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The Influence of *Aspergillus niger* Tiegh. on the Electrochemical Characterisation of a Polyaniline Modified Ni Electrode

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The strain L-10 of *Aspergillus niger* Tiegh. was cultivated on the malt extract agar (DIFCO OXOID). A polyaniline film was synthesized by potentiodynamic polymerization in an aqueous 0.3 M $H_2C_2O_4$ solution containing 0.1 M aniline on the Ni electrode. Electrosynthesis gives cohesive films. The intensive growth of the treated microorganisms was observed on the polyaniline modified Ni surface. After 7 days of exposure the influence of *A. niger* L-10 on polyaniline was evaluated by 4.5 points. Optical and scanning electron microscopy were used to examine the morphology of polyaniline surface. Elemental analysis of polyaniline coatings was carried out using X-ray photoelectron spectroscopy. The electrochemical behaviour was determined in a 0.05 M H_2SO_4 solution by the cyclic voltammetry method. The presence of *A. niger* L-10 increases the electrochemical activity of polyaniline film.

Keywords: influence, Aspergillus niger, electrochemical characterisation, polyaniline.

ITRODUCTION

The microbial influence on metals and polymers properties is widely studied. From the broad common microbial diversity *Aspergillus niger Tiegh.* is one of the most stable and one of the most investigated microorganism. The influence of *A. niger* on diversity of plastics and on metal corrosion has been elucidated recently [1, 2]. The wild strain *A. niger* acted either as a corrosion accelerator or an inhibitor depending on the metal it colonized. Relatively little is known about biofilms formation on the electrically conductive polymers surface. Electrochemical stability of an electrode modified with conducting polymers is the important quality index because of its various practical applications (energy storage, electrocatalysis, organic electrochemistry, bioelectrochemistry, photoelectrochemistry, sensors, electrochromic displays, corrosion protection, etc.) [3 – 8]. Polyaniline was chosen for this study owing to its easy syhthesis in aqueous medium and a low cost of the monomer. Electropolymerization on inexpensive non-platinum metal electrodes was more interesting than that on expensive platinum-metal electrodes. The electrodeposition of conducting polymers on oxidizable metals is indeed not easy because the metal dissolves before the electropolymerisation potential of the monomer is reached [5, 7, 9 – 11]. Electrochemical conditions, which lead to partial passivation of the metal and decrease its dissolution rate without prior electropolymerization, were determined. Such a result was obtained by using oxalic acid as a supporting electrolyte and what is more, oxalic acid is relatively strong and therefore easily allows a low pH to be attained for the growth of polyaniline [5, 7]. It has already been reported that the conductivity of several polymer films derived from aniline derivatives is increased by incorporation of certain aromatic species [12]. It was detected, that *A. niger* produces numerous organic acids, and among them oxalic acid [13]. As *A. niger* itself produces oxalic acid the formation of adhesion between the polyaniline surface and the microorganism may occur.

The aim of the present investigation was to study an influence of *A. niger* on the electrochemical characterisation of the polyaniline modified Ni electrode by the cyclic voltammetry method.

MATERIALS AND METHODS

All the reagents were of analytical grade or higher and deionized water was used for the solution. Aniline was further purified by distillation. The electrolyte solutions were deoxygenated by bubbling nitrogen gas before the experiments.The strain L-10 of *Aspergillus niger* was cultivated on the malt extract agar (DIFCO OXOID) during 10 days at a temperature of 26 °C ±2 °C.

Polyaniline films were synthesized by potentiodynamic polymerization in an aqueous 0.3 M $H_2C_2O_4$ solution containing 0.1 M aniline within the potential limits of -0.1 V and 1.3 V at a sweep rate of $0.05\ \mathrm{V\,s^{-1}}$. The procedure was performed 10 min in order to obtain approximately the same film thickness characteristic of all the electrodes.

The electrode was obtained by cutting a commercially pure 10 μm thick Ni plate into pieces with working surface dimensions 0.5 cm by 0.5 cm, embedded by a galvanic strip SC-1. The metal was polished to a smooth finish with AlO_2 M-2 powder lubricated with water.

The polyaniline electrodes were exposed to Petri dishes with a pure *A. niger* L-10 culture at 26 °C. The intensities of oxidizing reactions of electrodes were checked after 7, 14, 28 and 36 days.

The electrochemical activity of polyaniline film was studied in a 0.05 M H_2SO_4 solution by the cyclic voltammetry metod. Each sample, before measuring, after the contact with *A. niger* L-10, was rinsed with water. The reference polyaniline electrode without microbial treatment was examined after the same time of exposure. The potential range from -0.1 V to 1.1 V and a sweep rate of

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$0.02\ \mathrm{V\,s^{-1}}$ were used. The chacteristic of influence of *A. niger* L-10 on the polymer film properties was characterised by the value of the current density (*i*), potential (*E*) of the first anodic peak and the charge (*Q*) of the voltammogram (current density – potential *i*(*E*) curves).

