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Investigation of microbiologically influenced corrosion
2. EIS study of two-year exposure of zinc to *Penicillium frequentans*, *Aspergillus niger* and *Bacillus mycoides*

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The paper is a sequel of previous investigations in the field of the influence of microorganism strains isolated from Al, Cu, Zn and steel samples exposed to marine, rural and urban sites in Lithuania. The following strains were under investigation: *Penicillium frequentans*, *Aspergilus niger* and *Bacillus mycoides*. Zn samples were subjected to a two-year effect of the microorganisms under laboratory conditions in humid atmosphere at a temperature of ca. 26 °C. Electrochemical impedance spectroscopy (EIS) ascertained microbially influenced corrosion acceleration (MICA) on zinc samples. The data indicated a three-layer structure developed on zinc during microbial corrosion. The reasons for MICA lie mainly in diminishing the inner layer (next to the metal), whose passivating capacity is much higher as compared to that of the other layers.

INTRODUCTION

The action of microorganisms on metal surfaces includes numerous phenomena, *e.g.*, production of corrosive metabolites, chelation of metal cations, production of organic solvents (ethanol, propanol, butanol), etc. [1]. The microbial products that are of importance for microbially induced corrosion (MIC), include inorganic and organic acids, sulphide, ammonia, carbon dioxide, nitrogen oxides, hydrogenase, etc. Microorganisms, due to heterogeneous surface colonization, may cause formation of concentration cells, which results in localized corrosion attack. The corrosion process is affected also by favoured water uptake by the biofilm. In general, MIC is a complex interaction between the microbial population, the environment and the metal substrate, which will ultimately determine whether a corrosion process will be induced.

Microorganisms may cause either corrosion inhibition or acceleration. The remarkable protective effect of biofilms was observed for Al 2024 and brass (70Cu/30Zn) in artificial seawater and Luria Bertani medium [2–5]. It was shown that a pronounced pitting attack took place in blank medium, whereas in the solutions containing bacteria the pitting process was stopped after two days of exposure. The authors performed also experiments with genetically engineered bacteria capable of producing inhibitors – polyglutamate or polyaspartate. However, they came to a conclusion that even the bacteria that are not engineered to produce inhibitors passivated the surface. Numerous examples of microbially induced corrosion acceleration are well presented in survey [1]. The effects of microbial colonization on inert electrodes have also been investigated [6]. The microbial biofilms formation not associated with corrosion was studied by quartz crystal microbalance (QCM), confocal scanning laser microscopy (CSLM) and cyclic voltammetry (CV).

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Under outdoor conditions, different communities of microorganisms may coexist on metal surfaces. As reported previously, we have identified *ca.* 70 bacterial and fungal populations that colonized metal samples during a two-year exposure in urban, rural and marine conditions in Lithuania [7]. From this microbial diversity, however, the most stable populations appeared to be *Bacillus mycoides, Aspergillus niger* and *Pennicilium frequentans.*

Aspergilus niger requires ions of iron, copper, zinc, manganese, molybdenum, boron, vanadium, gallium and scandium [8–10]. Studies ascertained the function of zinc ions to be inactivating glucose-6-phosphate dehydrogenase. Under the influence of zinc ions, incomplete oxidation of hydrocarbons goes on and production of organic acid occurs. *A. niger* produces numerous organic acids: citric, oxalic, acetic, gluconic, 5-keto-D-gluconic, glutaric, *trans*-glutaconic, *cis*-aconitic, glycolic, glyoxylic, pyruvic, hydroxypyruvic, α-ketoglutaric, galactonic, D-mannonic, saccharic, L-ascorbic and L-xyloascorbic [8–10]. Enhanced formation of *A. niger* conidia was determined in the medium with aluminium ions at a concentration of 0.001 mg/l, whereas at higher concentrations the inhibition of fungal growth took place [11]. The *Penicillium frequentans* strain produces succinic, tartaric, citric, oxalic, fulvic and asteric acids [8, 12]. *Bacillus mycoides* (a gram-positive, non-mobile soil bacterium) forms enzymes such as lipases, proteases, xylanases [13].

This study addresses the influence of the strains *Bacillus mycoides, Aspergillus niger* and *Pennicilium frequentans* on zinc corrosion processes. The study of zinc corrosion is of great importance, because zinc and its coatings (hot dip and galvanized steel, zinc rich paints, etc.) are extensively used in numerous technical applications. Evaluation of MIC characteristics and properties was performed by electrochemical impedance spectroscopy (EIS) during a two-year exposure of the samples to microbial effect.

