but on working with low levels of activity the reliability of the results falls. The second method, although it makes the process more laborious, nevertheless provides the possibility of counting samples with higher efficiency at any concentrations of quenchants and with any levels of activity.

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PHOTOPHYSICAL AND PHOTOCHEMICAL PROPERTIES OF LIGNIN CHROMOPHORES IN AQUEOUS SYSTEMS

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The results are given of an investigation of the changes in the physicochemical properties of the chromophores of lignin substances of effluents by photopotential, luminescence, and ESR spectroscopy and the polarographic determination of oxygen as a function of the number of quanta of incident energy in the interval from 300 to 600 nm. It has been established that under the action of light a change in the redox properties, an increase in the rate of consumption of oxygen, the formation of radical intermediate products, and the appearance of excited triplet states of the lignin chromophores take place in the lignin substances. Action spectra of the photopotential, of the yield of EPCs of free radicals, and of the consumption of oxygen by the lignin substances in the interval from 300 to 600 nm have been obtained.

In nature few polymers are found in which there is such a diverse set of extremely dynamic and labile chromophoric systems as lignin preparations. Depending on the type of treatment to which the lignin is subjected in industrial processes during the production of cellulose, preparations of it differ considerably in their physicochemical properties.

An analysis of the products of the far-reaching breakdown of lignin under the action of lights and atmospheric oxygen has been the subject of a number of investigations. In the opinion of the authors concerned [1-5], an important role in the photooxidation of lignin under the action of light in the presence of oxygen is played by phenoxyl radicals, photosensitizers, singlet oxygen, and carbonyl groups in an excited state.

There have been far fewer investigations on the analysis of the photophysical properties of lignin chromophores and the chemical transformations preceding far-reaching oxidative breakdown. Results due to Sergeev and Chupka [6] show that the initial stages of the oxidation of lignin are accompanied by chemiluminescence in the spectra of which there are maxima at wavelengths of 490, 560, and 640 nm which are due to the de-activation of the triplet states of carbonyl groups and of various forms of singlet oxygen. It has been established that in lignin macromolecules, which have a globular form, there is an intensive migration of the energy of electronic excitation, as a result of which the lignin behaves, according to an expression of Swedish workers [7], as a "single chromophore." The most active accepters of energy are aldehydes, ketones, quinones, quinone methides, and stilbenes.

The fact that the lignin chromophores possess photoactivity and are labile under the action of light and atmospheric oxygen acquires particular importance in connection with the setting up of investigations to establish standards for the maximum acceptable concentrations

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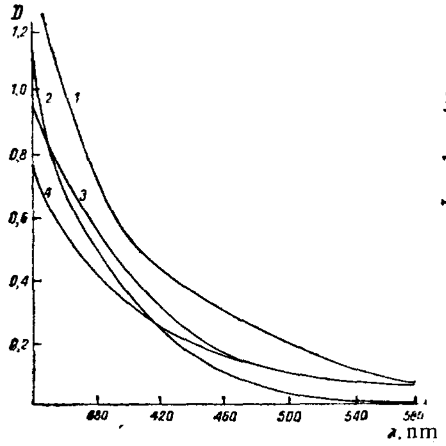

Fig. 1

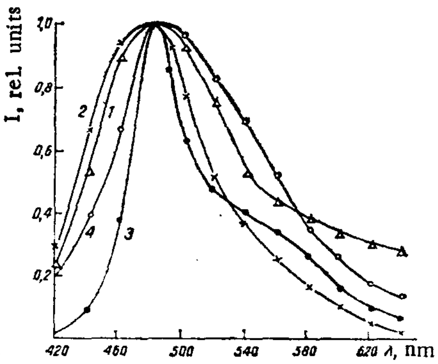

Fig. 2

Fig. 1. Absorption spectra of lignin substances: 1) industrial effluents from final purification stage of BLIC,* $D_{\lambda_{435}} = 0.42$, pH 7.2; 2) water-soluble fraction of sulfate liquors after a cook, $D_{\lambda_{435}} = 0.12$, pH 5.4; 3) chlorolignin, C = 0.2 g/liter, $D_{\lambda_{435}} = 0.25$, pH 7.2; 4) lignosulfonic acid, C = 0.2 g/liter, $D_{\lambda_{435}} = 0.22$, pH 7.2.

