30–50 minutes.

Thus, humic substances from lignite have a clearly expressed antiradical activity, which was determined by experimental studies of the interaction of HA and HmA with DPPH in the environment of two polar solvents of different nature (proton donor ethanol and aprotic DMSO).

According to the generally accepted methods of researching the inhibitory effect of some compounds [20], we chose ascorbic acid as an inhibitor for comparison, which, along with natural phenols, is used in the study of ARA of natural substances.

Based on the data obtained by us on the value of the optical density of DPPH solutions in DMSO depending on the concentration of humic substances ($[C]$), the amount of ascorbic acid equivalent to a certain concentration of HmA ($[AA]$) was calculated and the value of ARA for HA and HmA was obtained (Table 1).

ARA of humic substances was calculated as the tangent of the slope of the direct ratio of the value of the amount of humic substance to the corresponding equivalent value of ascorbic acid. That is, it is the same concentration at which the same amount of DPPH reacted.

Table 1

Antiradical activity (ARA) of humic substances

Humic substances, (ARA)	$[C]$, mg/L	$[AA]$, $\mu\text{mol/L}$
HA, (0.57)	54.0	76.85
	40.5	64.68
	36.1	43.29
	27.0	31.49
	18.1	18.88
HmA, (0.90)	13.5	9.85
	40.5	46.93
	36.1	44.10
	27.0	38.27
	18.1	22.96

From the obtained results, it can be seen that ARA for HmA is almost 2 times higher than for HA. This indicates that during the extraction of HmA, a significant proportion of the antiradical structures of HA is concentrated precisely in the ethanol extract.

2. Determination of the antioxidant activity of lignite HA.

As you know, compounds that exhibit general antioxidant properties are used to inhibit the oxidation processes of various consumer products. Reactions of oxidation inhibitors play an important role not only in modern technological processes, but also in the selection of effective antioxidants that protect living organisms from the destructive effects of ecotoxins, light, and radioactive radiation. HA contain a wide range of fragments that are oxidized at different potentials. When oxidized, they release protons and electrons and undergo irreversible further reactions. For example, phenolic antioxidants donating electrons and hydrogen can react with reactive oxygen species in a reaction to stop the oxidation process. The activity of many natural compounds with respect to DPPH is determined by the presence of various phenolic fragments and correlates with the total concentration of OH groups. Indeed, Aeschbacher, et al. [17] reported that phenolic fragments are the main electron-donating groups in humic substances.

On the other hand, the number of quinoid fragments is only part of the structural fragments of HA, which participate in the transfer of electrons. In addition to quinoid/semiquinoid fragments, phenolic groups oxidized to phenoxyl radicals may also contribute to the anti-oxidant activity of HA.

We emphasize again that the problem of choosing antioxidants is quite complex, since their effectiveness depends not only on the chemical structure of the compounds, but also on environmental conditions, the mechanism of their action, etc. [19].

That is why the study of the oxidation of hydrocarbons in the liquid phase is an informative method for evaluating the effectiveness of the inhibitor. And the use of molecular oxygen as an oxidant in the selected method for researching the antioxidant activity of hydrocarbons has its obvious advantages: it is an available, cheap and environmentally safe reagent of "green chemistry" [20].

In accordance with the previously outlined tasks, we conducted a study of the effect of HA and HmA on the oxidizing mixture of cumene – solvent – initiator. Fig. 4 shows the characteristic kinetic curves of oxygen absorption in the process of cumene oxidation in DMSO medium in the presence of various humic substances.

