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Investigation of microbiologically influenced corrosion
3. Two-year exposure of aluminium to *Penicillium frequentans, Aspergillus niger* and *Bacillus mycoides*

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Aluminium samples were for two years exposed to the microorganisms *Penicillium frequentans*, *Aspergilus niger* and *Bacillus mycoides*. The strains of microorganisms were isolated from Al, Cu, Zn and steel samples, which had been exposed to marine, rural and urban sites in Lithuania. Microbially influenced corrosion inhibition (MICI) of aluminium was determined using polarization resistance as a criterion. EIS data indicated a two-layer structure on the colonized aluminium samples: a native aluminium oxide and a layer that resulted from the oxide interaction with metabolism products of microorganisms. An increase in aluminium oxide layer resistance but a decrease in the layer thickness implied that microorganisms might act as passivity promoters at the sites of localized corrosion attack (passive layer defects, pores, microcracks, etc). This conclusion was supported by scanning electron microscopy (SEM) observations.

INTRODUCTION

The present study follows our previous investigations of microbiological corrosion induced by microorganisms isolated from samples exposed in the outdoor stations covering different environmental conditions in Lithuania [1, 2]. We identified *ca*. 70 bacterial and fungal populations, which colonized metal samples during a two-year exposure to urban, suburban, rural and marine conditions [1, 2].

Microbially induced corrosion acceleration was detected for zinc samples subjected for two years the influence of *Aspergillus niger*, *Bacillus mycoides* and *Penicillium frequentans* strains [2]. EIS data indicated a three-layer structure developed on zinc affected by the microorganisms. It was concluded that the reasons for microbial corrosion acceleration lie primarily in diminishing the thickness of the inner layer which had the greatest passivating capacity. The intermediate and outer layers of corrosion products had a several times lower passivating capacity as compared to a counterpart free of microorganisms.

The microbial products of importance for microbial induced corrosion (MIC) include organic and inorganic acids, hydrogen sulphide, carbon dioxide, hydrogenase, etc. MIC is a complex interaction among the microbial population, the environment and the metal substrate. However, the main factor which ultimately determines the character of corrosion is the kind of metal substrate.

The aim of the present study was to elucidate the influence of the microorganisms *Bacillus mycoides, Aspergillus niger* and *Pennicilium frequentans* on aluminium corrosion. Aluminium and its alloys, due to a low specific weight and high strength/weight ratio, are of increasing importance in a variety of technical applications such as food equipment, chemical processing, transport and structural fields, especially where seawater exposure is involved [3]. The corro-

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sion evaluation was performed by electrochemical impedance spectroscopy (EIS) during a two-year exposure of the samples to microbial influence.

EXPERIMENTAL

The main principles of microbiological studies were described in detail previously [1, 2]. The microorganisms were isolated from Al, Cu, Zn and carbon steel samples exposed in outdoor stations arranged according to the standard ISO 9223, 8565 in the places covering different environmental conditions in Lithuania: marine – the Curonian Spit, Preila (100 m from the Baltic See coast); rural – Kulioniø and Rûgðteliðkiø villages in Molëtø and Utenos districts; urban – the central part of Vilnius (Institute of Chemistry) and suburbs of Vilnius (Experimental Base of the Institute of Chemistry).