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*Bratsk lumber industrial complex

Fig. 2. Fluorescence spectra of chlorolignin (1), of lignosulfonic acid (2), of the water-soluble fraction of sulfate liquors after a cook (3), and of industrial wastes from the final purification stage of BLIC (4).

(MACs) for lignin in water bodies on the discharge of the effluents of pulp and paper factories when sunlight is taken into account. In view of this, it is necessary to determine how deep are the changes under the action of light of the physicochemical properties (photopotential, rate of consumption of oxygen, amount of highly reactive radical products and triplet states formed) of the chromophores of the lignin substances, which effect the toxicity of the effluents.

We have investigated the change in the physicochemical properties of lignin preparations under the action of light in aqueous solutions with pH 7.2-5.4 in the stages preceding far-reaching decomposition.

The absorption spectra of the lignin preparations investigated in the interval from 340 to 580 nm at pH 7.2-5.4 are given in Fig. 1. We were justified in expecting a change in the physicochemical properties of the lignin preparation over the whole range of absorbed light [8], since on passing into an electronically excited state on the absorption of light (or as the result of chemical generation), the chromophores sharply change their reactivities and their amphotericity in redox reactions [9], which leads to the occurrence (or marked acceleration) of processes that are slow or do not go at all in the ground state.

When lignin substances in solutions frozen in liquid nitrogen were excited with the light of a LGI-21 laser, $\lambda_{exc} = 337.5$ nm (region of absorption of carbonyl groups), phosphorescence was observed. The maxima in the phosphorescence spectra of the lignin substances of effluents were located at 480-500 nm (Fig. 2).

Kinetic curves of the quenching of the phosphorescence and their anamorphoses are given in Fig. 3. The lifetimes determined from the slopes of the anamorphoses were: for the water-soluble fraction of sulfate pulps after a cook — 0.36 sec, for chlorolignin — 0.2, for lignosulfates — 0.4 sec, and for the lignin substances of industrial effluents — 0.12 sec. The lifetimes of the chromophores of the lignin preparations correspond to the time characteristics of triplet states [10].

It may be expected that a change in the toxicity of the lignin substances in the illumination stage will be determined by its dependence of the redox properties on the intensity and wavelengths of the incident light which may be determined from the action spectrum of the

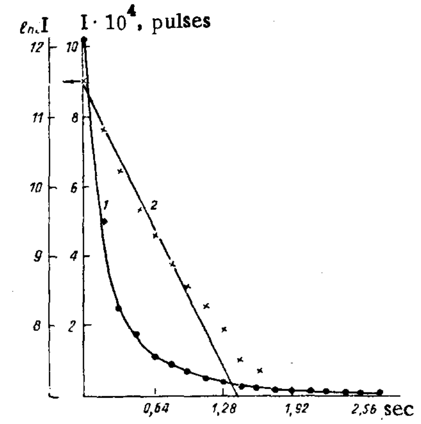

Fig. 3

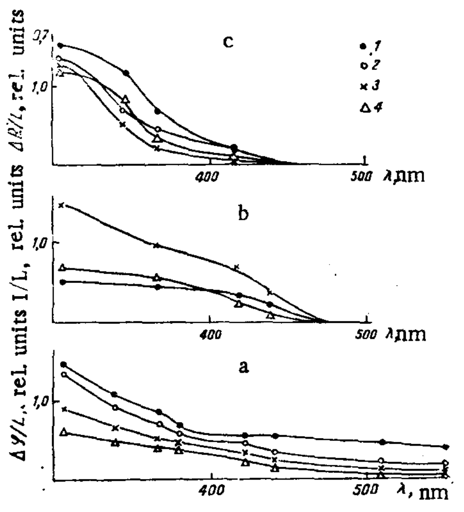

Fig. 4

Fig. 3. Kinetic curve of the quenching of the phosphorescence of the water-soluble fraction of sulfate liquors after a cook (1) and its semilogarithmic anamorphoses (2).

