ISSN 1070-3632, Russian Journal of General Chemistry, 2017, Vol. 87, No. 7, pp. 1476–1480. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © E.A. Kalinichenko, A.V. Kalinichenko, I.D. Odaryuk, L.V. Kanibolotskaya, A.N. Shendrik, 2017, published in Zhurnal Obshchei Khimii, 2017, Vol. 87, No. 7, pp. 1082–1087.

Emitters of Chemiluminescence Occurring During Autoxidation of Substituted Hydroquinones in Water

E. A. Kalinichenko*, A. V. Kalinichenko, I. D. Odaryuk, L. V. Kanibolotskaya, and A. N. Shendrik

Donetsk National University, ul. 600-Letiya 21, Vinnitsa, 21021 Ukraine
**e-mail: e.a.kalinichenko@gmail.com*

Received November 3, 2016

Abstract—A mathematical processing method for determination of spectral parameters of chemiluminescence emitters during the autoxidation of phenolic compounds in aqueous-alkaline media has been developed. The presence of a single luminescence emitter (the corresponding *p*-benzoquinone in the triplet state) has been demonstrated in the hydroquinone–oxygen–water system. The emitters spectra have been obtained.

Keywords: chemiluminescence, emitter, hydroquinones, autoxidation

DOI: 10.1134/S1070363217070052

The liquid-phase oxidation of phenolic compounds (including biologically important polyphenols and quinones: pyrocatechol, adrenalin, *p*-benzoquinone, pyrogallol, and gallic acid) with molecular oxygen in an aqueous-alkaline medium is accompanied by a super-weak chemiluminescence [1–6]. The appearance of the chemiluminescence emitter (*p*-benzoquinone) in the reaction between peroxyl and phenoxyl radicals has been detected during the inhibition of the liquid-phase oxidation of alkylbenzenes [7]. Being a primary product of the hydroquinone autoxidation in an alkaline medium (along with hydrogen peroxide), *p*-benzoquinone acts as the chemiluminescence emitter in this process as well [8]. Analysis of the published data has revealed that the spectral parameters of chemiluminescence emitters formed upon oxidation of polyphenols have been scarcely (if at all) studied.

This work aimed to investigate the spectral parameters of emitters of chemiluminescence occurring during autoxidation of hydroquinones in aqueous-alkaline media.

Oxidation of hydroquinone (*p*-QH_2), 2-chlorohydroquinone ($ClQH_2$), and 2-methylhydroquinone ($MeQH_2$) solutions with molecular oxygen was accompanied by chemiluminescence exhibiting a single maximum after the induction period (Fig. 1). This is due to the accumulation of intermediate products accelerating the process. The corresponding benzoquinone is likely to be such intermediate. That fact was confirmed by the shortening of the induction period after addition of *p*-benzoquinone (*p*-Q) at the initial stage of hydroquinone oxidation [6].

The formation of the radicals in the aqueous-alkaline hydroquinone–oxygen system can be explained by electron transfer from anionic forms of hydroquinones to oxygen or the corresponding benzoquinone [1, 3, 9, 10].

$$HQ^- + O_2 \xrightarrow{k_{1a}} Q^{\cdot -} + O_2^{\cdot -} + H^+, \quad (1a)$$

$$Q^{2-} + O_2 \xrightarrow{k_{1b}} Q^{\cdot -} + O_2^{\cdot -}, \quad (1b)$$

$$HQ^- + Q \xrightarrow{k_2} 2Q^{\cdot -} + H^+. \quad (2)$$

Here, HQ^-, Q^{2-}, and $Q^{\cdot -}$ are anionic and semiquinone forms of hydroquinone.

The formed semiquinone radicals $Q^{\cdot -}$ are in the acid-base equilibrium with the protonated form $HQ^{\cdot}$. The dissociation constant of semiquinone radical and its methyl derivatives can be expressed as $pK_a = 4.1 + 0.25n$, with n being the number of methyl groups [11]. The single-electron transfer to oxygen gives rise to superoxide radical, which is in the acid-base equilibrium with pK_a 4.7 [11] or 4.8 [12]. In a weakly alkaline solution, hydroquinone and its derivatives partially undergo the first stage of dissociation, since pK_{a1} for these compounds are as follows: 8.90 for $ClQH_2$ [13], 9.91 for *p*-QH_2 [14], and 10.05 for $MeQH_2$ (at the ionic strength 0.65 mol/L) [13]. The participation of

superoxide anion-radical was confirmed by the effect of addition of superoxide dismutase (EC 1.15.1.1), and *p*-Q and H_2O_2 are the only stable primary products of autoxidation of hydroquinone. Accumulation of these products at the first stage of the reaction was symbate [8].