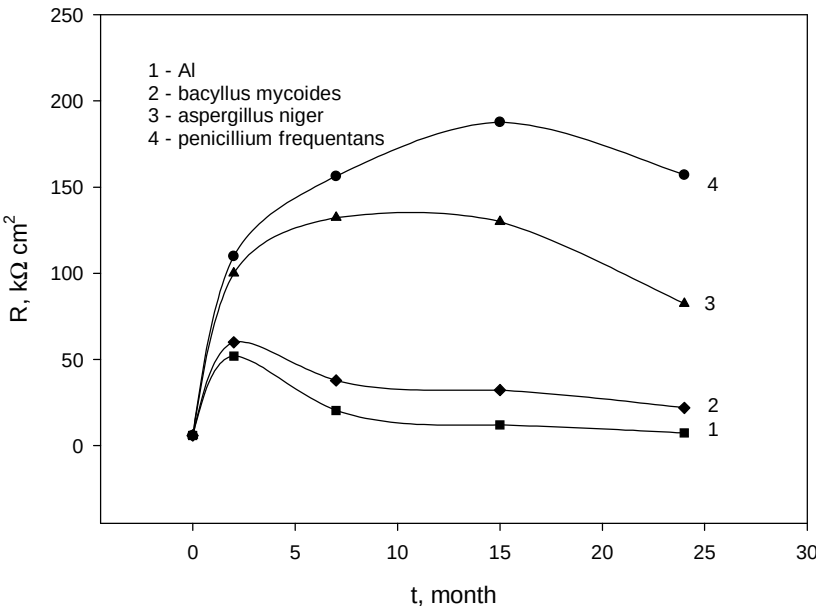

Fig. 1. Polarization resistance (R_p) change during two-year exposure of Al to humid atmosphere and of Al subjected to the effect of *Penicilium frequentans*, *Aspergilus niger* and *Bacillus mycoides*

Microorganisms were isolated from metal samples exposed in 2002 after 6, 9 and 12 months. The isolation was performed directly from corroding samples using a sterile metal loop or preparing suspensions of different dilution from the collected rainwater, which rinsed the samples. The microorganisms were inoculated on solid malt supplemented with antibiotics to suppress bacteria growth (for isolation of microscopic fungi) and solid meat peptone medium (for isolation of bacteria).

The microorganisms were grown at 26 ± 2 °C. Pure cultures were obtained from grown microorganisms and identified according to their physiological, cultural and morphological peculiarities, which were investigated by light and electron scanning microscopy.

The aluminium samples were subjected to the influence of microorganisms by exposing them to a humid atmosphere at a temperature of ca. 26 °C. This was performed in exposure vessels (volume 5) containing 1 l of saturated potassium sulphate solution (relative humidity *ca.* 97%). Al plates (2 cm^2 in size and 1 mm thick, purity >99.5%) were treated with emery SiC paper (grade 1200), washed with acetone and kept under ambient conditions for *ca.* 30 min and finally placed in a vertical position in the exposure vessels (four samples in each vessel). Then the samples were sprayed with a solution containing a strain of microorganisms and hermetically closed in the vessels, where they were kept at a temperature of 26 ± 2 °C. At

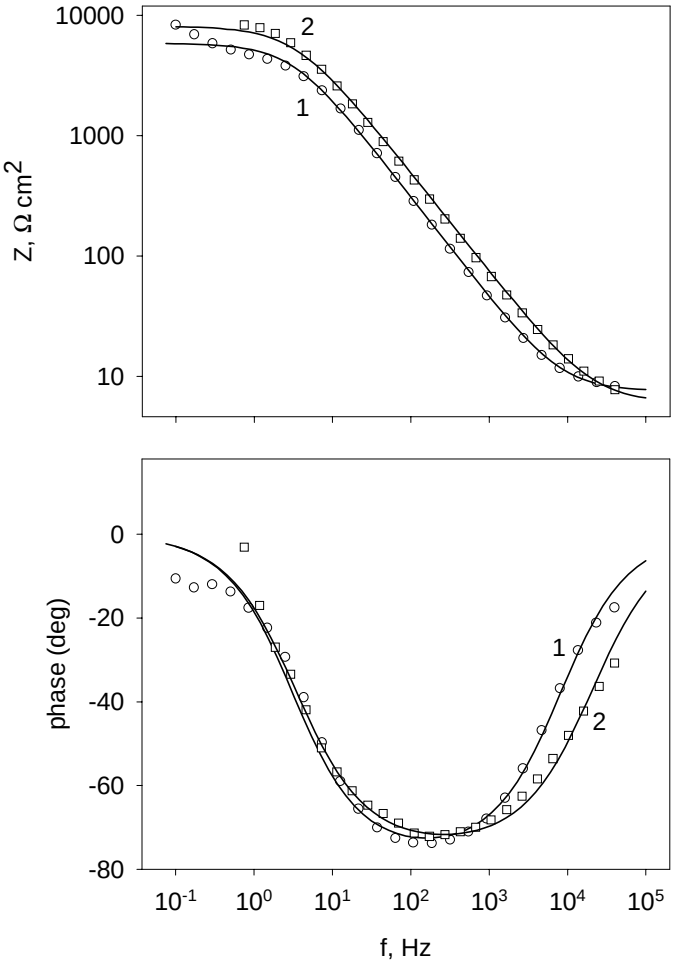

Fig. 2. EIS data for pristine Al sample and for that exposed for two years to humid atmosphere

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.

