3.2. Posters

P01 DECOMPOSITION OF ALKALINE COPPER (II) BY HYDROGEN PEROXIDE IS ACCOMPANIED BY FORMATION OF SINGLET OXYGEN

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Introduction

Interaction of copper(II) with hydrogen peroxide in neutral and mildly acidic solution has been a subject of many biochemical studies because of its ability to induce various biological damages. Much less information is available concerning catalytical decomposition of hydrogen peroxide by copper salt in alkaline medium, although this reaction is a popular source of reactive oxygen species in chemiluminescence (CL). The optimum pH for luminol chemiluminescence is 11.6, if more alkaline mixtures are used, less common chemiluminescence reactions e. g. that of xanthene dyes can be elicited. It is well known[1] that the decomposition of hydrogen peroxide in alkaline medium starts with the reduction of Cu^{2+} according to the Equation:
$Cu^{2+} + HOO^- \rightarrow Cu^+ + O_2^{\cdot -} + H^+$ (Eq. 1). Hydrogen peroxide can further react with Cu^+ via classical Fenton – like mechanism: $Cu^+ + HOO^- \rightarrow Cu^{2+} + O^{\cdot -} + OH^-$ (Eq. 2). The presence of hydroxyl radical in the $Cu(OH)_2$–H_2O_2 reaction mixture was indirectly proved by EPR[2].

Simultaneous presence of superoxide and hydroxyl radical should lead to generation of singlet oxygen: $O_2^{\cdot -} + O^{\cdot -} + H_2O \rightarrow {}^1O_2 + 2\ OH^-$ (Eq. 3). Furthermore, alternative pathway $Cu^{2+} + O_2^{\cdot -} \rightarrow Cu^+ + {}^1O_2$ (Eq. 4) for singlet oxygen formation is suggested in[3], based on the observation of the ECL emission accompanying electrochemical generation of superoxide in the presence of copper salt. Singlet oxygen is detectable by its dimolar chemiluminescence emission (λ_{max} = 650 nm). However in the same work the authors state the emission from the above reactions is too low to be observed using conventional apparatus, if 3.10^{-4} M Cu^{2+}, 0.05 M NaOH and 0.5 M H_2O_2 is used. We have found that well measureable emission can be observed at lower hydrogen peroxide concentrations.

Experimental

M a t e r i a l s

Sodium hydroxide (Lachema Brno Czech Republic), hydrogen peroxide (Sigma). Copper sulphate (Sigma) was recrystalized from hot water with the addition of small amount of nitric acid in order to remove traces of iron.

M e t h o d s

Two identical reaction mixtures were prepared, one of them was used for chemiluminescence measurement, the second one for EPR measurement. BioOrbit 1250 luminometer (Finland) was employed for chemiluminescence measurements. Desired amounts of copper sulphate and sodium hydroxide stock solutions were mixed in the chemiluminometric cuvette and filled up with water. The total volume of the reaction mixture in the cuvette was 2 ml. Reactions were initiated by a squirt of hydrogen peroxide. EPR spectra were recorded on Miniscope MS200 EPR spectrometer (Magnettech, Germany) equipped with Dewar flask for measurements in liquid nitrogen. Aliquots of 50 µl were transfered into EPR sample capillaries at ca 3 minutes intervals and frozen in liquid nitrogen at 77 K. EPR spectra of frozen samples were recorded at 9353 MHz, microwave power 20 mW and modulation amplitude 2 G.

Results and discussion

The typical CL intensity vs. time curve is shown on the Fig. 1. It can be seen that early and final stages of the decomposition of hydrogen peroxide by copper(II) in strongly alkaline medium are accompanied with singlet oxygen generation. Singlet oxygen is detectable by its dimolar chemiluminescence emission at the beginning of the reaction, when copper(II) is immediately reduced to copper(I), as evidenced by color change (from blue to ochre) and disappearance of Cu^{2+} EPR signal. Concomminantly, the EPR signal of superoxide anion radical arises, the intensity of which stays virtually constant until after a certain lag phase (during which hydrogen peroxide is consumed) the singlet oxygen chemiluminescence emission can again be observed. The formation of the second CL peak is accompanied by restoration of Cu^{2+} EPR signal (Fig. 2.). The observation of the second CL peak can be explained in a following way: after hydrogen peroxide is consumed, Cu^+ is oxidized by oxygen present in the reacion mixture into Cu^{2+}, which reacts according to Eq. 4 yielding