The observed chemiluminescence could be due to the relaxation of electron-excited form of benzoquinones (Q*) being formed via the recombination of the semiquinone radical with superoxide anion-radical [6] or hydroxyl radical [8].

$$Q^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow Q^* + H_2O_2, \quad (3a)$$

$$Q^{\cdot -} + HO^{\cdot} + H^+ \rightarrow Q^* + H_2O, \quad (3b)$$

$$Q^* \xrightarrow{k_4} Q + h\nu. \quad (4)$$

Quinones are formed via the laccase-catalyzed oxidations of phenols as well. The enzymatic process also includes the stage of electron transfer from the hydroquinones to oxygen yielding semiquinone radicals [15–17]. In this case, oxygen is reduced directly into water rather than into hydrogen peroxide in the case of the autoxidation.

The products of the enzymatic oxidation were identified by sampling the reaction mixture and comparison of its retention time and spectra with pure hydroquinones and quinones. Using the *p*-QH_2/*p*-Q pair as an example, we demonstrated the coincidence of the retention time and absorbance spectra of the individual compounds (Fig. 2). The accumulation of the corresponding *p*-benzoquinones (*p*-Q, MeQ, and ClQ) in the products of cooperative enzymatic oxidation of three hydroquinones with laccase was detected by means of chromatography. Consumption

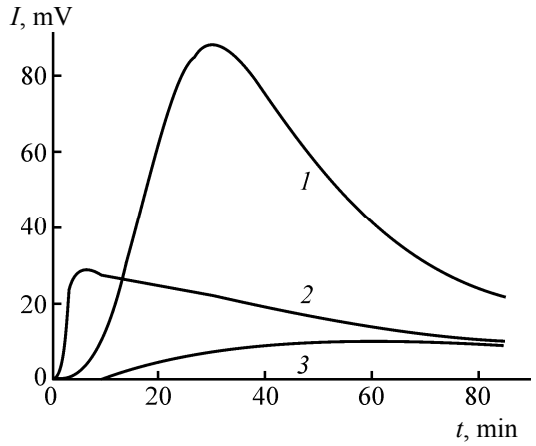

Fig. 1. Kinetic curves of chemiluminescence in the reaction of autoxidation of *p*-QH_2 (*1*), $ClQH_2$ (*2*), and $MeQH_2$ (*3*). c_0 = 5 mmol/L, pH = 8.0, T = 308 K.

of the hydroquinones and accumulation of *p*-benzoquinones were monitored by photometry at 290 and 245 nm, respectively (Fig. 3).

UV spectra of benzoquinones obtained via enzymatic oxidation of hydroquinones were identical to the spectra of the reaction mixtures sampled during autoxidation of those phenols. Hence, it could be assumed that the excited state of quinone formed via the autoxidation of the hydroquinones was the luminescence emitter.

The use of diffraction slits with a photomultiplier was not efficient for the investigation of spectral parameters of the emitter in the studied reaction due to low signal to noise ratio (i.e. superweak chemiluminescence). To elucidate the nature of the emitter in the reaction of the hydroquinones autoxidation, we

