

УДК 577.4,577.346

## **Bioluminescent Monitoring: Detoxification of Phenol Solutions by Humic Substances and UV-irradiation**

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Received 2.06.2008, received in revised form 25.08.2008, accepted 27.08.2008

*The study demonstrates redox activity of humic substances in solutions of organic reducers and oxidizers; a series of homologous 1,4-hydroquinones (phenols) and their oxidized forms (1,4-quinones) was taken as an example. The well-founded interrelation between redox potentials of the quinone-phenols pairs and detoxification coefficients in the humic solutions was ascertained. Photoinduced UV detoxification of the phenol solutions was revealed; combined effect of humic substances and UV irradiation in the phenol solutions was studied as well. Detoxification coefficients were calculated. A conclusion was made that UV-irradiation and treatment by humic substances reveal non-additive but antagonistic effect.*

*Keywords: bioluminescent assay, detoxification, humic substances, UV-irradiation, phenols and quinones.*

### **Introduction**

One of the important tasks of modern ecology and biotechnology is the search of methods to detoxify the environmental toxicants. A possible way to do this is to use Humic Substances (HS) – the products of oxidative transformation of organic matter in soil (Orlov, 1998, 2002; Shin et al., 1999). Toxicity of some compounds was shown to decrease in the presence of HS (Perminova et al., 2001; Gu et al., 2003; Zhilin et al., 2004). The mechanism of detoxification by HS is currently of great interest for researchers (Stom et al., 1977,

2004; Grainer et al., 1999; Vigneault et al., 2000; Perminova et al., 2001; Kulikova et al., 2002; Provenzano et al., 2004).

In (Donnelly et al., 1997; Weinstein et al., 1999), the detoxification effects of HS were studied using classic bioassays involving fish, algae, crustaceans, or plants. Detoxifying effect of HS can be easily evaluated by bacterial bioluminescent assay (Kudryasheva et al., 1998). The advantages here are rapidity, sensitivity, simplicity of analysis, and availability of the

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devices for toxicity registration (Kratasyuk et al., 1987).

Bioluminescent bacteria have been used as a bioassay for almost a half of a century. The bioassay was described in its current form in 1969 (Grabert et al., 1997). In the late 1980s the test was standardized in Germany as a method for pollutants' detection. In 1990 the bioluminescent system of enzymatic coupled reactions was suggested as a toxicity assay (Kratasyuk et al., 1990); its applications were developed (Kudryasheva et al., 2002, 2003). Now, the ecological bioassay is a traditional and important biotechnological application of the bioluminescence phenomenon (Girotti et al., 2008; Gitelson, et al., 2002; Roda et al., 2004; Kudryasheva 2006, 2006b, Lapota et al., 2008; Cali et al., 2008; Vlasova et al., 2007; Reis et al., 2007; Vetrova et al., 2007).

Testing of redox activity of HS in solutions of oxidizers and reducers is of great interest. Reduction ability of HS was supposed to be responsible for detoxification of oxidizers in water solutions (Tchaikovskaya et al., 2006; Fedorova et al., 2007). Phenolic, SH-, and other groups of HS macromolecules are responsible for reduction of the oxidizers.

Testing of oxidative activity of HS in solutions of organic reducers (phenols) is of applied interest. In the present study we chose a series of homologous phenols: tetrafluoro-1,4-hydroquinone, 1,4-hydroquinone, and 1,4-naphthohydroquinone. We compared them with a series of corresponding homologous oxidizers: tetrafluoro-1,4-benzoquinone, 1,4-benzoquinone, 1,4-naphthoquinone, (Fedorova et al., 2007).

In natural conditions, detoxification of solutions by HS might take place under UV and visible light. This is why the study of photoinduced detoxification of pollutants in the presence and absence of HS is of urgent need. In (Tchaikovskaya et al., 2006, 2007), the toxicity of phenol and p-cresol solutions has already been

monitored under combined effect of HS and UV-irradiation.