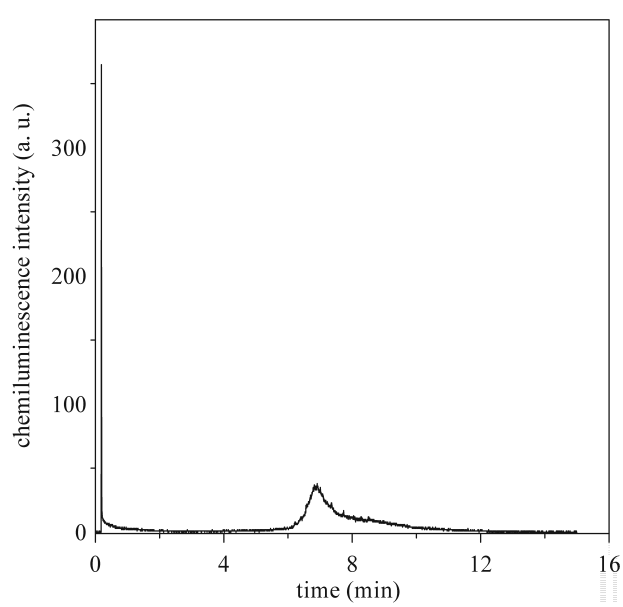

Fig. 1. **Chemiluminescence time profile of singlet oxygen emission accompanying the reaction between Cu^{2+} and H_2O_2: 0.5 M NaOH, 0.15 M H_2O_2, $1{\cdot}10^{-3}$ M Cu^{2+}**

singlet oxygen. During this process a bright green species is formed, presumably copperII peroxocomplex. The stepwise formation of several other differently colored copper species is observed during further decomposition.

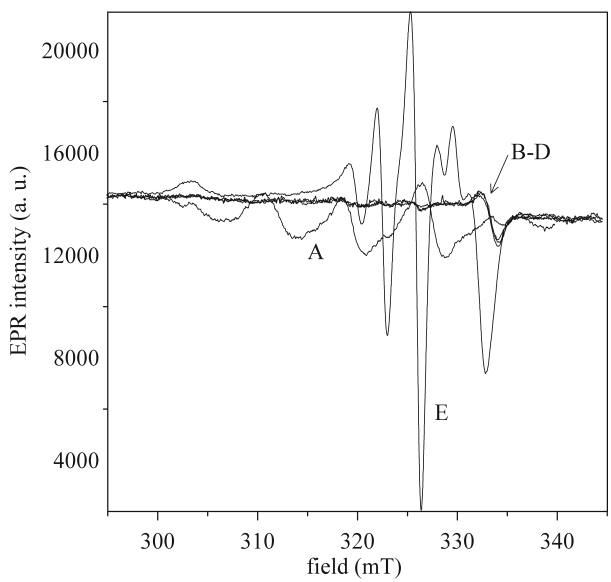

Fig. 2. **EPR spectra obtained during various stages of the H_2O_2 decomposition of Cu^{2+}. Trace A: Cu^{2+} and NaOH, B-E: approx. 1, 3, 5 and 7 minutes after H_2O_2 addition. Concentrations given in Fig. 1.**

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P02 THE EPR STUDY OF PROPERTIES OF SORBENT ON THE BASIS OF HUMATE

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Introduction

The serious problem of our modern civilization is production of a plenty of various wastes due to industrial production, energy, transport, agriculture and others. Enormous quantities of harmful substances get into the environment in high concentrations over their natural concentration limits or heterogeneous substances not occurring in the environment. Heavy metals belong to the most important well known toxic contaminants.

The ecologically significant harmful effects of heavy metals are due to their toxic and accumulation abilities. They are biologically un-demoted. High attention is devoted to the study of heavy metals. Common mechanism of their interaction with the natural organic mass has not been jointly described yet. Cu, Pb, Zn and Cd were chosen for this study.

The humates are the most extensive form of organic carbon in the nature. Most humic substances (next only HS) are chemically attached to inorganic components, {oxides and clay}, and a smaller part get dissolved in the solutions of the soil. An important feature of HS is that they can combine with metal ions, oxides and clay minerals to form water soluble or insoluble complexes. The complexes can exhibit interactions with the organic compound such as alkanes, fatty acids, capillary-active substances, pesticides and others. They also take place in transportation and binding ions complexes[1]. They can significantly reduce dangerous toxic materials in the environment. The sample of humate Fe (cheap sorbent utilized for remediation of contaminated soil and water treatment) containing high percentage of humic materials was used for this work.

Lately the most up-to-date spectroscopic technique with the aim to clarify as much as possible mechanism of interaction between metals and HL are the following:

The list of applied methods includes FTIR (infrared Fourier transform), EPR (electron paramagnetic resonance), NMR (nuclear magnetic resonance), NOQ (nuclear quadrupol interaction), fluorescent spectroscopy. The method EPR was chosen in this project, which enables thorough examination of structure of HL, their identification, interaction with heavy metals and rating of free radicals.