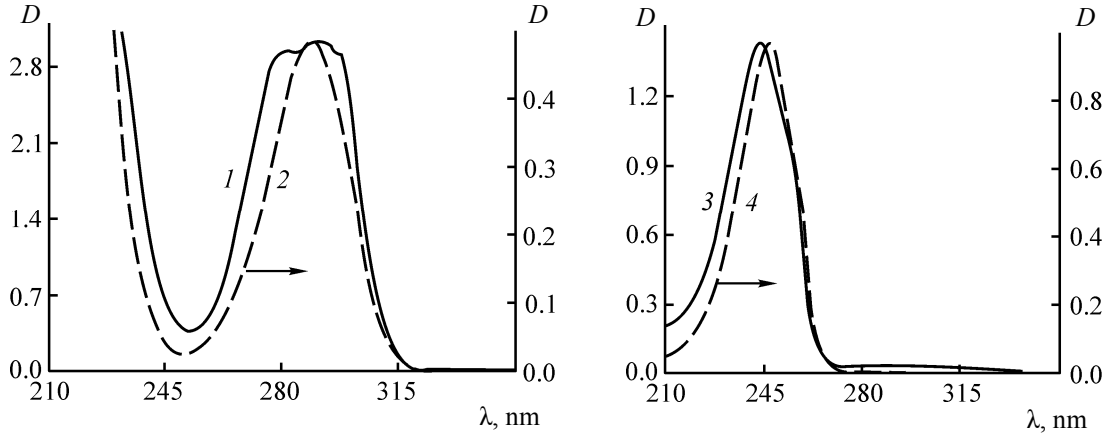

Fig. 2. UV absorbance spectra of the reaction mixture in the maximum of the chromatographic peak at t_R 3.66 (*1*) and 5.18 min (*3*), of 1.92×10^{-4} mol/L of hydroquinone solution (*2*), and of 4.7×10^{-5} mol/L *p*-benzoquinone solution (*4*).

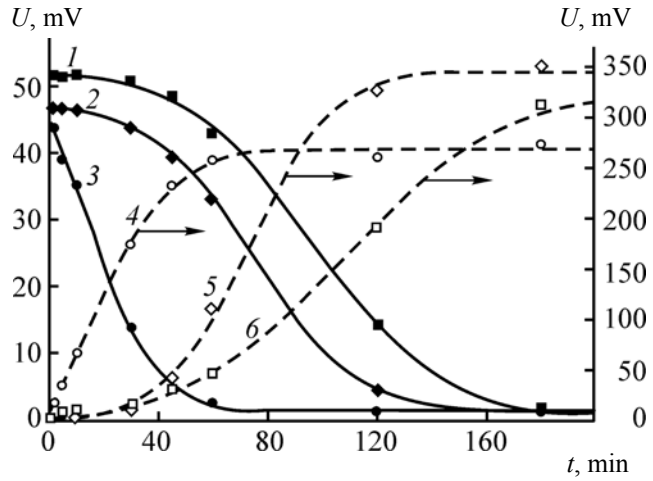

Fig. 3. Curves of simultaneous consumption of $ClQH_2$ (*1*), *p*-QH_2 (*2*), and $MeQH_2$ (*3*) and of accumulation of their enzymatic oxidation products MeQ (*4*), *p*-Q (*5*), and ClQ (*6*). c_0 = 2 mmol/L, laccase activity 0.27 units.

measured the integral intensities of the emission over the narrow spectral ranges limited by the color filters. The obtained data were processed as follows. Sensitivity of photocathode was conveniently expressed as the quantum yield K_λ (the ratio of the number of emitted electrons to that of the quants absorbed at each wavelength). Quantum yield of a FEU-38 photomultiplier as a function of the wavelength is found in Ref. [18]. Since the spectral response of FEU-38 within the range of 400–800 nm, the latter was used for the spectral data analysis. To account for the sensitivity of the photomultiplier for the used color filters, each of the light transmission values of the discrete set $T_{LF,\lambda1}$, $T_{LF,\lambda2}$, …, $T_{LF,\lambda n}$ (recorded with 1 nm step) should be multiplied by the corresponding $K_{\lambda i}$ value.

Certain wavelengths λ_i corresponding to higher transmission values $T_{\lambda i}$ should have higher statistical weight, and it was reasonable to use the weight-average value (λ^*) for the λ_1, λ_2,..., λ_n set, determined with Eq. (5).

$$\lambda^* = \frac{\Sigma_{i=1}^{n} \lambda_i K_{\lambda i} T_{\lambda i}}{\Sigma_{i=1}^{n} K_{\lambda i} T_{\lambda i}}. \quad (5)$$

Here, $T_{\lambda i}$ and $K_{\lambda i}$ are the light transmittance and the photomultiplier quantum yield at wavelength λ_i.

We obtained the kinetic curves of chemiluminescence during autoxidation of hydroquinones (Fig. 4a) using the color filters with different spectral characteristics (Fig. 4b) installed between the reactor and the photomultiplier. The similar shape of the chemiluminescence curves suggested the presence of a single luminescence emitter in the system, and the ratio between the emission intensities with the color filter (I^{LF}) and without it (I) could be used as the parameter of the intensity in the chemiluminescence spectrum (L).

Emission spectra of the chemiluminescence during autoxidation of hydroquinones were fitted with Gaussian function (Fig. 5). Their maxima for *p*-QH_2, $MeQH_2$, and $ClQH_2$ were found in the green range of the spectrum (see the table). Since the average energy of the excited state of the emitter was strongly correlated with the $T_1 \rightarrow S_0$ transition energy of *p*-benzoquinone (222 kJ/mol) [9], we suggested that the corresponding *p*-benzoquinone molecule in the electron-excited triplet state was the luminescence emitter during autoxidation of the hydroquinones.