Fig. 4. Action spectra of the photopotential (a), of the absorption of oxygen (b), and of the EPCs of the free radicals (c) of the lignin substances of effluents: 1) chlorolignin; 2) lignosulfonic acids; 3) water-soluble fraction of sulfate liquors after a cook; 4) industrial effluents from the final purification stage of BLIC.

photopotential, the change in the rate and amount of oxygen absorbed, and the action spectrum reflecting the yield of free radicals.

The results showed that when a solution of the lignin substances of effluents (pH 7.2-5.4) was irradiated the photopotential φ increased. In the dark stage, there was a reversible decrease in the photopotential to values close to the initial ones. Depending on the quantum of energy supplied, the maximum acceptable value of $\Delta\varphi$ changed: with an increase in the size of a quantum of excitation the photopotential rose. The rate of accumulation of electrode-active products was also determined by the energy of a quantum. Figure 4a shows action spectra of the photopotential plotted as the dependence on the excitation wavelength of the ratio of the maximum acceptable photopotential to the intensity of the irradiation of the source for a given wavelength. The minimum energy of the appearance of potential-determining products was determined from the "red edge" [11] and was located in the 500-550 nm region, which corresponds to an energy of 2.46-2.24 eV. Thus, under the action of light, the redox properties of the lignin chromophores change with an efficiency which depends on the excitation wavelengths.

A proof of the fact that the photoinitiated oxidation of lignin takes place under the action of light is provided by the results reflecting the dependence of the consumption of oxygen on the wavelengths of the incident light (Fig. 4) and by the marked increase in the rate of consumption of oxygen by various lignin preparations. Below, we give the rate constants (K) of the consumption of oxygen by the lignin substances of the effluents on excitation with an energy E = 2.95 eV ($\lambda_{50\%}$ = 418 nm):

	K, h^{-1}
Industrial effluent from the final purification stage of BLIC	0.06
Water-soluble fraction of sulfate liquors after a cook	0.16
Chlorolignin	0.082

The water-soluble fraction had the highest value of K. The amount and the rate of the absorption of oxygen depended on the quantum of energy supplied.

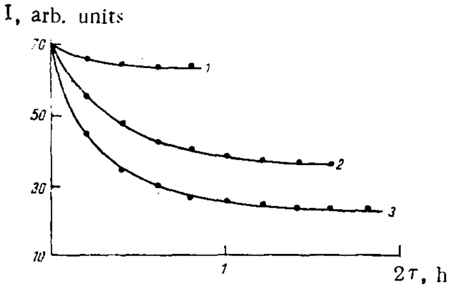

Fig. 5. Change in the rate of consumption of oxygen by the water-soluble fraction of sulfate liquors on irradiation with light having different wavelengths: 1) $\lambda_{50\%}$ = 418 nm; 2) $\lambda_{50\%}$ = 365 nm; 3) $\lambda_{50\%}$ = 308 nm.

Figure 5 shows the kinetic curves of the consumption of oxygen by the water-soluble fractions of sulfate liquors after a cook on irradiation with quanta having different energies. The results obtained unambiguously show that under the action of light in the visible region of the spectrum the amount and rate of consumption of oxygen by the lignin preparations in water bodies rise sharply, which, of course, impairs the oxygen balance of aquatic ecosystems. The most dangerous feature is the presence of the water-soluble lignin fractions in the effluent, since they are characterized by the highest increase in the consumption of oxygen on passing from the dark to the light stage. The "red edge" in the action spectra of the consumption of oxygen was located at 460 nm.