Experimental

M a t e r i a l s

All chemicals were obtained from Sigma (Aldrich), redestilled water and calibration standard (Galenus, GmbH,

Berlin, FRG). The sample of humate Fe was obtained from Severočeské Doly (North-Bohemian Mines), Bilina, Czech Rupublic, where it is produced by a precipitation of wastewaters containing humic substance with iron salts. The sorption of heavy metals in humate Fe was carried out at the Faculty environmental in Ústí nad Labem.

A p p a r a t u s

EPR spectra were recorded on E-540 spectrometer (Bruker, Rheinstetten, Germany); magnetic field was measured on a 1H NMR magnetometer, and microwave frequency on a frequency counter. The following conditions were used while recording the spectra: microwave power 2 mW, modulation amplitude 0.02 mT, attenuation 25 dB, time constant 0.5 s, scan speed 0.3 mT min^{-1}, calibration standard Cr^{3+}/MgO and room temperature. The programs used for spectra recording, handling and evaluation were CDAQ and CPRO (Galenus, Germany) and WINEPR (Bruker, Germany). 5,5-Dimethyl-1-pyrroline-Noxide (DMPO) was used as a radical trapping agent. The design of the EPR experiments is described in the legend to the respective figures.

Results and discussion

The presented work deals with the study of sorption and complex-forming ability of humic materials with heavy metals. The research was focused on the analysis of the samples of humate Fe and humate Fe with fixed Cu, Zn, Pb, Cd, both in solid state and solute. The influence of UV and ozone on the samples was observed. It was ascertained, that humate Fe has anisotropic EPR spectrum because of present Fe^{3+}, which occurs in two different forms, that is the octahedral and in the tetrahedral site. Both forms of Fe^{3+} are able to bound to humic functional groups (carboxylate and polyphenol groups) in organic compounds through inner-sphere complexes. For humic substances of various origins, either terrestrial or aquatic were identified[2]. Furthermore, in humate Fe froms organic radical, which is induced by stable radical on the humate anion. At high field around 3300 G is observed the signal corresponding to complex Cu^{2+}. It is incorporated in octahedral surrounding low-spin Fe^{3+}. The EPR spectrum Cu^{2+} in soluble shows slight tetragonal circumflexion in Fig. 1. This phenomenon called Jahn-Teller is caused by the[3] bond Cu^{2+}–O.

From measured EPR spectrums of humate Fe and humate Fe with fixed Cu, Pb, Zn, Cd, it follows that these metals do not show signal but probably influence the spectrum of the humate Fe. It is documented by the following spectrum shown in the mentioned Fig. 1. These metals are bound to complex humate.

This work was aimed at basic sorption abilities and characterization of samples of humate Fe. Intended research should elucidate detailed characteristics of bond Cu^{2+}-humate Fe (in dependence on metal concentration and pH). Further topic intended is the investigation of influence of ozone on various humates. In the future the project needs other modern spectroscopic methods (NMR).

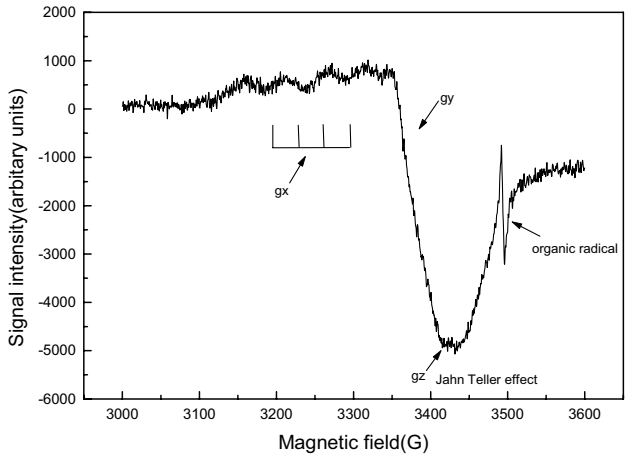

Fig. 1. **Spectrum humate Fe with Cu in solution**

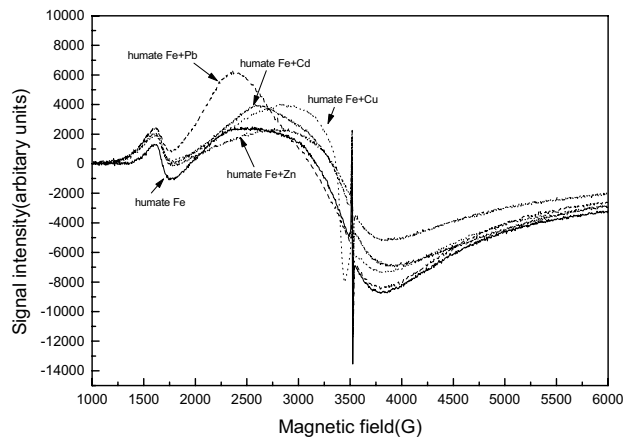

Fig. 2. **Comparison of spectrums IH, IH+Cu, Pb, Zn, Cd**

Conclusions

Information on HL sorption abilities of particular metals resulted. Copper and lead are most adsorbed by Fe-humate. Moreover, we found that higher amount of ozone causes the loss of organic radical and partial precipitation of Fe^{3+} from humates endangering the bond metal-humates. As a sequel negative influence of ozone can cause worsening of soil quality. One of the possible unwanted effects could be the decrease of production of crops negatively affecting the agriculture.