In summary, the corresponding *p*-benzoquinone in the triplet state is the only luminescence emitter during autoxidation of hydroquinones in aqueous media. The developed method of the spectral data processing allows determination of the position of the emitter chemiluminescence band for the systems without any chemiluminescence promotor, and it can be used for spectral study of other systems exhibiting superweak emission.

EXPERIMENTAL

Hydroquinone (99%, Aldrich), 2-chlorohydroquinone (85%, Aldrich), 2-methylhydroquinone (98%, Aldrich), and *p*-benzoquinone ("chemical pure") were purified by vacuum sublimation.

Kinetic studies of autoxidation of 5 mmol/L solutions of the hydroquinones with molecular oxygen were performed at 308 K and atmospheric pressure in 0.1 mol/L phosphate buffer (pH 8.0) prepared from $Na_2HPO_4 \cdot 2H_2O$ (≥99%, Fluka) and $NaH_2PO_4 \cdot H_2O$ (≥98%, Sigma Aldrich) used as received [19]. The solutions were prepared just before the experiment using deionized water with resistance of at least 18.2 MΩ cm.

Chemiluminescence intensity was measured using a FEU-38 photomultiplier. The signal was amplified with an elecrometric amplifier and postprocessed using an L-305 analog-to-digital converter (LCARD, Russia). The data collection and processing were performed

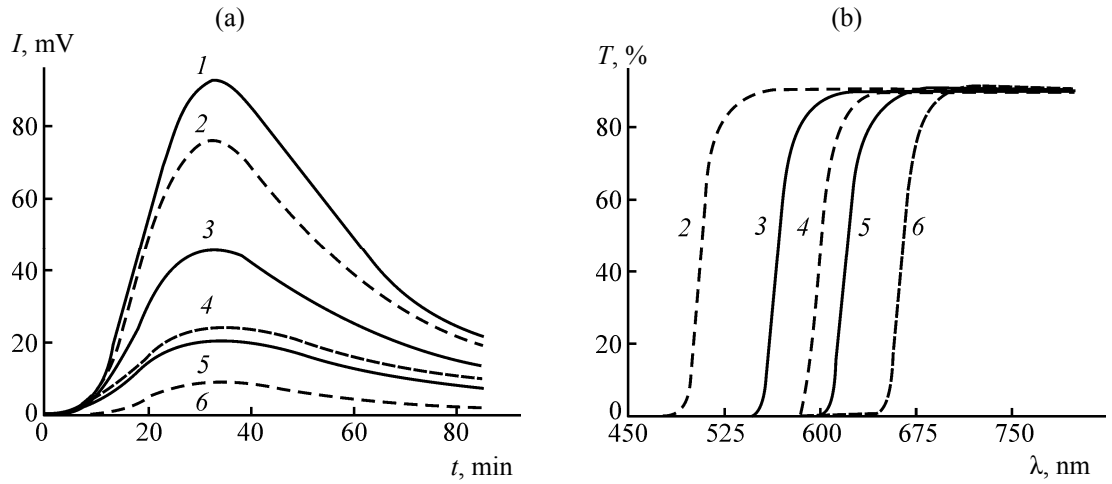

Fig. 4. (a) Kinetic curves of luminescence intensity during autoxidation of hydroquinone solution (c_0 = 5 mmol/L, pH = 8.0, T = 308 K) without color filter (*1*) and with ZhS-18 (*2*), OS-13 (*3*), KS-10 (*4*), KS-11 (*5*), and KS-17 (*6*) color filters; (b) light absorbance spectra of the color filters.