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

RESULTS AND DISCUSSION

The polarization resistance (R_p) change during two years of exposure of aluminium samples is shown in Fig. 1. As commonly known, the corrosion current density is in inverse proportion to R_p. The corrosion activity of the blank sample does not change significantly during the exposure (curve 1). It is also obvious that the samples colonized by microorganisms exhibit a much lower activity (curves 2–4). An especially great inhibiting influence is characteristic of *Penicilium frequentans*: the highest R_p value (after 15 month exposure) is more than twenty times higher as compared to the counterpart free of microorganisms (curves 4 and 1, respectively). The data show the following sequence of the inhibiting capability of the strains studied: *Penicilium frequentans* > *Aspergilus niger* > *Bacillus mycoides.*

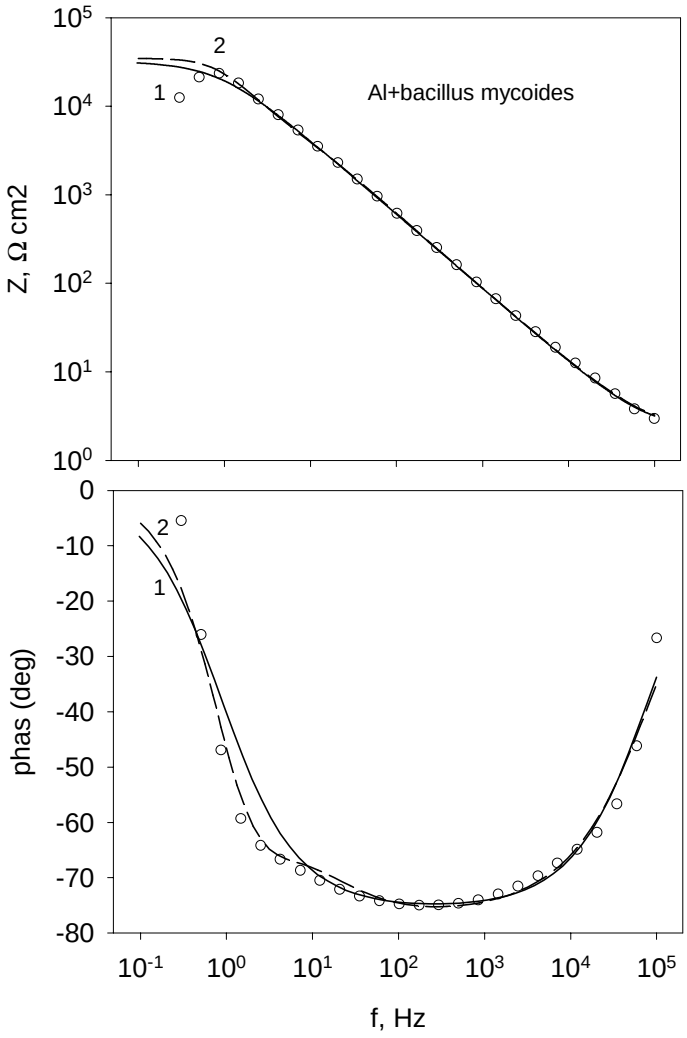

Fig. 3. EIS data for Al sample with *Bacillus mycoides* exposed for two years to humid atmosphere. The dotted line represents the data fitting assuming two RC elements and the continuous line shows the result with one RC element assumed

The EIS diagrams (the impedance Z dependence on frequency f) for pristine Al and that exposed to a humid atmosphere for two years are given in Fig. 2. The data were fitted using a one time constant model, *i.e.* an equivalent circuit consisting of one R_t-*CPE* element (charge transfer resistance – constant phase element) and the uncompensated ohmic resistance (R_Ω) in series. This model assumes one passive layer to be developed on aluminium (the fitting parameters are given in Table 1). The impedance values at a low frequency domain ($Z_{\omega\to 0}$), which characterizes the polarization resistance, show a high degree of stability: the sample activity does not change significantly during a two-year exposure to a humid atmosphere. At the same time, a divergence in phase angle data appears during the exposure.

Aluminium is known to corrode not uniformly because of preferential attack on grain boundaries. The localized corrosion of Al alloys was indicated by EIS as an appearance of a second time constant in the low frequency region [4, 5]. The authors have pointed out that the effect is usually partially masked by the scatter of the data at low frequencies. The data in Fig. 2 suggest the possible contribution of the localized corrosion phenomena, which is evidenced by the typical phase angle divergence at low frequencies (symbols) from the one time constant model (line). A typically observed low-frequency minimum is partially masked by the data scatter below 0.1 Hz. It was difficult to interpret the analogous data obtained for the exposed sample (curve 2, Fig. 2) due to a great data scatter of the experimental data below 1 Hz (the scattered data are not shown in the figure).