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P03 EPR STUDY OF HUMAN HAIR

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Introduction

Human hair contain biopolymer melanin. Melanin is in hair, in skin, in cornea, and other parts of body like nails etc. There are free radical centers on the surface of melanin particles. Many human diseases are caused by the formation of free radicals and other reactive oxygen species. Biopolymers are macromolecular compounds formed in organisms as a part of living bodies. Melanin is an important biopolymer contained in hair, skin, and eye pigments. The basic units of melanin are quinones. The important properties of melanin are due to active free radical centres on the surface of melanin particles.

We studied melanin samples from human hair. Relations between EPR spectra and health condition of examined persons were revealed[1]. During some dermatological disorders or illness the changes in observed EPR spectra have been observed. The risk of such disorders including allergies, skin cancer and hair disorders is increasing. Our original results have been published recently[1–3].

Melanin is a biopolymer forming granules with multiple radical centres on their surface. There are natural melanins found in plant and animal tissues and synthetic melanins. Melanin is paramagnetic and EPR spectroscopy makes possible to study the generation, reactions, and decay of melanins. We studied concentration changes of melanin in the samples of human hair using EPR spectroscopy and we observed the influence of irradiation, ozonization, and some medicines. UV irradiation was carried out using mercury discharge lamp, ozonization by O_2–O_3 mixture. We found that melanin in hair is highly sensitive to colouring and discolouring of hair. UV irradiation causes increase the occurrence of radical centres on the melanin granules surface, further irradiation causes destruction and decay of melanin. Changing melanin content in hair causes changes of melanin concentration in nails. We isolated melanin form human dark hair by alkali extraction and precipitation for its characterisation by EPR spectroscopy. Application of our experience in EPR spectroscopy to measurements of sorption of toxic substances on the surface of granules of melanin considered as a biopolymer with long-term stable radical centre. Gaseous substances (SO_2, NH_3, nitrogen oxides, toxic combustion products), radicals (products of burning and ecotoxic substances including tobacco smoke) and liquid substances (model mixtures of aerosols of petroleum derivatives and organic compounds).

Experimental part

EPR spectra were recorded on Elexsys E-540 spectrometer (Bruker, Rheinstetten, Germany): microwave power 20 mW, modulation amplitude 0.02 mT, attenuation 20 dB, time constant 0.5 s, scan speed 0.3 mT min, calibration standard Cr^{3+}/MgO and room temperature. 5, 5-Dimethyl-1-pyrroline-Noxide (DMPO) was used as a radical trapping agent. The reaction system of the EPR experiments is described in the legend to the respective figures.

Results and discussion

The open skin and hair are directly endangered by UV irradiation, which is the strongest external source of free radicals (FR). In both cases it is the natural pigment melanin, which protects human skin and hair from FR, by its scavenger reaction: melanin eliminates the UV radiation but finally it changes into FR too. The amount also the function of melanin depends on the function of melanocytes. In case of hair illness the disruption occurs of cycles of melanogenesis, which is tight coupled to the hair growth cycle. We focussed our attention on this process with the amplitude of FR signal.

Eight samples of human hair, from seven women and one man, all the patients of dermatological clinic were delaminated by the optimal method for identification and estimation of FR, which is the electron paramagnetic spectroscopy (EPR).

1. Samples were studied using X-Band EPR spectroscopic method, UV irradiation by mercury discharge lamp, ozonization by mixture of O_3–O_2. We have found the sensitivity of hair melanin to colorisation and decolorisation of hair. There is and increase of free radical concentration on the melanin particles by irradiation of hairs. The long-term irradiation causes the degradation of melanin. Long-term therapy of some pharmaceuticals causes the deposit of active substances in hair melanin and also the degradation of melanin. The changes of hair melanin correspond to the change of melanin in nails.

2. The open skin and hair is directly endangered by UV irradiation (as a part of sun light) which is the strongest external source of free radicals. In both cases it is the natural pigment melanin, which protects human skin and hair from FR, by its scavenger reaction: melanin eliminates the UV radiation but finally it changes into FR as well. The amount and also the function of melanin depend on the function of melanocytes. In case of hair illness the disruption occurs in cycles of melanogenesis, which is tight coupled to the hair growth cycle. We focused our attention on possible connection of this process with the amplitude of FR signal.