The morphological peculiarities of fungi were studied employing an optical and scanning electron microscope EVO 50 XVP (Carl Zeis SMT AG, Germany). Elemental analysis of the polyaniline coatings was carried out using X-ray photoelectron spectroscopy (XPS).The XPS spectra of C1s, O1s, N1s were obtained by a VG ESCALAB MK II spectrometer (VG Scientific UK), using MgK$_\alpha$ anode radiation (1253.6 eV and a constant pass energy of 20 eV). The pressure in the analysis chamber was maintained at $1.33{\cdot}10^{-7}$ Pa or lower during the measurments. The binding energies were calibrated with respect to the C1s peak at 284.6 eV. No less than five spectra were recorded for every element. The carbon (C1s), oxygen(O1s) and nitrogen (N1s) spectra were fitted to Gaussian-shaped components of equal full-width at half maximum (FWHM) for a particular species.

Cyclic voltammograms were recorded using a PI-50-1 model programming potentiostat connected via an AD-converter RS-232 to a PC. The reference electrode was a silver-silver chloride wire with a saturated KCl solution. The electrolyte temperature was maintained in the range of 25 °C – 26 °C.

RESULTS AND DISCUSSION

Fig. 1 shows the cyclic voltammogram (CV) of the electropolymerization of aniline on Ni in an oxalic medium. Three oxidation current peaks (in the 0.2 V – 0.8 V range) and a larger reduction (in the 0.3 V – 0.6 V range) current are seen and electrodeposition of a green-blue film is visually observed. Electrosynthesis gives cohesive films. The nature of CV during deposition of polyaniline from an $H_2C_2O_4$ aqueous medium are nearly similar to those from H_2SO_4 presented in [7]. A more pronounced difference is seen in the potencial region between 0.6 V and 1.3 V, where the oxidation of aniline takes place.

Fig. 2 presents the XPS survey spectrum of polyaniline film that is polimerized in the presence of oxalic acid on the Ni surface. In the survey spectra the Ni2p peaks were not observed, indicating that the surface was completely covered by a PANI film. After deconvolution the carbon 1s spectrum displays three peaks centered at 284.5 eV – 285.7 eV and 287.5 eV. The main peak at 284.5 eV is attributed to the C–C / C–H groups present in the polymer. The peaks at 285.7 eV and 287.5 eV are attributed to C–N groups and O–C=O groups due to the carboxylic groups of oxalic acid, which is incorporated in the film as dopant (the quantity of oxalic acid ions is about 8.7 at.%). The binding energy peak of N1s at 399.2 eV can be assigned to the benzenoid aniline (–NH–) structure. The residual high binding energy tails (at 401.5 eV) were probably due to some surface oxidation products like weakly charged-transfer complexed oxygen [5, 6].

In the preliminary microbial reactions study the morpholgy investigations on polyaniline layers exposured to the influence of *A. niger* L-10 were performed. After 7 days of polyaniline exposure, the influence of *A. niger* L-10 was evaluated by 4.5 points from 5. Such intensive surface growth can testify good adhesion of the treated microorganisms [14]. Fig. 3 illustrates the images of reference and treated polyaniline samples by scanning electron microscopy (SEM) after 14 days of microorganisms growth. The growth of a new generation of *A. niger* L-10 was observed on the polymer surface. A further investigation up to till 36 days has not shown any noticeable changes in the general view of *A. niger* L-10 growth.

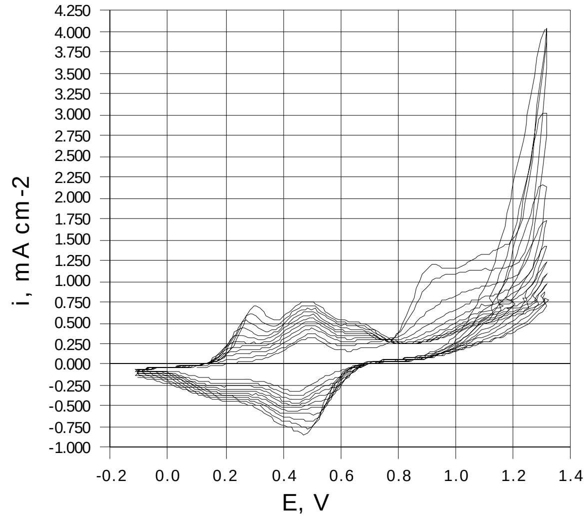


Fig. 1. CV for electrodeposition of polyaniline on Ni from 0.3 M $H_2C_2O_4$, containing 0.1 M aniline (potential cycling between $E = -0.1$ V and 1.3 V, 0.05 $\mathrm{Vs^{-1}}$, $t = 10$ min), vs. Ag/AgCl