Figures 3–5 show the EIS diagrams obtained for samples colonized by microorganisms. The data for each sample were fitted assuming one and two R_t-*CPE* elements and R_Ω in series. There are some distinctive zones on the phase angle diagrams, which

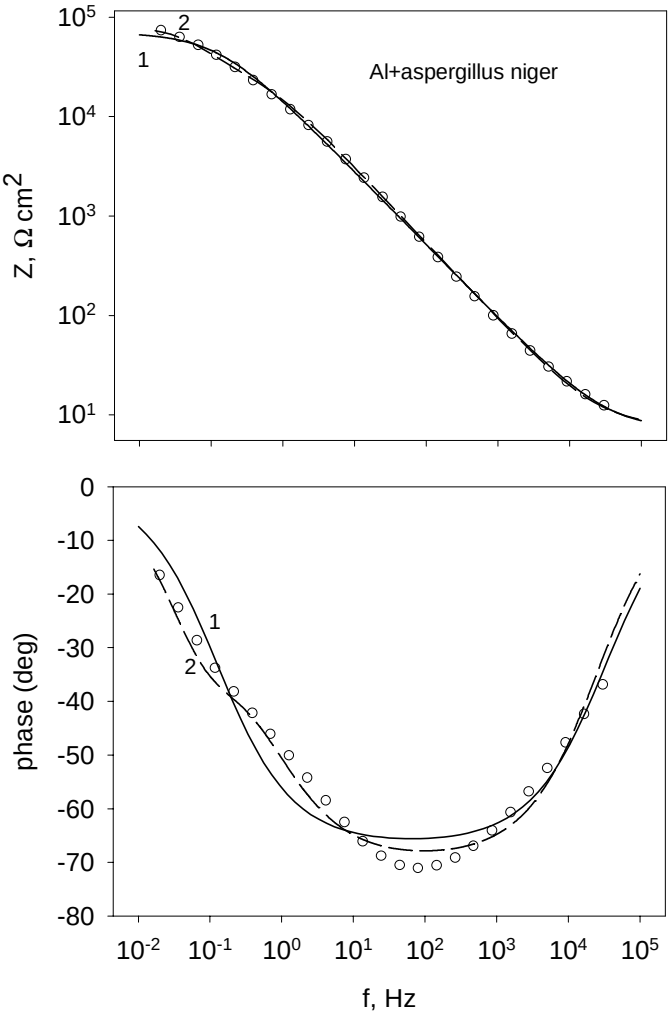

Fig. 4. EIS data for Al sample with *Aspergilus niger* exposed for two years to humid atmosphere. The dotted line represents the data fitting assuming two RC elements and the continuous line shows the result with one RC element assumed

are in a better agreement with the model of two R_t-*CPE* elements. Thus, EIS measurements indicate a two-layer structure to be developed on Al during MIC. The first layer should be composed of an aluminium oxide and the second one should result from the oxide interaction with microorganisms (the oxide/biofilm interface). Each layer is characterized in Table 1 by the charge transfer resistance (R_1 and R_2) and capacitance (C_1 and C_2). The latter define the layer thickness, which is in inverse proportion to the capacitance (strictly speaking, this is true assuming that the relative permittivity does not differ significantly for different layers).

The studies of the samples performed by X-ray diffraction (XRD) indicated a mostly thick corrosion product layer on the sample with *Penicilium frequentans*, while the thinnest layer was characteristic of the sample without microorganisms [6]. These results are in agreement with those of EIS investigations, which led to analogous conclusion (Table 1).

It is interesting to note that even a three-layer structure was identified by EIS on the aluminium upon exposure to the atmosphere [7]. The structure consisted of a native oxide, a corrosion layer on the oxidized aluminium and a surface contamination layer (mainly sulphate and chloride species).

The R_1 value for the colonised samples was much higher than that for blank Al (R_1 = 31÷153 kΩ cm² *vs.* R_1 = 8.2 kΩ cm², respectively). At the same time, the inner layer on the samples with microorganisms was several times thinner (*Bacillus mycoides* and *Aspergillus niger*) or had a similar thickness (*Penicilium frequentans*).