3. Relations between skin diseases and free radicals and EPR spectra: We have found the hair loss is connected to the changes of EPR spectra of hair. The breakdown of free radical centres and destruction of melanin follow.

4. We studied effects of UV irradiation on changes in EPR spectra of hair. We have found that radical concentrations increase according to different kinetics during the UV irradiation. It depends on colour used for colourisatiation and decolourisation of human hair. We have found much different spectra of hair in patients with hepatitis type C.

The type of spectra could be used as an indicator of some health problems.

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P04 THE FREE RADICAL GENERATION IN HUMIC ACIDS INDUCED BY THE ISOTHERMAL HEATING

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Introduction

Humic substances (HS) are ubiquitous substances primarily derived from the degradation of litter fall from vascular plants. In spite of different properties of precursor organic carbon, they show similar chemical/physical characteristics. A significant biological role involving HS has been discovered[1]. It has been recently described that quinone moieties are the important electron-accepting groups for microbial reduction of HS[2]. It is well known, that HS consist of number of organic molecules possibly possessing unpaired electron that implies the lack of detailed hyperfine structures in EPR spectra. Nevertheless, experimental data clearly showed the dominancy of semiquinones as the main organic radicals[3]. A perusal of literature provides information on content variability of free radicals; the effects of physical and chemical factors including pH, ionic strength and state of aggregation, hydrolysis and alkylation, redox conditions and irradiation on free radical in HS has been evaluated[3]. On the other hand, there is a lack of information on the mechanism, kinetics and characteristics of free radical generated by heating of HS. Therefore, the main purpose of this paper is to contribute to such oriented research.

Experimental

Humic acids (HA) extracted from South Moravian lignite by standard IHSS method[4] were milled, put into glass cuvette and measured in solid state by means of EPR spectrometer SpectraNova and Bruker E-SRC. The same sample was heated in oven at isothermal conditions whereas the EPR spectrum was continuously measured (at certain time period in dependency on temperature). Temperatures of isothermal heating were chosen as follows 105, 120, 150, 180 and 210 °C, in respect to the previous thermoanalytical results, i. e. in temperature region in which no significant heat evolution or weight loss have been registered[4]. Experiments were realized in presence of oxygen. The relative area of EPR signals was obtained by double integration and compared with EPR measurements of standard sample of known spin per gram content. For evaluation of g-factor, the method of inner standard was employed.

Results and discussion

EPR spectra of original HA resulted in a singlet peak without any hyperfine structure. In all cases in point, the thermal treatments lead to gradual increase of total area under double integrated singlet peak as long as it reached maximal value τ_{max}, then it started to decrease slowly; g-value alteration has not been observed. Likely the increase of free radical content can be ascribed both to weight loss caused by moisture evaporation and chemical changes of the sample in the course of heating. Since g-value has not been changed (2.0036), the development of new chemical structures probably has not occurred. All this findings can be rationalized by the one-electron step reaction in solid phase given in Fig. 1.

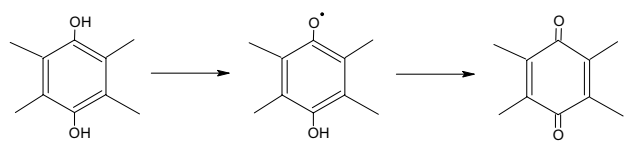

Fig. 1. **Mechanism of radical evolution in the course of isothermal heating**

Although the suggested mechanism seems to be valid for all temperatures, the humic moieties involved in such transformation are likely different. The starting number of spins per gram of humic samples has been determined $4.64 . 10^{17}$ whereas at τ_{max} for 105, 120, 150, 180 and 210 °C was $6.65 . 10^{17}$, $17.9 . 10^{17}$, $19.6 . 10^{17}$, $29.3 . 10^{17}$ and $58.9 . 10^{17}$, respectively.

Reactions in solid phase represent a complex problem. Depending on the reaction conditions, reaction can be both chemically and diffusion controlled. Since TA measurement did not indicate any heat effects it can be assumed that the reaction likely took part on the particle surface therefore the diffusion influence could be neglected. Thus parameters of reaction rate describe solely the kinetics of the chemical reaction. As it has been stated previously, the period of reaching the free radical content maximum is represented by time τ_{max}; it is inversely proportional to the rate coefficient (constant) k of quinone radical development; i. e. $\tau_{max} \sim 1/k$ or α/k where α is proportionality constant. Employing Arrhenius equation one can receive $\tau_{max}(T) = \alpha/(A . \exp(-E_a/RT)) = \alpha/A . \exp(-E_a/RT)$ where A is frequention factor, T is tem-

perature and E_a stands for activation energy. Fig. 2. shows dependency of τ_{max} on the reciprocal value of temperature. Obviously, parameters of the equation can be used directly for Arrhenius parameters calculation.