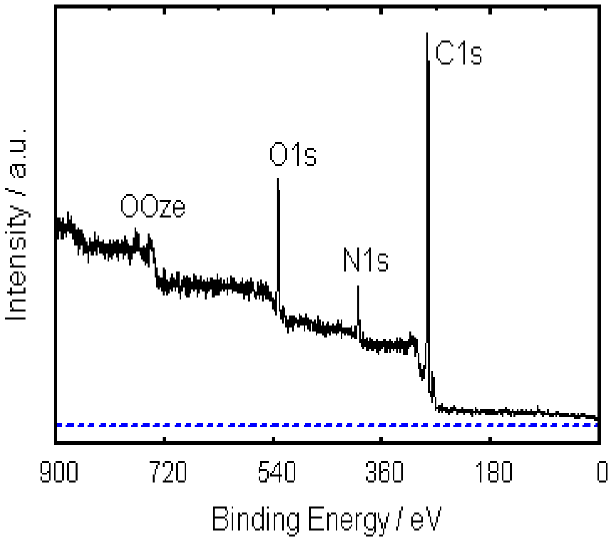
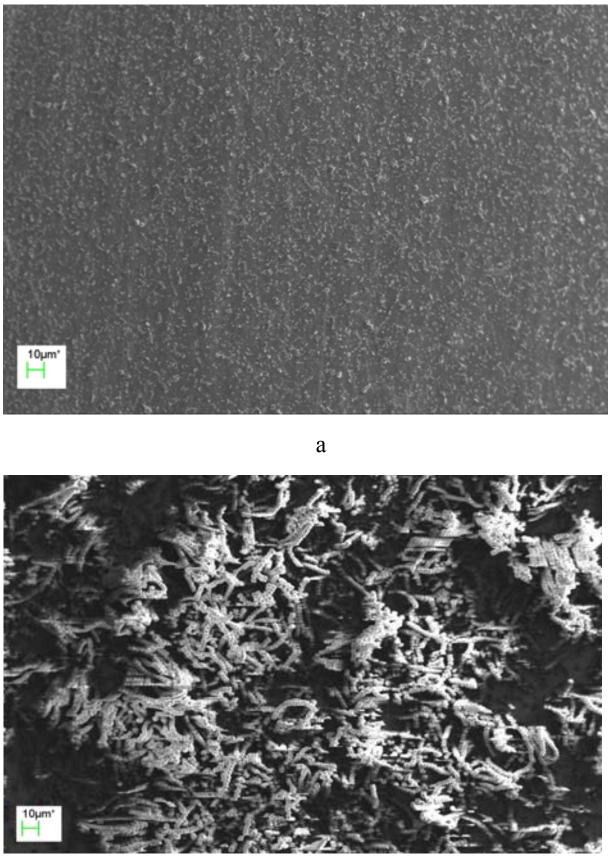


Fig. 2. XPS wide-scan spectra of the polyaniline modified Ni electrode, electrodeposited in 0.3 M $H_2C_2O_4$ solution containing 0.1M aniline (potencial cycling between $E = -0.1$ V and 1.3 V, 0.05 $\mathrm{Vs^{-1}}$, $t = 10$ min), vs. Ag/AgCl

To investigate the influence of *A. niger* L-10 on the activity of polyaniline films, the CV of the samples in a 0.05 M H_2SO_4 solution were recorded. The CV of a



a

b

Fig. 3. SEM micrographs of polyaniline film ($Q = 13.72 \cdot 10^{-3}$ C cm^{-2}): a – reference; b – after 14 days of exposure under the influence of *Aspergillus niger Tiegh.* L-10

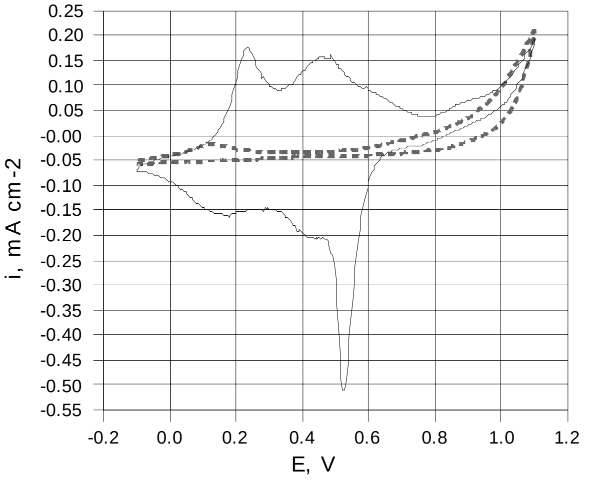


Fig. 4. CV for: (solid line) polyaniline film and (broken line) Ni recorded in 0.05 M H_2SO_4 solution (potencial cycling between $E = -0.1$ V and 1.1 V, 0.02 Vs^{-1}), vs. Ag/AgCl