In the photolysis of lignin preparations in effluents, highly reactive free radicals arise, and it may be assumed that these are phenoxyl radicals [12]. The formation of radicals was determined from the disappearance of a label — a stable nitroxyl radical, which does not react with peroxide radicals [13]. The action spectrum of the formation of free radicals on the photolysis of lignin preparations presented in Fig. 4c, show that the highest yield of radicals was observed for the chlorolignins. The "red edge" of the yield of free radicals was located in the 440 nm region. The yield of free radicals rose with an increase in the magnitude of the quantum of supplied energy from 2.8 to 4.1 eV.

On the oxidation of lignin in solution, carbonyl groups in the triplet electron-excited state are also formed, which, as is well known, possess well-marked dehydrogenating properties [14], together with various forms of $^1O_2$ which is an extremely powerful oxidizing agent [15], and, as results obtained recently have shown, the superoxide radical anion $O_2^{-\cdot}$ and $OH^{\cdot}$ [16], which are also toxicants for aquatic organisms [17].

EXPERIMENTAL

The following products were analyzed: chlorolignin obtained by treating sulfate lignin with aqueous solutions of chlorine; lignosulfonic acid isolated from the liquors of the industrial effluents of the Amur pulp and paper combine (TsBK); the water-soluble fraction of sulfolignin isolated from the black liquors of the Bratsk lumber industrial complex (BLIC); and the effluents of the BLIC taken from the last sampling point of the final purification stage before their discharge into water body.

Buffer mixtures were used to prepare solutions of the lignin substances with given pH values [18]. pH values were monitored by means of a MV-88 precision pH-meter. Absorption spectra were obtained on a Specord M-40 instrument. The source of the photoexcitation of the lignin substances in the effluents was a DRSh-500 superhigh-pressure mercury lamp. Cut-off glass filters were used to isolate individual lines of the emission spectrum of the lamp [19]. The photopotentials of the solutions of the lignin substances of the effluents were recorded on a N-306 recorder. Platinum was used as the working electrode, and the comparison electrode was silver chloride. The oxygen in the system was monitored by a polarographic method on a LP-7 instrument with a sealed Clark electrode [20]. Solutions of the lignin substances from the effluents were placed in a thermostated optical cell with a volume of 25 ml and were irradiated. The temperature of the solutions investigated was 298 K.

The formation of radicals in the lignin substances of the effluents on irradiation was determined with the aid of the spin trap method [21]. The stable iminoxyl radical 2,2,6,6-tetramethyl-4-oxopiperidine-oxyl was used as the trap. A mixture of a solution of a lignin substance and the stable radical were placed in a quartz capillary and irradiated. On the irradiation of the solution of the stable radical the concentration of the electron paramagnetic centers (EPCs) did not change during the time interval investigated. The change in the concentration of EPCs was recorded by means of a EPA-2M radiospectrometer. The procedure for calculating the concentration of radicals has been given in [22].

To investigate the phosphorescence of the lignin substances of effluents we used kinetic spectroscopy apparatus designed for measuring the time of extinction and for obtaining time-resolved luminescence spectra (instantaneous spectra). The source of excitation was a LGI-21 nitrogen laser (λ_{exc} = 337.5 nm) with 10-nsec pulses. The frequency of the sequence of pulses was set by a GV-26 pair pulse generator. The radiation fell via a Specol monochromator onto a FEU-79 photoelectron multiplier. The amplified signal was recorded in the memory of a AI-1024 pulse time analyzer. Samples of the solutions of lignin substances to be investigated were taken in a quartz capillary with a diameter of 3 mm, were frozen in a cell containing liquid nitrogen (T = 77°K), and were placed in an optical Dewar for investigation.

SUMMARY

The results obtained unambiguously show that, depending on the light conditions and the types of manufacture which lead to the passage into water bodies of lignin preparations with different initial compositions, a sharp change takes place in their physicochemical properties and reactivity in redox reactions, which is shown in a change in the photopotential, an increase in the amount of oxygen absorbed, and an accumulation of highly reactive free radicals, which, as a result, has an adverse action on hydrobionts.

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