QCM study of microbially induced corrosion of aluminium exposed to Aspergillus niger Tiegh.

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INTRODUCTION

The action of microorganisms on metals includes numerous phenomena, e. g. the production of corrosive metabolites (inorganic and organic acids, sulphide, ammonia, carbon dioxide, nitrogen oxides, etc.), chelatization of metal cations, production of organic solvents (ethanol, propanol, butanol), etc. Due to a non-uniform surface colonization, microorganisms may cause the formation of concentration cells, which results in localized corrosion. The corrosion process is affected also by favoured water uptake by the biofilm [1].

In general, microorganisms may cause either a microbially influenced corrosion acceleration (MICA) or an inhibition (MICI). MICA has recently been widely studied [1–13], while relatively little is known about the MICI process. A remarkable protective effect of the biofilms was observed in solutions for aluminium alloys and brass (70Cu/30Zn) [13–17]. It has been shown that a pronounced pitting attack took place in a sterile medium, whereas in the solutions containing bacteria, the pitting process was stopped after two days of exposure. The authors have also performed experiments with genetically engineered bacteria capable of producing inhibitors – polyglutamate or polyaspartate. However, they came to the conclusion that even the bacteria that were not engineered to produce inhibitors passivated the surface.

MIC is a complex interaction between the microbial population, environment and metal substrate; the latter could be of the ultimate importance on the character of MIC. Most of the studies of microbially influenced corrosion (MIC) were performed in aqueous solutions (artificial seawater, Luria-Bertani medium, etc.) in the course of a relatively fast formation of the biofilm. Recently the MICI effects have also been determined under atmospheric conditions [18, 19]. The conclusions were drawn from two-year studies of metals influenced by single wild strains (e. g., Penicillium frequentans, Bacillus mycoides), which were isolated from a wide variety of bacterial and fungi populations (over 170) on metals exposed to outdoor conditions [20, 21]. From this microbial diversity, Aspergillus niger appeared to be one of the most stable populations.

In our previous studies [18, 19], electrochemical impedance spectroscopy (EIS) ascertained the microbially influenced corrosion inhibition of Al. An increase in the aluminium oxide layer resistance with a simultaneous decrease in the thickness of the layer implied that MICI primarily affected the sites of the localized corrosion of Al (pores, micro-cracks, etc.). X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) studies indicated that the bioproducts (i. e. organic acids) did not form crystalline phases with corrosion products of Al. The study suggested that microorganisms could be used as corrosion protectors instead of toxic chemicals, the application of which tends to be increasingly restricted.
The present study aims at getting a deeper insight into the influence of *A. niger* on aluminium corrosion using quartz crystal microgravimetry (QCM) modified with glued foil electrodes and applied to long term exposures to atmosphere. The QCM provides the possibility to obtain sensitive (ng cm\(^{-2}\)) and continuous mass change data in-situ as well as the possibility to combine the mass measurements with other techniques (voltammetry, EIS, optical spectroscopy, etc.). The results with the glued foil electrodes reported here suggest the possibility to expand the scope of materials to be studied by QCM, as the conventional QCM studies are usually limited to evaporated, sputtered or electroplated materials. There are only few works in literature where glued foil QCM was applied for electrochemical studies [22, 23]. The subject under investigation was aluminium, i.e., a metal of great technical importance. Localised corrosion is characteristic of aluminium; the passive layer on it is thin (in the order of nanometers) with a high insulating capability. Aluminium is also of interest because it plays an important role in the metabolism processes of *A. niger*. An enhanced formation of *A. niger* conidia was determined in the medium with aluminium ions at a concentration of 0.001 mg l\(^{-1}\), whereas at higher concentrations the inhibition of fungal growth took place [24].

**EXPERIMENTAL**

Micromycetes of *Aspergillus niger* species were isolated from Al, Cu, Zn and steel samples exposed to atmosphere at 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 shore of the Baltic Sea); rural – Kulionys and Rūgštelėskiai villages in Molėtai and Utena districts; urban – the central part of Vilnius (Institute of Chemistry) and its suburb (Experimental Base of the Institute of Chemistry). Climatic parameters, air composition and water adsorption on the metal samples were measured continuously at these sites (for more details, see [21]).

The microorganisms were isolated from metal samples after 6, 9, and 12 months of exposure. The isolation was performed in two ways: 1) directly from the corroded samples using a sterile metal loop and 2) preparing suspensions of different dilution from rainwater which rinsed the samples. The microorganisms were inoculated on solid malt supplemented with antibiotics to suppress bacterial growth, for isolation of microscopic fungi.