EXPERIMENTAL

Microbiological studies

Microorganisms were isolated from Al, Cu, Zn, and low carbon steel samples were exposed in outdoor exposure sites which covered different environmental conditions in Lithuania. The stations were arranged according to the standard ISO 9223, 8565. They were located in the following places: marine – the Curonian Spit, Preila (100 m from the shore of the Baltic Sea); rural – Kulioniai and Rûgðteliðkiai villages in Molëtai and Utena districts; urban – the central part of Vilnius (Institute of Chemistry) and a suburb of Vilnius (Experimental Base of the Institute of Chemistry). Climatic parameters, air composition and water adsorption on metal samples were measured continuously at these sites.

The purity of the samples was Zn > 98.5%, Al > 99.5%, Cu > 99.5%, carbon steel C 0.05–0.11%, Mn 0.25–0.5% Si < 0.05%, Cr 0.1%. Fifteen samples of each metal (10 cm × 15 cm in size and 1–3 mm in thickness) were exposed unsheltered in stands at 45° to the horizon and the south orientation. The samples were degreased and disinfected before exposure with acetone and alcohol. To collect the rainwater that had rinsed the sample surface, special vessels were placed under each sample.

Microorganisms were isolated from the metal samples exposed in 2002 after 6, 9 and 12 months. The isolation was performed in two ways: 1) directly from corroding samples using a blank metal loop and 2) preparing suspensions of different dilution from the rainwater that had rinsed the samples. The microorganisms were inoculated on two media: 1) for isolation of microscopic fungi – on solid malt supplemented with antibiotics to suppress bacteria growth; 2) for isolation of bacteria on a solid meat peptone medium.

The microorganisms were grown at a temperature of 26 ± 2 °C. The grown colonies of microorganisms were counted in the following way: bacteria after 2–3 days and fungi after 3, 5 and 7 days. Pure cultures were obtained from grown microorganisms and identified according to their physiological, cultural and morphological peculiarities. Fungal morphological peculiarities were investigated by light and electron scanning microscopy. Fungal species were identified according to various manuals [8, 14–19].

Metal sample preparations and EIS studies

The samples were subjected to the effect of microorganisms by exposing them to humid atmosphere at a constant temperature. The exposure vessels (volume 5 l) contained 1l of saturated potassium sulphate solution, which maintained the relative humidity at *ca.* 97%. Zn plates (2 cm^2 in size and 1mm in thickness) were treated with emery SiC paper (grade 1200), washed with acetone and kept under ambient conditions for *ca.* half an hour. Then they were put on a ceramic grid in vertical position and placed in exposure vessels (four samples in each vessel). The samples were infected with microorganisms by spraying them with a solution containing: $NaNO_3$ – 2g, KH_2PO_4 – 0.7 g, KCl – 0.5 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, $FeSO_4 \cdot 7H_2O$ – 0.01 g, distilled water – 1000 g and a corresponding strain of microorganisms (the solution pH was 6.0–6.5). After spraying, the vessels were hermetically closed and kept in a glass box at a temperature of 26 ± 2 °C. At time intervals of 3, 7, 15 and 24 months the samples were sprayed repeatedly. Before that, some samples had been taken from the exposure vessels for visual evaluation, EIS investigations and vitality test of the microorganisms.

The EIS measurements were carried out using an electrochemical glass cell equipped with holes for metal specimen as well as Ag/AgCl reference and Pt counter electrodes. The metal sample was mounted in a special holder, placed in the cell filled with 3.5% NaCl, and EIS measurements were started after 5 min of exposure. The measurements were performed at open circuit potential applying a signal of an amplitude of ΔE = ± 5–10 mV. An IM6 apparatus by Zahner (Germany) was used. After measurements the samples were rinsed with distilled water, dried and newly infected with microorganisms as described above.

RESULTS AND DISCUSSION

As the criterion of the effect of microorganisms on corrosion rate served the polarization resistance (R_p), which is in inverse proportion to the corrosion current density (j_{corr}). The R_p value was obtained from the equivalent circuit parameters used for experimental data fitting. Determination of R_p was shown to be an informative approach for MIC studies [5].

Figure 1 shows R_p change during a two-year exposure of blank zinc and of zinc colonized with different microorganisms. A considerable increase in R_p with the exposure time of pure zinc indicates a decrease of corrosion activity, which is due to surface passivation by corrosion products. Pure zinc is known to corrode slowly and uniformly under atmospheric conditions (deep pitting usually is not observed) with formation of a thick and porous corrosion product layer.