The contribution of the outer layer to charge transfer kinetics was less as compared to that of the inner one. For instance, the outer layer in case of *Pennicilium frequentans* had the resistance R_2 = 4.5 kΩ cm², while the resistance of the inner layer was R_1 = 153 kΩ cm². This difference is quite understandable considering that the outer layer may have a disordered and porous structure due to influence of microorganisms.

Thus, a conclusion may be drawn that the reasons for the inhibiting action of microorganisms lies primarily in the increase of the charge transfer resistance through the inner oxide layer. It is important to stress that this effect cannot be attributed to the increase of the layer thickness.

As already noted, aluminium usually corrodes not uniformly because of preferential attack on grain boundaries, which results in a symptomatic feature – appearance of a second time constant in the low frequency region of the phase angle diagram. The determined increase in R_1 and decrease or similarity in d imply that microorganisms may affect the weak

Table. 1. **Fitting parameters for the EIS data (Figs. 2–5) obtained for pristine Al sample and those exposed to humid atmosphere for two years**

Sample	C_1, μF cm^{-2}	C_2 μF cm^{-2}	R_1 kΩ cm^2	R_2 kΩ cm^2
Al, pristine	3.6	–	5.9	–
Al, exposed	2.2	–	8.2	–
Al + *Bacillus mycoides*	7.5	2.3	31.3	3.5
Al + *Aspergillus niger*	14.7	2.0	59.9	22.5
Al + *Penicilium frequentans*	1.7	40.3	152.6	4.5

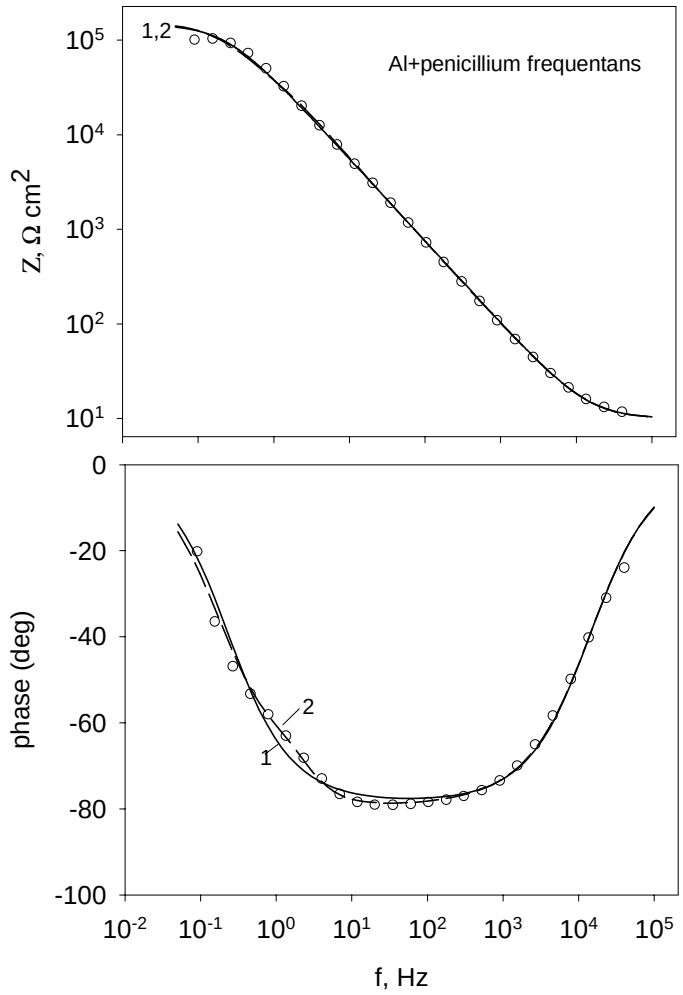

Fig. 5. EIS data for Al sample with *Penicilium frequentans* exposed for two years to humid atmosphere. The dotted line represents the data fitting assuming two RC elements and the continuous line shows the result with one RC element assumed

sites at the metal/oxide interface (defects, pores, microcracks, etc.) where localized corrosion may take place. Microorganisms colonize these sites preferentially and the induced MIC process causes development of aluminium oxide which, being a valve metal oxide, has a great barrier effect on electron transfer. In other words, microorganisms may act as promoters of a "healing" process at the sites of localised corrosion attack.