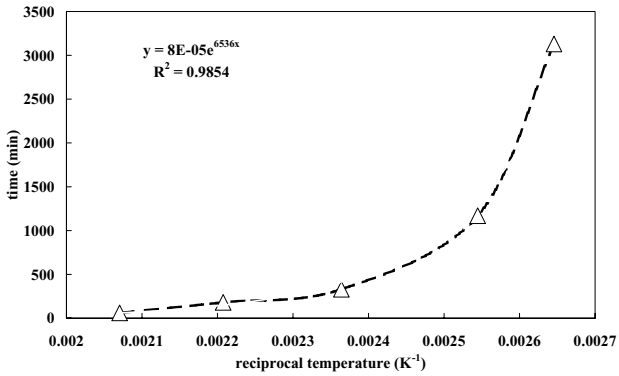

Fig. 2. **Dependency of τ_{max} on the reciprocal value of temperature**

Accordingly, E_a of process under study is 55 kJ mol^{-1}. Kinetic parameters concerning suggested reaction mechanism are useful for prediction of humic matter behavior and stability; nevertheless additional correlation should be done to find an interrelationship between basic humic functions and those parameters.

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P05 INFLUENCE OF EXOGENOUS STRESS ON ANTIOXIDANT AND RADICAL SCAVENGING ACTIVITY OF YEASTS

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Introduction

The properties of antioxidants formed by yeasts can be substantially influenced by the environment surrounding the cells. Therefore, there is a necessity to develop the reliable methods to investigate total radical scavenging and antioxidant capacity of samples without distinguishing the contributions from individual compounds[1]. Simultaneously, the analysis of the yeast's metabolites composition and their comparison to the total antioxidant capacity of yeasts can help us to identify compounds that are the most efficient radical scavengers and antioxidants in the investigated samples.

Applying different EPR experiments, the suitable procedure was recently developed[2] to investigate the radical scavenging and antioxidant properties of pigments localized on the surface of cell walls and inside of the cell bodies of yeasts grown on exogenous stress. For comparison the total antioxidant capacity was additionally investigated using spectrophotometric methods including an ABTS assay[3], which confirmed the conclusions based on EPR spin trapping experiments as will be described below.

Experimental

A Bruker EMX EPR spectrometer (Germany) was used in EPR experiments. UV-Vis spectra were recorded on a spectrometer PC2000 (Ocean Optics). All strains grew under a non-lethal and maximally tolerated concentration of metal ions (for details of sample preparation see[2]). All solutions were prepared using redistilled water and spectroscopic grade dimethylsulfoxide (DMSO) from Lachema (Czech Republic).

Results and discussions

The studies were focused on pigment forming yeast Rhodotorula glutinis CCY 20-2-26. The corresponding yeast was stressed at different experimental conditions including the presence of metal ions (Zn^{2+}, Cu^{2+}) and H_2O_2. Spectroscopic assay based on ABTS reagent was used in order to characterize total antioxidant capacity of yeast extracts more precisely. ABTS is a spectroscopic method for the screening of antioxidant activity. It is based on a decolorization assay applicable to both lipophilic and hydrophilic

antioxidants. The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) is generated by oxidation of ABTS with potassium persulfate (Fig. 1.).