The isolated microorganisms were cultured at 26 ± 2 °C. The grown colonies of fungi were counted after 3, 5 and 7 days. A pure culture was obtained from the grown microorganisms and was identified according to its physiological, cultural and morphological peculiarities by light and electronic scanning microscopy. The fungal species were identified according to various manuals [24–29].

“Plano-convex” AT-cut quartz discs with the fundamental frequency of 2.4 MHz and 14 mm in diameter were used. The “convex” sides of the resonators were magnetron-sputtered by a 3 mm Au layer. The “plane” sides of the resonators were covered with Al foil. Aluminium electrodes were prepared from 15 µm thickness Al foil of >99.5% purity. Both foil sides were thoroughly cleaned with acetone of high purity and “keyhole-shaped” electrodes 12 mm in diameter were cut off. The Al electrodes were glued with epoxy resin to the “plane” side of the quartz discs. After that, the discs were mounted to a special holder and were kept under 16 kg cm\(^{-2}\) pressure at ambient temperature for 24 hours.

According to the Sauerbray’s equation, the mass to frequency factor of the glued Al resonators for fundamental frequency of 2.4 MHz was 0.078 µg Hz\(^{-1}\) cm\(^{-2}\) [30].

The prepared Al resonators were mounted into a special chemically resistant plastic holder between two silicone rings. Stainless steel wires were used to provide electrical contacts of electrodes with a frequency measurement set. The exposed area of the Al electrodes was 0.5 cm\(^2\).

Measurements were carried out in ~2.5 l vessels. The vessels were capped with special chemically resistant plastic covers with holes designed for quartz holders. The holders with Al were impermeably mounted in the vessels. The working surface of the resonators was oriented horizontally downward. A saturated K\(_2\)SO\(_4\) solution was poured to the bottom of the vessel in order to maintain constant humidity. The open area of solution was ~35 cm\(^2\). The relative humidity settled inside the vessel was ca. 97% at a temperature of ca. 25 °C.

Before the beginning of the exposition (experiment), the frequencies of the dry Al resonators were measured, and after that approximately half of the electrode area was directly overlayered with a 3% glucose solution with *Aspergillus niger* conidia. The spore concentration was ca. 1 × 10\(^6\) cfu ml\(^{-1}\) (cfu – colony forming units), which was determined using a special counting chamber. Alongside the “infected” vessel an “abiotic” one was also prepared. Both experimental sets were placed inside a box where a temperature of 26 ± 2 °C was maintained. Throughout the exposure, the atmospheric pressure inside the box was monitored.

The frequencies of the exposed Al resonators were periodically measured by using an oscillator device constructed according to a scheme with an operational amplifier [31]. During the exposition, the vessels were not moved, and the resonators were not withdrawn from it. Before each measurement, the oscillator set was calibrated. The changes in frequency of the resonators were recalculated into the ones of the electrode mass [30].

After the experiments, the resonators were extracted from the vessels and the Al electrodes surfaces were examined on an optical microscope MB-9. The vitality of the microorganisms was checked by a replica method (more details are presented below).
RESULTS AND DISCUSSION

A partial coverage of the surfaces of the electrodes by a 3% glucose solution with/without Aspergillus niger provided the opportunity to evaluate the fungus growth and its self-spreading on the studied metal during exposure. The optical photograph of the Al electrode surface after 90 days of exposure is shown in Fig. 1a. As it was determined after the electrode surface examination, the colony of A. niger was formed and it self-outspread over the electrode surface. It was evident that micelium had originally been formed on the “glucose-coated” surface and only later it colonized the residual electrode part. The micelium was not dense and the dimensions of the fungus fruitbodies were not uniform throughout the surface. The A. niger fruitbodies formed on the “glucose-coated” electrode area were considerably larger than those formed on the free area: ~0.2 mm in diameter. It is important that the corrosion pits were promptly formed under large fruitbodies. These damages most probably occurred due to the enzymes and organic acids secreted by A. niger: citric, oxalic, acetic, etc [32]. For the “glucose-free” Al area, the above phenomena were not determined. In addition, one of the samples was checked for the vitality of microorganisms by taking replicas from its surface on a beef-extract agar (by pressing the sample surface into the agar). The replica was exposed to the temperature of 26 ± 2 °C for 5 days and then was inspected by optical microscopy (Fig. 1b). As one can see, the colonies of A. niger were grown from the replica, which proved the vitality of the inoculated microorganisms. For the abiotic medium the absence of any microorganisms was detected by a vitality test.