The microorganisms accelerate zinc corrosion: the effect especially great at the final stages of exposure (Fig. 1). The data obtained during two-year studies do not provide unambiguous information concerning the dynamics of the accelerating capability of the microorganisms, although during the fifteen-month exposure a higher activity of *Aspergillus niger* and *Bacillus mycoides* is evident.

The impedance Z dependence on frequency f for a pristine zinc sample and that exposed for two years to humid atmosphere are given in Fig. 2. The phase diagram for pristine surface exhibits two clearly pronounced time constants, which are typical of a pure zinc electrode [20]. Curve 1 was modelled by an equivalent circuit consisting of two R_t-*CPE* elements (charge transfer resistance – constant phase element) and the uncompensated ohmic resistance (R_Ω) in series. Some discrepancy between experimental and calculated data is observed at the high frequency end of the phase diagram, which indicates a contribution of the third R-CPE element to be possible. Indeed, the experimental data are better described taking into account the third R_t-*CPE* element. The fitting parameters are summarized in Table 1. According to them, a three-

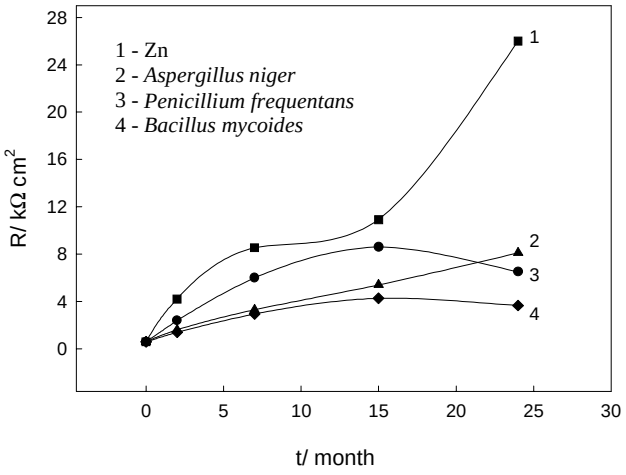

Fig. 1. Polarization resistance change determined from EIS data during two-year exposure of blank Zn sample and samples colonized by microorganisms. The samples were exposed to humid atmosphere at 26 °C. Strains of microorganisms are indicated in the figure

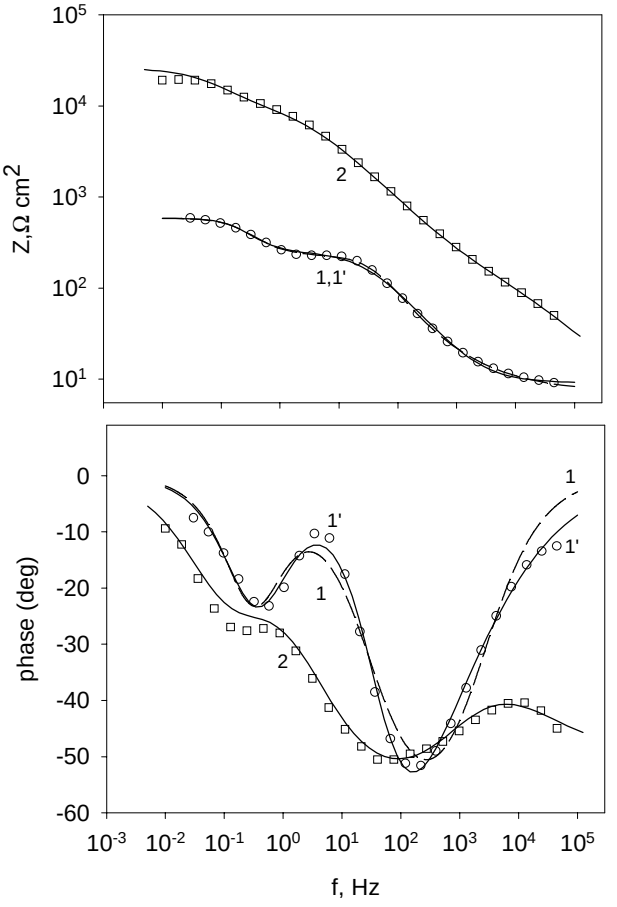

Fig. 2. EIS diagrams for pristine Zn sample (1) and samples exposed for two years to humid atmosphere at 26 °C (2). Curve 1 represents the data fitting assuming two R-CPE elements and curve 1' is a result obtained assuming three R-CPE elements

layer structure is present on a pristine zinc sample: internal (C_1R_1), intermediate (C_2R_2) and external (C_3R_3).