The aim of the present study was to reveal detoxification of the homologous phenol (1,4-hydroquinones) solutions by HS, UV-irradiation, and combination of HS + UV-irradiation. Bioluminescent system of the coupled enzyme reactions was used as a bioassay.

## Materials and Methods

A series of hydroquinone preparations was used: tetrafluoro-1,4-hydroquinone (Aldrich, 95% purity), 1,4-hydroquinone, and 1,4-naphthohydroquinone (ChimReactiv, Russia, analytical grade).

Humic preparation Humate-80 (OOO "Humate", Irkutsk, Russia) was applied as a source of HS. It was produced by non-extracting treatment of coal with alkali treatment. The preparation includes more than 70% of potassium humate, providing high biological and chemical activity (Levinsky, 2000). Concentrations of HS that inhibit bioluminescence by less than 20% (< 0.3 g/L) were used.

Quartz lamp OUFQ-1 (OOO 'Solnishko', Russia) was applied as a source of UV-irradiation. 1 ml of a phenol solution was irradiated during 30 min in 1x1 cm<sup>2</sup> quartz cell.

Absorption spectra were recorded with a double-beam spectrophotometer UVIKON-943 (KONTRON Instruments, Italy).

Toxicity of the hydroquinone water solutions was assessed by bioluminescent assay based on a NADH:FMN-oxidoreductase-luciferase (R+L) coupled enzyme system from *Photobacterium phosphoreum*. To construct the assay system, 0.1 mg/ml L + R, 5·10<sup>-4</sup> M FMN, 10<sup>-4</sup> M NADH, and 0.002% tetradecanal solutions were used. The assay was performed in 0.1 M phosphate buffer (pH 6.8) at room temperature.

Maximal luminescent intensity was determined in control (without hydroquinones

–  $I_{contr}$ ) and experimental ( $I$ ) samples. Relative intensity  $I^{rel} = I / I_{contr}$  was used to evaluate the effect of the hydroquinones on bioluminescence. Dependence of  $I^{rel}$  on hydroquinone concentration was developed. Hydroquinones' concentration inhibiting bioluminescence to 40% of the control intensity ( $C_{40}$ ) was chosen. It was used in the experiments for studying the detoxification processes in hydroquinone solutions.

Symbols of bioluminescence intensity ( $I^{rel}$ ) in solutions of the hydroquinones under treatment by HS, UV-irradiation, and HS+UV were:  $I_{HS}^{rel}$ ,  $I_{UV}^{rel}$ , and  $I_{HS+UV}^{rel}$ , respectively. Detoxification coefficients  $K_{HS}$ ,  $K_{UV}$  and  $K_{HS+UV}$  were calculated as the intensity ratio in the presence and the absence of detoxification factor, with the latter equaling 0.4 ( $I^{rel} = 0.4$ ):

$$K_{HS} = I_{HS}^{rel} / I^{rel} = I_{HS}^{rel} / 0.4 \quad (1)$$

$$K_{UV} = I_{UV}^{rel} / I^{rel} = I_{UV}^{rel} / 0.4 \quad (2)$$

$$K_{HS+UV} = I_{HS+UV}^{rel} / I^{rel} = I_{HS+UV}^{rel} / 0.4 \quad (3)$$

## Results and discussion

Dependence of  $I^{rel}$  on hydroquinone concentration was investigated in the solutions of tetrafluoro-1,4-hydroquinone, 1,4-hydroquinone, and 1,4-naphthohydroquinone. Fig. 1 presents the experimental dependence with 1,4-naphthohydroquinone taken as an example. The figure shows the decay of the experimental curve. The phenol concentration that inhibited bioluminescence to 40% of the control intensity ( $C_{40} = 2.5 \cdot 10^{-5} M$ ) is shown in this figure, as well.

### 1. Detoxification by HS

Detoxification ability of HS was compared in solutions of 1,4-hydroquinones and corresponding 1,4-quinones. Fig. 2 presents these redox quinone-hydroquinone pairs. Their standard redox potentials are given in Table 1a.