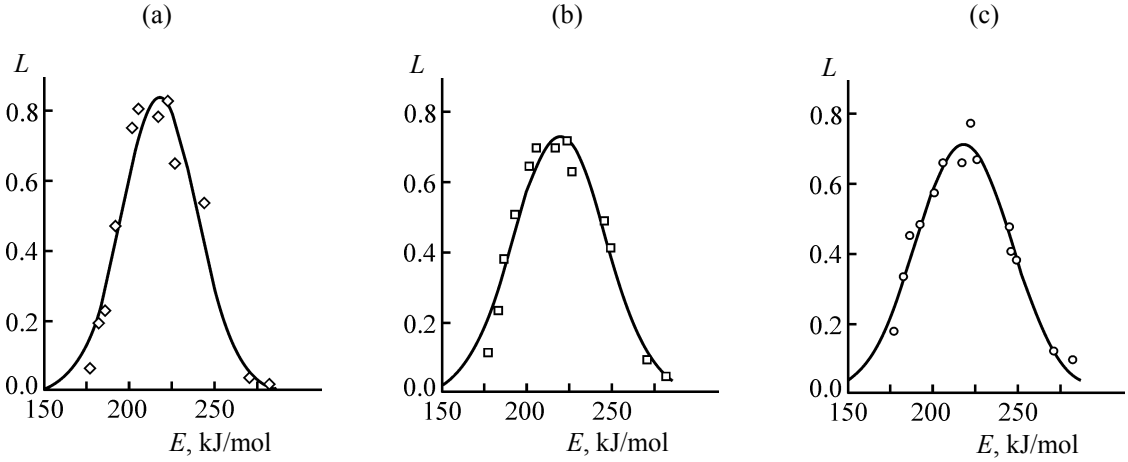

Fig. 5. Emission bands of chemiluminescence during autoxidation of *p*-QH_2 (a), $ClQH_2$ (b), and $MeQH_2$ (c).

using PowerGraph v.2.1 software. The kinetic regime of the reaction was ensured by the choice of the stirring rate and air feed. Kinetic curves of chemiluminescence were corrected for the measured average dark current ($I = I_{exp} - I_0$).

To determine the position of the chemiluminescence maximum, a set of 100 standard color filters (according to GOST 9411-91 for optical colored inorganic glass) was used, the filter was installed between the reactor and the photomultiplier. Spectral characteristics of the color filters were measured using an SF-2000 spectrophotometer (Russia) over the 200–1100 nm wavelength range.

Enzymatic oxidation of hydroquinones was performed by bubbling air at atmospheric pressure and 35°C through a mixture based on citrate buffer system (pH 4.6) prepared from as-received sodium citrate dihydrate (≥99%, Merck) and citric acid monohydrate (≥99.9998%, Fluka) [19]. 0.2 mL of a solution of the substrate in acetonitrile and 4.8 mL of a laccase solution in the buffer system (EC 1.10.3.2, *Trametes*

Energy parameters of the $T_1 \rightarrow S_0$ transition for *p*-benzoquinones (phosphate buffer, pH = 8.0, T = 308 K, confidence interval 3σ)

Substrate	E_{max}, kJ/mol	λ_{max}, nm
p-QH_2	218±6	548±14
$MeQH_2$	219±3	547±8
$ClQH_2$	220±4	545±10

versicolor, Sigma Aldrich) were introduced in the reactor. The enzyme activity in the reaction medium was 0.27 U. Inactivation of laccase and inhibition of the autoxidation was performed by transferring a sampled aliquot of the mixture in 1 mol/L solution of HNO_3 (TraceSelect®, Fluka).

Chtomatographic separation was performed using a NUCLEOSIL® 100-5 C_{18}HD column (Macherey-Nagel) with length 250 mm and inner diameter 4.0 mm equipped with the corresponding precolumn (eluent flow rate 0.8 cm^3/min, column thermostat temperature 40°C, injected volume 10 mm^3; linear gradient elution: 0–8 min from 85 to 50%, 8–10 min from 50 to 40% of the mobile aqueous phase). To prepare the mobile aqueous phase, 1.50 g of sodium octanesulfonate monohydrate (≥97.0%, Aldrich) and 2.50 g of KH_2PO_4 (≥99.5%, Merck) were dissolved in 960 cm^3 of water. pH of the solution was adjusted to 2.5 with orthophosphoric acid (≥85.0%, Merck) monitoring the pH with a HANNA HI9813 pH-meter, and then 40 cm^3 of acetonitrile (≥99.9%, CHROMASOLV®, for HPLC) was added. The prepared solution was thoroughly stirred and filtered through a Supelco Nylon66 Membranes 0.45 μm membrane filter. The buffer was freshly prepared before each experimental series. Acetonitrile was used as the mobile organic phase. The absorbance spectra were recorded at 190–700 nm using an SPD-M20A diode matrix detector of an LC-20AD Prominence high-performance liquid chromatograph (Shimadzu, Japan). Retention time for (t_R) hydro- and benzoquinones *p*-QH_2, $MeQH_2$, *p*-Q, $ClQH_2$, MeQ, and ClQ were 3.66, 4.62, 5.18, 5.71, 7.33, and 7.93 min, respectively.