The above suggestion supports observations by scanning electron microscopy (SEM) (Fig. 6). The micrograph clearly shows that the aluminium sample is colonised by *Aspergillus niger* preferentially at the microcracks of the oxide layer.

The results presented here with Al samples and those dealing with Zn samples presented previously [2] demonstrate that the microorganisms under study may act both as corrosion accelerators and inhibitors, depending upon the kind of metal they colonize. Pure zinc corrodes more or less uniformly (no deep pitting observed in many cases) with development of a thick and porous corrosion product layer of a low passivating capacity. By contrast, localised corrosion is characteristic of aluminium; its passive layer is very thin (several nanometers), but has a high insulating capacity. The microorganisms are capable of disintegrating the corrosion product layers on zinc, while the aluminium oxide layer is generally stable against microorganisms. If local MIC attack occurs, it leads to passivation of these weak sites due to a local development of aluminium oxide – a highly insulating barrier to charge transfer.

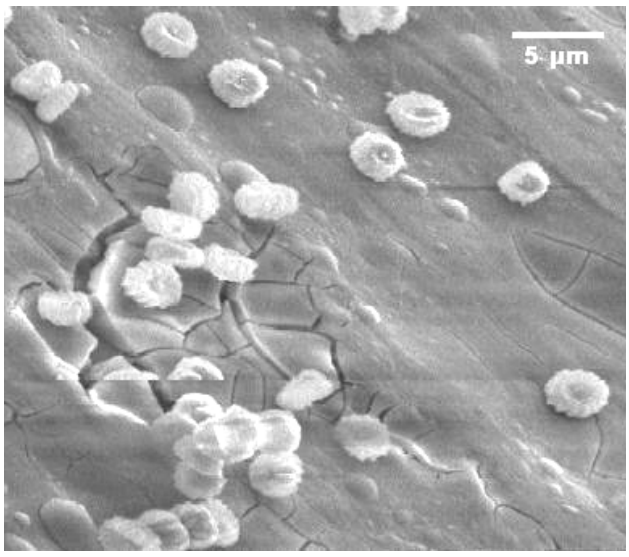

Fig. 6. SEM micrograph of an Al sample with *Aspergilus niger*, which shows colonization at surface microcracks to be preferential

CONCLUSIONS

Microbially induced corrosion inhibition was determined for aluminium samples. The microorganisms were in the following sequence of inhibiting capability: *Penicilium frequentans* > *Aspergilus niger* > *Bacillus mycoides.* EIS measurements indicated a two-layer structure developed on Al during MIC: the inner aluminium oxide layer and the outer one, which develops due to the oxide interaction with metabolism products of the microorganisms. An increase in charge transfer resistance but not in layer thickness implies that microorganisms promote locally the passivity at sites of microdefects (pores, cracks, etc.). A preferential colonization of microcracks is evident from SEM investigations.

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MIKROBINËS KOROZIJOS TYRIMAI
3. *PENICILIUM FREQUENTANS, ASPERGILLUS NIGER* IR *BACILLUS MYCOIDES*
MIKROORGANIZMØ DVEJØ METØ POVEIKIS ALIUMINIO KOROZIJAI

Santrauka

Dvejus metus laboratorinëmis sàlygomis Al bandinius veikë mikroorganizmai *Penicilium frequentans*, *Aspergillus niger* ir *Bacillus mycoides.* Šie mikroorganizmai buvo išskirti nuo Al, Cu, Zn ir anglinio plieno pavyzdþiø, eksponuotø jûrinio klimato, kaimo bei miesto sàlygomis Lietuvoje. EIS tyrimais nustatyta, kad ant paveikto mikroorganizmais Al pavirðiaus susiformavo du sluoksniai: natûralusis aliuminio oksido sluoksnis ir kitas sluoksnis, susidaræs sàveikaujant oksidui su mikroorganizmø metabolizmo produktais. Naudojant poliarizacijos varþà kaip kriterijø nustatyta mikrobinio poveikio sukelta Al korozijos inhibicija. Ið aliuminio oksido sluoksnio poliarizacinës varþos padidëjimo ir ðio sluoksnio storio sumaþëjimo seka, kad mikroorganizmai gali veikti kaip pasyvacijos skatintojai lokalizuotos korozijos vietose (pasyvaus sluoksnio defektai, poros, mikroplyðiai ir t. t.). Ði iðvada buvo patvirtinta skenuojanèios elektroninës mikroskopijos duomenimis.