Table 1 a, b demonstrates  $K_{HS}$  in the solutions of 1,4-hydroquinones and corresponding 1,4-quinones. It is evident that  $K_{HS}$ -values are in a

direct dependence on  $E^0$ -values in the series of both, hydroquinones and quinones. This reveals HS' reductive (antioxidant) activity in the solutions of the organic oxidizers, as well as HS' oxidative activity in the solutions of organic reducers.

### 2. UV-photoinduced detoxification

Effect of UV-irradiation of phenol solutions was studied. Table 1b presents  $K_{UV}$  values in the hydroquinone solutions. They appeared to be  $>1$ , indicating photoinduced detoxification of the solutions. In addition, the values of  $K_{UV}$  were similar for the solutions of all three phenols (Table 1b).

Fig. 3a-c presents the absorption spectra of 1,4-hydroquinones before and after UV-irradiation. It is seen that the optical density (O.D.) of tetrafluoro-1,4-hydroquinone solution increases under UV-irradiation in the region of the spectral maximum (260-290 nm) (Fig. 3a). Detoxification of the compound was probably due to its dehalogenation; this process can be highly effective in haloid organic compounds under UV-irradiation (Shigorin et al., 1993). UV maximum of 1,4-hydroquinone (Fig. 3b) does not change significantly under UV-treatment. However, wide and structureless band appears in a visible wavelength range of 350-600 nm. Usually, this band is attributed to the charge-transfer complexes in solutions of 1,4-benzoquinone and 1,4-hydroquinone. Fig. 3c shows the changes in spectra of 1,4-hydronaphthoquinone. The optical density of spectrum 2 does not reach zero in the visible spectral range, thus revealing the contribution of light scattering to this spectrum. It seems to be a result of the formation of hydrophobic aggregates in the process of photolysis of 1,4-hydronaphthoquinone. Thus the changes in the absorption spectra of solutions of 1,4-hydroquinones are an evidence of photochemical transformation of these phenols in the solutions.

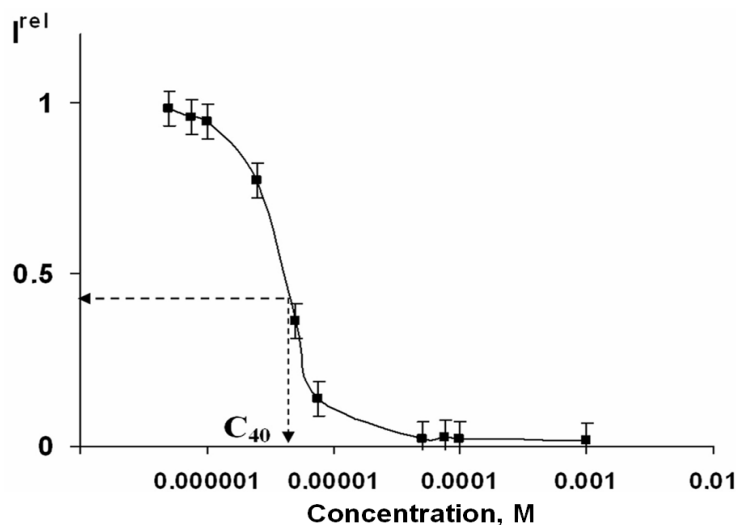


Fig. 1. Bioluminescence intensity vs. concentration of 1,4-naphthohydroquinone

Table 1. Detoxification of solutions: (a) 1,4-quinones (Fedorova et al., 2007) (b) 1,4-dihydroquinones.  
 $K_{HS}$  – detoxification coefficient under HS treatment,  $K_{UV}$  – detoxification coefficient under UV-irradiation,  
 $K_{HS+UV}$  – detoxification coefficient under combined effect of HS and UV-irradiation,  $E^0$  – standard redox potential  
of quinone-hydroquinone pairs,  $C_{40}$  – phenol concentration inhibiting bioluminescence to 40% of control intensity