polyaniline film (synthesized on the Ni electrode in a 0.3 M $H_2C_2O_4$) in a 0.05 M H_2SO_4 solution is shown in Fig. 4, by a solid line. Two oxidation peaks and a sharp reduction peak ($E = 0.5$ V) are observed. This can be attributed to the proton elimination-addition and anion doping-undoping reaction on the polyaniline surface. Electrochemical incorporation of anions into the polymer matrix of a conjugated polymer increased the conductivity, activity and special catalytic properties [3, 15 – 17]. Ni electrode (broken line) in a 0.05 M H_2SO_4 solution does not show electrochemical activity in relation to polyaniline electrode (solid line).

The experiments have shown the activity of polyaniline films to be higher for microbially treated films as compared with those of the reference ones (Fig. 5). The CV of polyaniline films after 28 days of exposure to the influence of *A. niger* L-10 (Fig. 5, curve a) in comparison to the CV of reference polyaniline films (Fig. 5, curve b) show a great difference in the potencial region between 0.6 V and 1.1 V. A more evident difference in the film activity is observed after 36 days of microorganisms growth. The polymer film charge is higher after 36 days of exposure to the influence of *A. niger* L-10 $Q = 6.5 \cdot 10^{-3}$ C cm^{-2} (Fig. 5, curve c), than that of reference polyaniline films $Q = 3.9 \cdot 10^{-3}$ C cm^{-2} (Fig. 5, curve d). The value of the first anodic peak current in Fig. 5 (curve c) is about 46 % higher ($i = 11.86 \cdot 10^{-5}$ A cm^{-2}) than that of the corresponding peak ($i = 8.08 \cdot 10^{-5}$ A cm^{-2}) in reference polyaniline film (curve d). Under the influence of *A. niger* L-10 the films degradation decreases. Ref. [2] suggests that the microorganisms could be used as surface destruction protectors and this work has proved it.

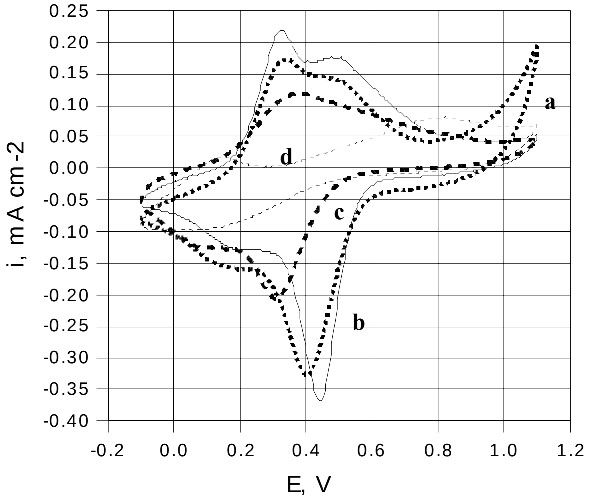


Fig. 5. CV for polyaniline recorded in 0.05 M H_2SO_4 : a – after 28 days of exposure to the influence of *A. niger* L-10; b – reference polyaniline film; c – after 36 days of exposure to the influence of *A. niger* L-10; d – reference polyaniline film (potencial cycling between $E = -0.1$ V and 1.1 V, 0.02 Vs^{-1}), vs. Ag/AgCl

As can be seen from Fig. 5 the polyaniline film undergoes oxidation during the influence of *A. niger* L-10. The potential of the first anodic peak attributed to the emaraldine/pernigraniline transition shifts from 0.34 V (curve a) to 0.38 V (curve c).

Inasmuch as the charge transfer complex between polyaniline and hydroquinone may be formed [12] the redox reaction H_2Q/Q was used in this study. The preliminary CV investigation has shown a slight increase in electrochemical activity of the polyaniline modified Ni electrode in comparison to that of Ni.

CONCLUSIONS

1. A polyaniline film synthesized in an oxalic medium on the Ni surface shows good adhesion of the treated

A. niger L-10 microorganizm. After 7 days of exposure the influence of *A. niger* L-10 on polyaniline was evaluated by 4.5 points from 5.

2. The polyaniline films activity that was examined from cyclic voltammograms (the magnitude of current density and the charge) in 0.05 M H_2SO_4 is higher for microbially treated films as compared to the reference ones.

3. The presence of *A. niger* L-10 moderates the films degradation.

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