UV absorbance spectra of solutions of pure hydroquinone and *p*-benzoquinone (200–350 nm) were obtained using a SPECORD S 300 spectrophotometer.

ACKNOWLEDGMENTS

Authors acknowledge the assistance in the study from the management of Luks company (Donetsk) and the head of laboratory N.N. Skripka.

REFERENCES

1. Brunmark, A. and Cadenas, E., *Free Rad. Biol. Med.,* 1989, vol. 7, p. 435. doi 10.1016/0891-5849(89)90126-3
2. Zhang, L., Bandy, B., and Davison, A.I., *Free Rad. Biol. Med*., 1996, vol. 20, p. 495. doi 10.1016/0891-5849(95)02089-6
3. Roginsky, V. and Barsukova, T., *J. Chem. Soc. Perkin Trans*., 2000, vol. 2, p. 1575. doi 10.1039/B000538J
4. Roginsky, V.A., Pisarenko, L.M., Bors, W., and Michel, C., *J. Chem. Soc. Perkin Trans*., 1999, vol. 2, p. 871. doi 10.1039/A807650B
5. Jarabak, R., Harvey, R.G., and Jarabak, J., *Arch. Biochem. Biophys*., 1997, vol. 339, p. 92. doi 10.1006/abbi.1996.9858
6. Shendrik, A.N., Odaryuk, I.D., Kanibolotska, L.V., Kalinichenko, E.A., Tsyapalo, A.S., Beznos, V.V., and Kanibolotsky, A.L., *Int. J. Chem. Kinet*., 2012, vol. 44, no. 6, p. 414. doi 10.1002/kin.20592
7. Shendrik, A.N., Kachurin, I.O., and Opeida, I.A., *Theor. Exp. Chem*., 1994, vol. 30, p. 85. doi 10.1007/BF00530592
8. Shendrik, A.N., Kanibolotskaya, L.V., and Odaryuk, I.D., *Ukr. Khim. Zh.*, 2009, vol. 75, nos. 11–12, p. 90.
9. Schafer, Fr.Q. and Buetner, G.R., *Free Rad. Biol. Med*., 2001, vol. 30, p. 1191. doi 10.1016/S0891-5849(01)00480-4
10. Dohrmann, J.K. and Bergman, B., *J. Phys. Chem*., 1995, vol. 99, p. 1218. doi 10.1021/j100004a023
11. Buettner, G.R., *Arch. Biochem. Biophys*., 1993, vol. 300, no. 2, p. 535. doi 10.1006/abbi.1993.1074
12. Benrahmoune, M., Therond, P., and Abedinzaden, Z., *Free Rad. Biol. Med*., 2000, vol. 29, no. 8, p. 775. doi 10.1016/S0891-5849(00)00380-4
13. Akutagawa, T. and Saito, G., *Bull. Chem. Soc. Japan*, 1995, vol. 68, no. 7, p. 1753. doi 10.1246/bcsj.68.1753
14. Speight, J.G., *Lange's Handbook of Chemistry*, New York: McGraw-Hill, 2005, p. 1623.
15. Kunamneni, A., Camarero, S., Garcia-Burgos, C., Plou, F.J., Ballesteros, A., and Alcalde, M., *Microbiol. Cell Fact*., 2008, vol. 7, no. 32, p. 1. doi 10.1186/1475-2859-7-32
16. Guillén, F., Muñoz, C., Gómez-Toribio, V., Martínez, A.T., and Martínez, M.J., *Appl. Environ. Microbiol*., 2000, vol. 66, no. 1, p. 170. doi 10.1128/AEM.66.1.170-175.2000
17. Morozova, O.V., Shumakovich, G.P., Shleev, S.V., and Yaropolov, A.I., *Appl. Biochem. Microbiol*., 2007, vol. 43, no. 5, p. 523. doi 10.1134/S0003683807050055
18. Shlyapintokh, V.Ya., Karpukhin, O.N., Postnikov, L.M., Zakharov, I.V., Vichutinskii, A.A., and Tsepalov, V.F., *Khemilyuminestsentnye metody issledovaniya medlennykh khimicheskikh protsessov* (Chemiluminescent Methods for Studying Slow Chemical Processes), Moscow: Nauka, 1966.
19. *Data for Biochemical Research*, Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, M.K., Eds., Oxford: Oxford University Press, 1986.