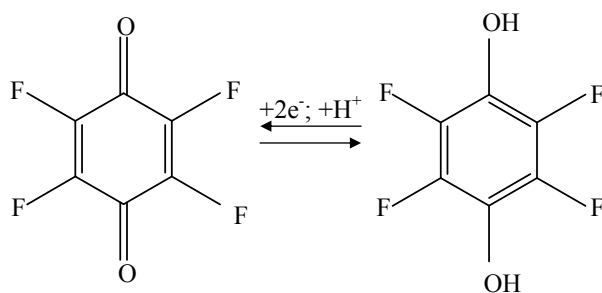
(a)

quinones	tetrafluoro-1,4-benzoquinone	1,4-benzoquinone	1,4-napthoquinone
$C_{40}, M$	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$4 \cdot 10^{-4}$
$K_{HS}$	$2.11 \pm 0.03$	$1.73 \pm 0.02$	$0.92 \pm 0.02$
$E^0, V$	0.838	0.712	0.480

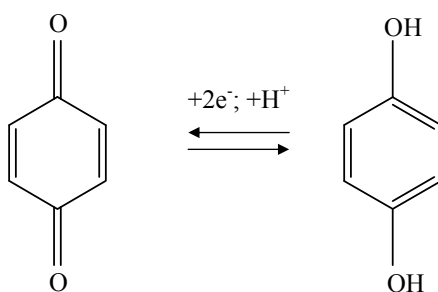
(b)

phenols	tetrafluoro-1,4-hydroquinone	1,4-hydroquinone	1,4-naptho-hydroquinone
$C_{40}, M$	$6 \cdot 10^{-7}$	$2.5 \cdot 10^{-2}$	$2.5 \cdot 10^{-5}$
$K_{HS}$	$1.00 \pm 0.04$	$1.18 \pm 0.03$	$2.10 \pm 0.02$
$K_{UV}$	$1.4 \pm 0.04$	$1.25 \pm 0.02$	$1.30 \pm 0.02$
$K_{HS+UV}$	$1.7 \pm 0.04$	$1.01 \pm 0.02$	$1.46 \pm 0.03$

a)



b)



c)

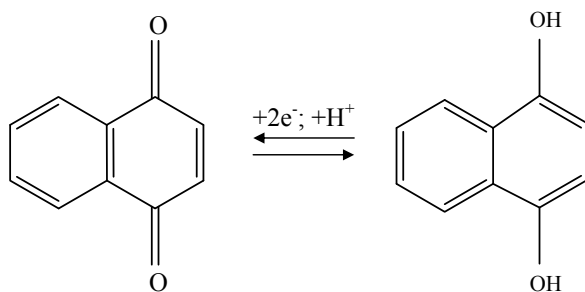


Fig. 2. Redox pairs quinone-phenol: (a) tetrafluoro-1,4-benzoquinone – tetrafluoro-1,4-hydroquinone, (b) 1,4-benzoquinone – 1,4-hydroquinone, (c) 1,4-naphthoquinone – 1,4-naphthohydroquinone