For the most correct evaluation of A. niger contribution to the overall Al corrosion process, QCM measurements with Al and Pt in an abiotic humid medium were performed. Figure 2 shows the mass change of the above metals exposed to humid (97%) atmosphere. The initial mass growth on the inert metal Pt is attributable to water adsorption on the metal surface. The entire mass of the adsorbed water on the Pt surface was about 5 µg cm⁻². This is about five times as high as the analogous value determined on the Ni-Cr-Mo alloy in a highly humid Ar atmosphere: ca. 1.1 µg cm⁻² after 2.5 h of exposure [33]. Lee and Staehle, who studied water adsorption on gold at different humidities at 25 °C, determined very close values [34].

The mass change for Al is higher because it may account for water adsorption plus corrosion due to the interaction of aluminium with water and the formation of Al₂O₃ layer. This layer has a high insulating capability, thus, it may be assumed that after a certain initial time, the surface gets passivated, as it is obvious from a very slow increase in the mass: dm/dt ≈ 0.018 ng s⁻¹ cm⁻².

The Al mass changes during a long-term exposure in A. niger environment and in an abiotic environment are shown in Fig. 3. It is clearly seen that the increase in the mass of the biotic sample is much higher than that of the abiotic sample. While the increase in the mass of the abiotic sample is attributed to the corrosion and accumulation of oxide Al₂O₃, the effect for the biotic sample is due to the colonization of the surface with A. niger and associated phenomena such as the increase in water.
uptake, bio-corrosion due to the action of metabolites, etc. The dotted line gives the atmospheric pressure change, which has some influence on water adsorption and, consequently, on mass, the data which are detected as slow oscillations on the mass curves. The atmospheric pressure at the “initial point” of the experiment was ca. 101.8 kPa (ca. 764 mm Hg). The maximum value of the pressure registered during the exposure was ca. 102.2 kPa, the minimum one – ca. 99.2 kPa. As it can be seen, the mass changes of the studied electrodes were synchronous with inverse pressure fluctuations. It is known that the increase in the atmospheric pressure leads to the decrease in the water vapour concentration in air and to a corresponding decrease in the moisture adsorbed onto the metal surface [35]. The electrode mass decrease during the first ~15 days was reliably stipulated by water evaporation from the glucose solution layer. The influence of the atmospheric pressure on the QCM data will be discussed in more detail hereafter.

The obtained data clearly show that QCM may be successfully applied to study a development of a microbial population on metal surfaces. Moreover, the performed experiments with the glued foils suggest some progress in the field because the foils may be prepared from bulk materials of great technical importance, for instance, various alloys used in medicine, construction, computer technology, aircraft industry, etc.

CONCLUSIONS

Quartz crystal microgravimetry was applied as a sensitive detector of mass change to study development of the microbial population of wild strain *Aspergillus niger* Tiegh. on aluminium. The dynamics of the formation of a biofilm was clearly detected by a comparison of QCM data for abiotic and biotic samples. The influence of atmospheric pressure on the QCM data during long lasting exposures was demonstrated. The QCM was modified with glued foil electrodes, which suggested the expansion of the scope of the materials to be studied by QCM.

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ASPERGILLUS NIGER TIEGH. POVEIKIO ALUMINIO MIKROBINEI KOROZIJAI TYRIMAS KKM METODO

Santrauka
Aspergillus niger Tiegh. rūšies mikromicetų buvo įskirti nuo metalų paviršių, eksponuotų prie jūros, kaime ir miesto vietove. Kljuojo ant kvarco Kristalų rezonatorių sąnes nepertraukiamai eksponuoti modelinėje atmosferoje, kurioje buvo palaikoma temperatūra 26±2°C ir santykinis oro drėgnumas ~97%. Duomenų, gautų kvarco kristalų mikrogravimetrines metodus Aspergillus niger užkristi ir sterilioje aplinkoje, palyginimas parodė, kad pirmuoju atveju, dėl susidarančių biosluoksnių ir mikrobinės korozijos, elektrodų masės augimą daugiau gausiai kaip daugiausiai nematetų atmosferinėje korozijai ir nenutrūkstamai registruoti proceso eiga ilgalaikės ekspozicijos metu.