As can be seen from Fig. 4, HA inhibits oxygen absorption almost three times more effectively than HmA. At the same concentration of these substances, within 60 minutes of the process, there is a significant but different decrease in the volume of absorbed oxygen, namely, for HA, oxygen absorption is 0.8 mL, for HmA, it is about 2.4 mL, without inhibitors this value is more than 4 mL. It can be seen that

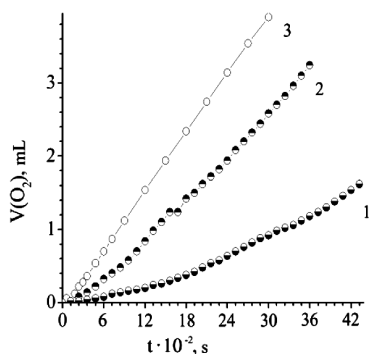


Fig. 4. Change in the amount of oxygen absorbed by the oxidizing mixture with the time of the chemical reaction:

1 – [HA] = 2.0 g/L; 2 – [HmA] = 2.0 g/L; 3 – without inhibitors

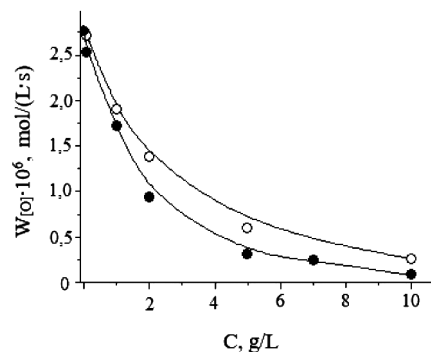


Fig. 5. Dependence of the rate of oxygen absorption ($W_{[O_2]}$) in the process of cumene oxidation in the presence of different concentrations of humic substances (C):

○ – HmA; ● – HA

the effect of reducing oxygen absorption due to the addition of humic substances to the selected system depends on their content, increasing with increasing concentration of humic substances (Fig. 5).

An increase in the concentration of natural antioxidants from 0 to 10 g/L leads to a decrease in the rate of oxygen absorption by more than 34 times (for HA) and by more than 10 times when using hyamatomelanic acids. The $W_{[O_2]}^*/W_{[O_2]}$ parameter, i. e., the ratio of the rate of oxygen absorption by the reactive mixture without humic acids to the similar one with humic substances, depending on the concentration, was calculated on the basis of the kinetics data. From the data in Table 3, the antioxidant activity (AOA) of the studied humic substances was calculated, the value of which for HA is 1.68 relative units, which is almost three times higher than this indicator for HmA – 0.50 relative units. The obtained value of AOA can be a characteristic of the general antioxidant activity of inhibitory substances, which allows comparing it with literature data.

As can be seen, the dependence of $W_{[O_2]}^*/W_{[O_2]}$ is directly proportional, i. e. the parameter increases if the concentration of the studied acids in the reaction mixture increases. The value of antioxidant efficiency for humic acids was calculated from the ratio $W_{[O_2]}^*/W_{[O_2]}$ taking into account the concentration.

According to the obtained data (Table 2), HA is a more effective antioxidant than HmA in the proposed model.

Conclusions. Based on the obtained experimental data of the study of the influence of humic substances from brown coal on the process of initiated liquid-phase oxidation of cumene, it can be concluded that the addition of humic substances causes a significant inhibition of the oxygen absorption process. The observed effect intensifies with an increase in the concentration of humic and hyamatomelanic substances in the studied system.

Table 2

[C], g/L	Parameter $W_{[O_2]}^*/W_{[O_2]}$	
	$W_{[O_2]}^*/W_{[O_2]}$	
	HA	HmA
0.1	1.09	1.02
1.0	1.60	1.45
1.5	2.44	1.71
2.0	2.97	2.00
3.0	4.98	3.42
5.0	8.90	6.73
6.0	10.27	7.45
7.0	11.50	8.30
8.0	19.37	9.08
10.0	34.50	10.62

Therefore, it was established that humic acids exhibit more pronounced antioxidant properties in the processes of radical-chain oxidation of hydrocarbons compared to humatmelanic acids.

On the other hand, it has been experimentally proven that the antiradical activity of humatmelanic acids is more pronounced than that of humic acids. Similar results were obtained for the first time.