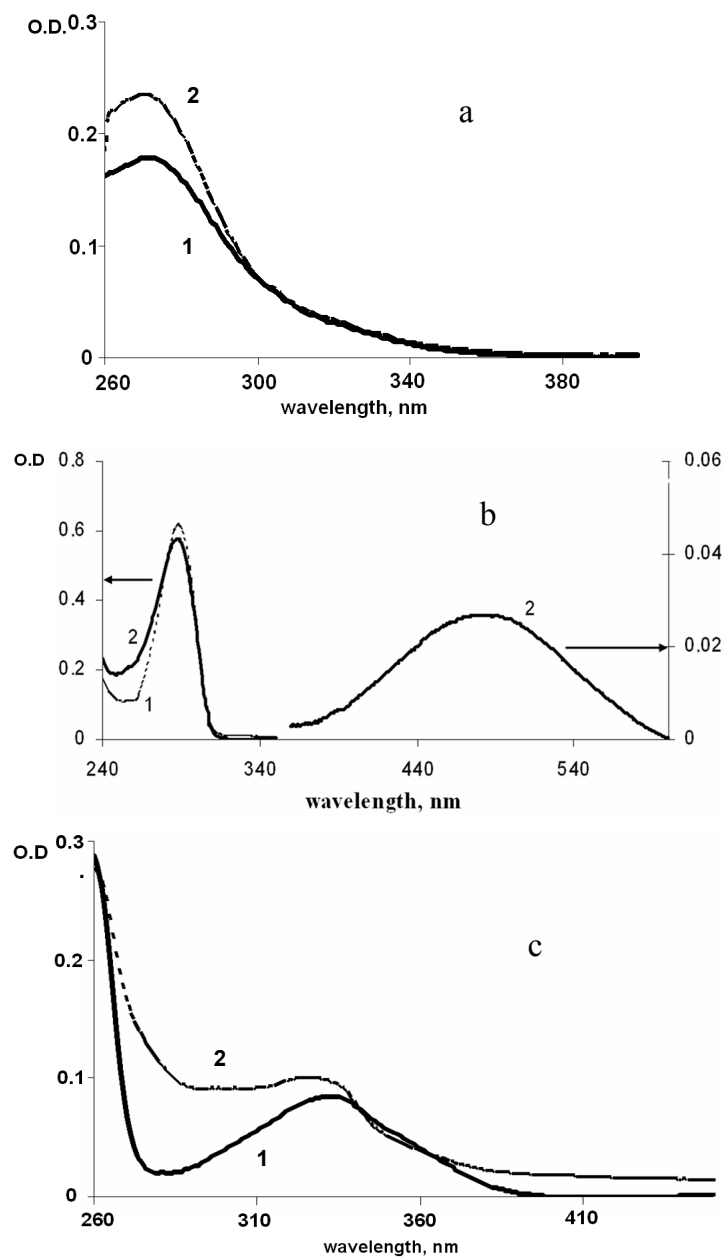


Fig. 3. Absorbance spectra of phenol solutions: (1) before UV-irradiation, (2) after UV-irradiation: (a) tetrafluoro-1,4-hydroquinone ( $C = 6 \cdot 10^{-7} M$ ); (b) 1,4-hydroquinone ( $C = 1.25 \cdot 10^{-3} M$ ); (c) 1,4-naphthohydroquinone ( $C = 2.5 \cdot 10^{-5} M$ )

### 3. Combined effect of HS and UV-irradiation

Table 1b presents  $K_{HS+UV}$  of 1,4-hydroquinones. Comparison of  $K_{HS+UV}$ ,  $K_{HS}$ , and  $K_{UV}$  values brings to conclusion that the combined effect of the two detoxifying factors (HS+UV) is not additive. The complex photoinduced processes taking place in quinone solutions in the presence of HS might account for this fact. Non-additivity of HS and UV effects was already found in the solutions of phenol and p-cresol (Tchaikovskaya et al., 2006, 2007).

### Conclusion

The effects of two detoxifying factors (HS and UV-irradiation) as well as the combined effects (HS+UV) on solutions of 1,4-hydroquinones were studied. Detoxification coefficients were calculated.

The reduction activity of HS was demonstrated in the solutions of organic oxidizers – 1,4-quinones, while the oxidative activity

of HS was demonstrated in the solutions of organic reducers – 1,4-hydroquinones (phenols). The interrelations between redox-potentials of quinone-hydroquinone pairs and detoxification coefficients in solutions of 1,4-quinones and 1,4-hydroquinones were revealed.

Photoinduced detoxification of 1,4-hydroquinones solutions was demonstrated. UV-irradiation and treatment by HS revealed non-additive but antagonistic effect in the phenol solutions.

### Acknowledgements

The work was supported by: Award No. RUX0-002-KR-06 of the U.S. Civilian Research & Development Foundation (CRDF) and RF Ministry of Education and Science, BRHE Program; Grant “Molecular and Cellular Biology”, program of Russian Academy of Sciences; Innovation program for young scientists of Siberian Federal University.

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