Information concerning the composition and structure of corrosion products was obtained by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) [21]. The diffraction patterns for the blank Zn sample as well as for samples with microorganisms, which had undergone a two-year exposure, indicated hydroxide, carbonate and chloride species to be developed on the surface. The width of the XRD peaks clearly showed that the grain size of the chloride species was much greater than that of the carbonate ones. According to XPS analysis, the surfaces contained *ca.* 13–29 at. % of carbon; this content decreased in deeper levels (*ca.* 10 at. % after 12 min sputtering with ionised argon, which corresponded ca. 2–3 nm depth).

The external layer develops as a result of the hydroxide interaction with the environment, *i.e.* atmospheric and biogenic carbon dioxide as well as NaCl solution in the measurement cell during EIS measurements. The first layer (next to metal) is typically composed of zinc oxide, and the intermediate layer is built by hydroxide. The oxide–hydroxide structure is confirmed also by other authors [22–24]. A complex corrosion product layer consisting of zinc oxide, hydroxide, and $4Zn(OH)_2$, $ZnCl_2$ was detected on zinc exposed to NaCl solutions by FTIR and *in situ* Raman spectroscopy [23, 24].

The layer thickness (d) may be characterised by the layer capacity, which is in inverse proportion to the thickness. The thickness of the entire structure on pristine Zn consists mainly of the internal and external layers, while the intermediate layer is very thin (C_1 = 12 μF cm^{-2}, C_2 = 988 μF cm^{-2}, C_3 = 19 μF cm^{-2}). (Strictly speaking, such comparison is correct assuming that there is no great difference in the relative permittivities of the films. However, this aspect may be neglected due to a great difference between C_2 and C_1, C_3).

A two-year exposure of a Zn sample leads to a clear appearance of a third time constant at high frequency domain of the phase diagram. This may result from the interaction of the external corrosion product layer with humid air. According to the capacitance values, the inner layer of both the pristine and the exposed samples are of similar thickness, whereas the thickness of the intermediate layer increases during exposure by three orders of magnitude. Likewise, the thickness of the external layer also increases *ca.* twenty times. It is important to note that the inner layer on the pristine surface has a much lower passivating capacity as compared to that on the exposed counterpart (R_1 = 0.035 kΩ cm^2 and 17.5 kΩ cm^2, respectively). Apparently the initial oxide layer has not a compact structure, which

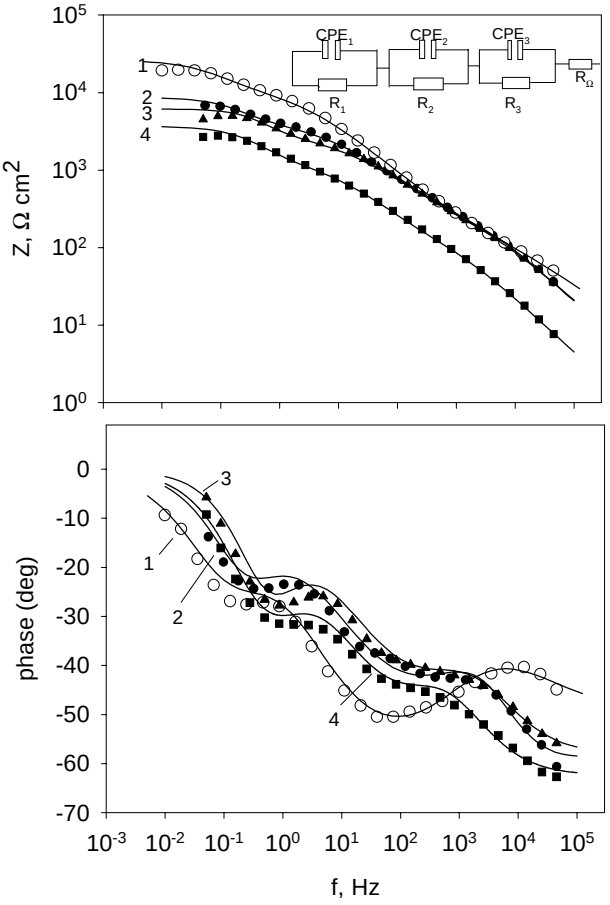

Fig. 3. EIS diagrams for blank Zn sample (1) and samples colonized by microorganisms (2–4). Samples were exposed for two years to humid atmosphere at 26 °C. An equivalent circuit, which was used for experimental data (symbols) fitting (lines), is demonstrated. *1* – blank Zn, *2 – Aspergillus niger*, *3 – Penicillium frequentans*, *4 – Bacillus mycoides*