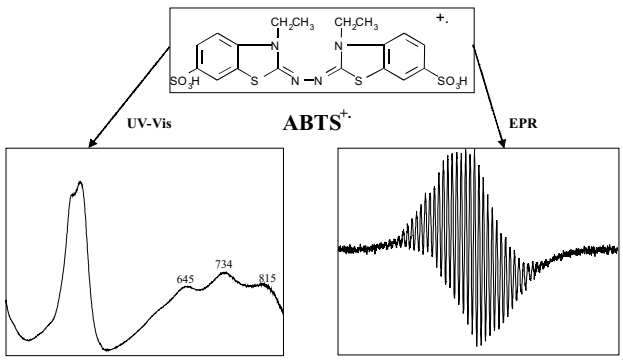

Fig. 1. **Structure, UV-vis and EPR spectra of ABTS cation radical in DMSO**

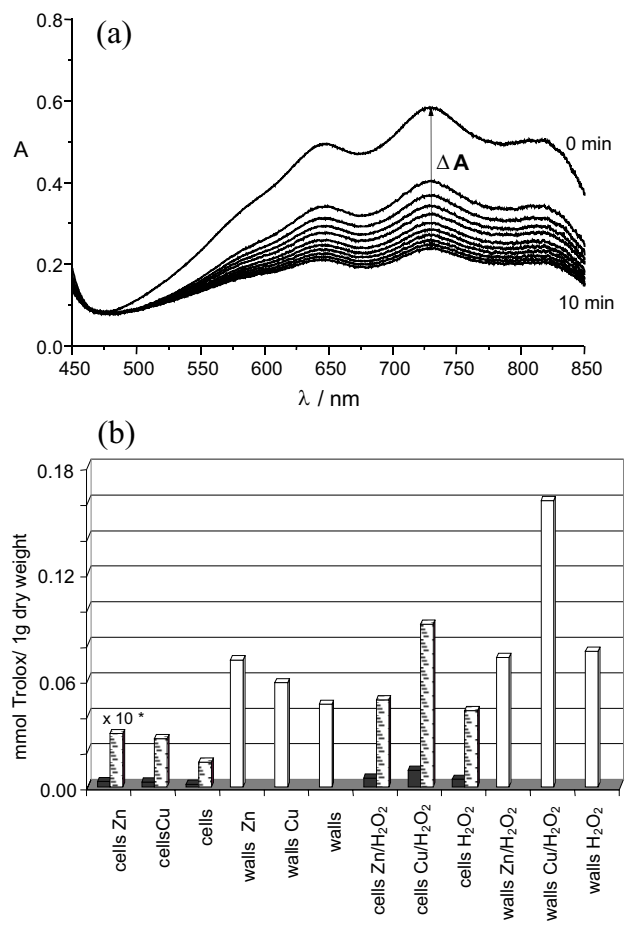

Fig. 2. **a) UV-vis spectra of $ABTS^{+\bullet}$ measured during first 10 minutes after addition of yeast extract. b) TEAC of the investigated samples evaluated by the ABTS assay (* expanded for clarity)**

In this assay the decrease in absorbance was followed to monitor the consumption of the colored ABTS radical and absorbance changes at 730 nm were taken as a measure of antioxidant activity, which was then calibrated to Trolox to evaluate TEAC (Trolox equivalent antioxidant capacity). The calibration curves of Trolox showed good linearity. Addition of yeast extract (20 μl) resulted in decreased absorbance after 10 min. incubation (Fig. 2a). The blank incubation contained ABTS reagent (400 μl) and DMSO instead of sample (20 μl), and no changes (except of negligible dilution) were observed in UV-Vis spectra. Different antioxidant capacity was estimated for variously stressed yeasts similarly as observed using EPR spectroscopy[2]. These further studies confirmed that antioxidants present in fibrilar part of cell (walls) showed much higher ability to scavenge free radicals than those from cytosol (cells). More effective antioxidant capacity was found in yeast samples grown under presence of Zn^{2+} ions. This phenomenon could be probably explained by both the presence of Zn^{2+} ions in "walls" and "cells" and by processes where Zn^{2+} ions during cultivation might induce more efficient scavenging and antioxidant capacities of yeasts metabolites compare to the cultivation in the absence of exogenous metal.

The yeasts treatment with metals caused substantial change in the carotenoid composition of the yeast[2]. For instance the level of luteine was very low in *R. glutinis* CCY 20-2-26 and increase amount of α-carotene was found in "walls" of the strains incubated with Zn^{2+} ions. However, changes in carotenoids composition do not correlate exactly with ABTS studies described above. This illustrates the complexicity of the investigated systems and the contribution of further compounds to the total antioxidant capacity except of carotenoids.

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P06 REDOX REACTIONS OF PYRIDOINDOLES (CV, EPR AND UV-VIS STUDIES)

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Introduction

Numerous studies in the literature were so far focused on the antioxidant properties of pyridoindoles, especially on stobadine[1,2]. However, less attention was oriented to the electrochemical and spectroelectrochemical studies of such systems. Only few reports can be found dealing with electrochemical oxidation of e. g. stobadine and its derivatives in aqueous solutions[3]. In our contribution optical, cyclovoltammetric and spectroelectrochemical studies of such systems in organic media are presented in order to explain in more details the redox processes of different novel synthesized pyroindoles (Fig. 1a).

Experimental

Acetonitrile (AN) and tetrabuthylammonium perchlorate (TBAP) purchased from Fluka were used as received. The cyclic voltammograms were recorded using a platinum wire as working and auxiliary electrodes, with saturated calomel electrode (SCE) as reference. HEKA PG 284 (Germany) served as potentiostat. UV-Vis spectra were recorded on a spectrometer PC2000 (Ocean Optics). EMX EPR spectrometer (Bruker) was used in EPR experiments.