This conclusion can be confirmed based on literature data [17], which demonstrate a proportional dependence (even correlation) of the activity of natural compounds to the death of the DPPH radical on the presence of phenolic fragments of different structures and the number of hydroxyl groups in these fragments. That is why the anti-radical activity of HmA (which have a higher content of OH groups) exceeds the antiradical efficiency of HA.

The experimental results obtained for the first time prove that the humic substances of domestic lignite exhibit clearly pronounced antioxidant properties, that is, they are effective inhibitors of radical-chain oxidation of hydrocarbons.

The conducted research expands the idea of the possible areas of application of lignite humic components, as they make it possible to predict and optimize oxidation processes with the participation of these valuable natural substances.

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Антиоксидантні властивості буровугільних гумінових речовин

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Гумінові речовини (ГР) належать до доволі поширеного у природі класу органічних сполук, що є складовою ґрунтів, торфу, бурого вугілля й сапропелів. Деякі властивості гуматів (солей гумінових кислот) дають змогу використовувати їх у різних галузях – рослинництві, тваринництві, при бурінні свердловин і структуванні ґрунтів. Біологічна й хімічна активність гумінових речовин добре відома. Вона обумовлена наявністю у складі макромолекул ГР таких функціональних груп як карбоксильних, гідроксильних, карбонільних, азот- і сірковмісних. Цей факт також слугує підґрунтям для більш розширеного вивчення інших властивостей ГР, а саме антиоксидантної активності.

Мета. Поглиблення й розширення уявлень про біологічну та антиоксидантну активність буровугільних гумінової (ГК) і гіматомеланової (ГмК) кислот, визначення її природи та оцінка перспектив використання в малодосліджених процесах окиснення органічних і споживчих речовин.

Методика. У роботі використовували фотоколориметричний метод визначення антирадикальної активності (АРА) двох складових гумінових речовин вітчизняного бурого вугілля – гумінових і гіматомеланових кислот. Антиоксидантну активність (АОА) гумінових і гіматомеланових кислот із бурого вугілля визначали за допомогою газоволюмометричного методу, оцінюючи швидкість гальмування реакції на прикладі ініційованого рідинно-фазного окиснення кумолу киснем.

Результати. Встановлено, що гумінові кислоти виявляють більш виражені антиоксидантні властивості у процесах радикально-ланцюгового окиснення вуглеводнів порівняно з гіматомелановими кислотами. Загальна АОА ГК становить 1,68 відносних одиниць, що майже втричі перевищує цей показник для ГМК (0,50). Натомість, експериментально доведено, що гіматомеланові кислоти мають більш виражені антирадикальні властивості, ніж гумінові кислоти. Загальна АРА ГМК – 0,90 відносних одиниць, що приблизно втричі більше аналогічної величини для ГК (0,57).

Наукова новизна. Попри те, що наразі розроблена низка методів визначення антирадикальної та антиоксидантної активності низькомолекулярних антиоксидантів і їх сумішей, методика визначення цих показників для

високомолекулярних нативних сполук досі не створена. Вперше запропоновано та експериментально апробовано метод визначення антирадикальної дії гумінових речовин за допомогою стабільного радикала ДФПГ. Розроблена методика визначення антиоксидантної активності гумінової та гіматомеланової кислот у процесах окиснення вуглеводнів (на прикладі кумолу). Показана зручність і задовільна відтворюваність розроблених методик.

Практична значимість. Актуальним напрямом стабільного розвитку суспільства є розробка й застосування згідно із принципами «зеленої хімії» препаратів на основі нативних і модифікованих гумінових речовин як полімерів із регульованими окислювально-відновними властивостями (редокс-полімери). Ці речовини є основою для отримання нових лікарських препаратів, засобів захисту рослин, нових сорбентів, препаратів для рекультивациі територій, забруднених радіонуклідами, важкими металами, органічними екотоксикантами, нафтопродуктами тощо.

Ключові слова: антирадикальна активність, антиоксидантна активність, інгібітори, гіматомеланові кислоти, гумінові кислоти

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