Table. **Fitting parameters for the EIS data (Figs. 2 and 3) obtained for pristine Zn sample and that exposed for two years to humid atmosphere with and without microorganisms**

Sample	C_1, μF cm^{-2}	C_2 μF cm^{-2}	C_3 μF cm^{-2}	R_1 kΩ cm^2	R_2 kΩ cm^2	R_3 kΩ cm^2
Pure Zn, pristine	12.1	988.5	19.1	0.035	0.36	0.19
Blank Zn, exposed	11.9	0.5	0.8	17.5	0.12	8.8
Bacillus mycoides	51.6	2.6	3.2	2.60	0.05	1.0
Aspergillus niger	59.5	0.5	0.7	4.70	0.84	3.9
Penicilium frequentans	70.1	0.8	0.6	3.45	2.58	0.1

is also penetrated by solution ions during EIS measurements (due to thin and permeable external and intermediate layers).

A three-layer structure develops under the influence of microorganisms, which is evident from the phase diagrams in Fig. 3. The phase diagrams are qualitatively similar for all three kinds of microorganisms (curves 2–4), but the data differ distinctly from those obtained for the blank sample. The experimental data were well fitted by an equivalent circuit, which consisted of three R-CPE elements and solution resistance (R_Ω) in series (Table).

The EIS data provide unambiguous information that corrosion acceleration by the microorganisms lies primarily in the reduction of thickness of the internal layer (C_1R_1), which has the greatest passivating capacity. The capacitance of the first layer C_1 in the presence of microorganisms is about 4 to 6 times higher (the layer is thinner) as compared to the blank counterpart (Table 1). The R_1 value for the samples with microorganisms is accordingly less. At the same time, the corrosion products developed during MIC have a relatively low passivating capacity. This is evident from the value $R_2 + R_3$, which in case of MIC is several times lower as compared to that of the blank counterpart.

CONCLUSIONS

Microbially induced corrosion acceleration was ascertained for zinc samples by polarization resistance measurements during a two-year exposure to the influence of an ambience with constant humidity and temperature and containing *Aspergillus niger*, *Bacillus mycoides* and *Penicillium frequentans* strains. The data did not provide a clear sequence of the accelerating capability of different strains.

The EIS data indicated a three-layer structure developed on zinc under the influence of microorganisms. The reasons for the microbially induced corrosion acceleration lie primarily in the diminishing of thickness of the internal layer, which is next to metal and has the greatest passivating capacity. The corrosion products developed during MIC (intermediate and external layers) have a several times lower passivating capacity as compared to that of the counterpart free of microorganisms.

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MIKROBINËS KOROZIJOS TYRIMAI
2. EIS TYRIMAS VEIKIANT CINKÀ DVEJUS METUS *PENICILIUM FREQUENTANS, ASPERGILLUS NIGER* IR *BACILLUS MYCOIDES*

S a n t r a u k a

Toliau tirta anksèiau pradëtø ávairiø mikroorganizmø rûðiø (iðskirtø nuo eksponuotø jûrinio klimato bei miesto ir kaimo sàlygomis Lietuvoje Al, Cu, Zn ir anglinio plieno bandiniø) átaka metalø korozijai: *Penicilium frequentans, Aspergillus niger* ir *Bacillus mycoides* kultûrø átaka cinkui. Laboratorinëmis sàlygomis esant santykinei drëgmei ~98,5% ir ~26°C temperatûrai Zn bandiniai 2 metus buvo veikiami ðiø mikroorganizmø. Elektrocheminës impedanso spektroskopijos metodu nustatyta mikrobinio poveikio sàlygota Zn korozijos akceleracija. Remiantis gautais duomenimis nustatyta trisluoksnë struktûra, susidaranti ant Zn mikrobinës korozijos metu. Pagrindinë mikrobinio poveikio sàlygota Zn korozijos akceleracijos prieþastis – vidinio sluoksnio (artimiausio metalo pavirðiui) sumaþëjimas. Ðio sluoksnio pasyvacinë geba yra daug didesnë, nei kitø dviejø sluoksniø.