Results and discussion

Fig. 1b represents typical cyclic voltammogram obtained in the oxidation and reduction of investigated compounds **I** and **II** (R_1: H, CH_3 or OCH_3; R_2: H; R_3: H, CH_3 or $CH_2C_6H_5$) in acetonitrile solution containing 0.1 M TBAP (illustratively for compound **A**). Additionally, characteristic UV-vis spectra simultaneously taken under in situ oxidation are shown in Fig. 1c. The oxidation results in the formation of new EPR silent product with the transition at about 420 nm. All compounds exhibited an irreversible oxidation with relative complex pattern in the potential region from 0.4 to 1.6 V vs. SCE. Stobadine derivatives (**I**) exhibit lower first oxidation potentials (~0.4 V) and simultaneously more redox steps as their precursors (**II**) (~0.8 V). Additionally only for stobadine derivatives (**I**) EPR spectra of stable nitroxyl radicals (Fig. 2.) were observed under its oxidation with PbO_2/tBuOOH system[4]. Indole nitrogen centered radical is probably primarily formed due to the reaction of pyridoindole with free radicals and converting then to nitroxide intermediate which is transformed to nitrone[5]. Nitrone then traps another free radicals and its adduct is monitored using EPR. On the exact nature of the oxidation products formed, on the influence of subsituents on redox behavior of pyridoindoles and the estimation of their antioxidant capacity will be focused our future studies.

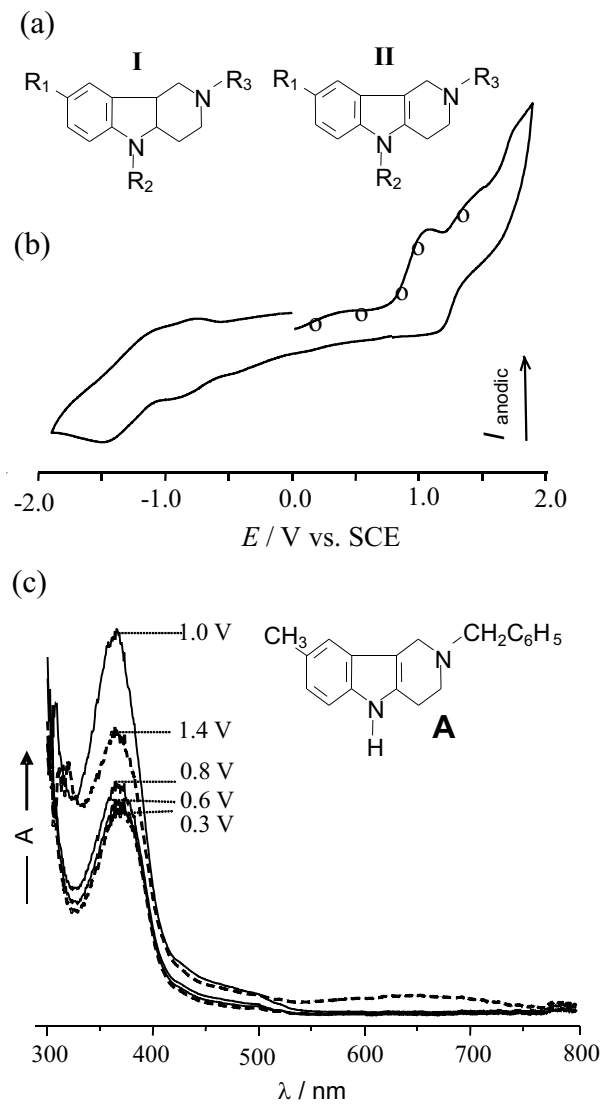

Fig. 1. **a) Investigated compounds (with R as specified above), b) cyclic voltammogram of** A **in AN/0.1 M TBAP and c) UV-vis spectra simultaneously taken under in situ oxidation of** A

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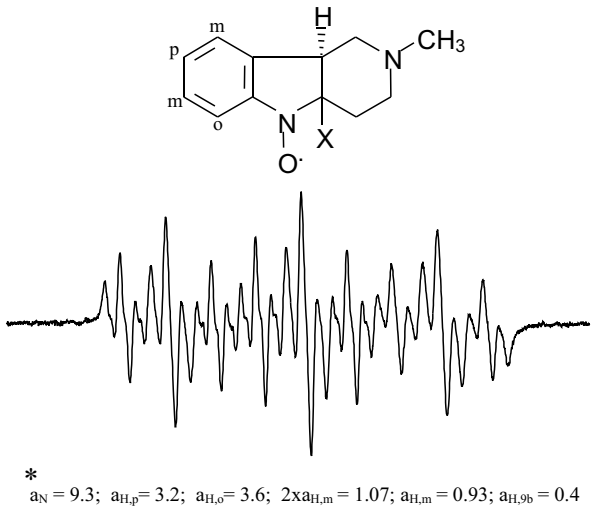

Fig. 2. **EPR spectrum of stable nitroxyl radical observed in the oxidation of stobadine derivative** I **(with R_2 = H) with PbO_2/tBuOOH system in acetonitrile (*splitting constants in Gauss)**

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