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ORIGINAL PAPER

QCM study of microbiological activity during long-term exposure to atmosphere—aluminium colonisation by *Aspergillus Niger*

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Abstract The study demonstrated a possibility to sense the activity of microorganisms on metals in situ under atmosphere conditions using a quartz crystal microbalance (QCM) as a sensitive mass change detector. Other innovative aspects of the QCM application include long-term monitoring (over month), taking count of the influence of atmospheric pressure and application of Al-glued foil electrodes. The research subject was aluminium colonisation by *Aspergillus niger* Tiegh., a filamentous ascomycete fungus. The difference between the QCM data for abiotic and biotic samples reflected microbiological activity, which resulted in exponential mass gain during exposure. The increase in mass was due to various phenomena, i.e. development of biomass, secretion of metabolites, water uptake by the colony and microbially induced corrosion. The glued foil method demonstrated a possibility to expand the scope of the QCM studies from evaporated, sputtered or electroplated materials to those, from which thin foils may be produced.

Keywords QCM · Microgravimetry · Aluminium · Microbiological corrosion · *Aspergillus niger*

Introduction

Numerous phenomena take place when microorganisms colonise metal surface, e.g. production of corrosive metabolites (inorganic and organic acids, sulphide, ammonia, carbon dioxide, nitrogen oxides, etc.), chelatisation of metal cations, production of organic solvents (ethanol, propanol, butanol), etc. Due to non-uniform surface colonisation, microorganisms may cause localised corrosion, which is affected also by favoured water uptake by the biofilm [1]. In general, microorganisms may cause either microbially influenced corrosion acceleration (MICA) or inhibition (MICI). Although MICA has been widely studied [1], relatively little is known about the MICI process.

The protective effect of biofilms was observed on aluminium alloys and brass (70 Cu/30 Zn) [2–6]. It has been shown that a pronounced pitting attack took place in sterile medium, whereas in the solutions containing bacteria, the pitting process was stopped after 2 days of exposure. The studies of the microbial influence deal with the processes in solutions (artificial seawater, Luria-Bertani medium, etc.), where relatively fast formation of biofilm occurs. However, MICI effects were recently also determined under atmospheric conditions [7–9]. The conclusions were drawn from 2-year observations when exposing the metals to wet atmosphere under influence of wild strains *Penicillium frequentans*, *Bacillus mycoides* and *Aspergillus niger*. Electrochemical impedance spectroscopy (EIS) ascertained that MICI affected primarily the sites of localised corrosion of Al (pores, microcracks, etc.). X-ray diffraction and X-ray photoelectron spectroscopy studies indicated that bioproducts did not form crystalline phases with corrosion products of zinc.

The advantages of quartz crystal microgravimetry (QCM) or quartz crystal nanogravimetry include high sensitivity (ng cm^{-2}), continuous data in situ, possibility to monitor the processes nondestructively, as well as to combine the measurements with other techniques (voltammetry, EIS, optical spectroscopy, etc.). The QCM corrosion

Dedicated to Professor Dr. Algirdas Vaškelis on the occasion of his 70th birthday.

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sensors have attracted considerable attention in recent years to study the stability of magnetron-sputtered materials [10–12] and photo-corrosion [13].

Applications of microgravimetry for microbiological studies on inert electrodes (gold) in aqueous environments were reported [14–16]. A monitoring of formation of *Pseudomonas capacia* in a flow cell during 2 days was performed by Nivens et al. [14]. The detection limit of the used technique was determined to be 3×10^5 cells per square centimetre. Bressel et al. [15] used the QCM in combination with optical and electrochemical methods to monitor (over 3 days) the colonisation of inert surface in flow cells. The microbial-mixed culture from a drinking water biofilm was studied. QCM monitoring (over 6 days) of formation of biofilm of *P. aeruginosa* on gold was recently reported by Reipa et al. [16]. The authors combined QCM measurements with reflectance of white light, which allowed studying viscoelastic properties of biofilm.

The present study focuses on the QCM sensing of microbiological activity under atmosphere conditions. The results reported here include several new aspects: (1) long-term exposures (over a month); (2) influence of atmospheric pressure on QCM data; (3) QCM sensing with Al-glued foil electrodes.

Application of QCM in material science has a drawback due to limited choice of materials, which are limited by evaporated, sputtered or electroplated coatings. A progress in the field was suggested by Bucur et al. [17], which introduced a QCM with glued foil electrodes. Later, such approach was used to study anodic dissolution of stainless steel [18]. The glued foil method was applied also in the present study to produce aluminium QCM sensor.

A. niger on aluminium was chosen as a subject of investigation. Our previous studies showed that *A. niger* acts as an inhibitor of Al corrosion and suggested a hypothesis that microorganisms could be used as corrosion protectors instead of toxic chemicals, application of which, due to environmental regulations, tends to be increasingly restricted [7, 8]. Aluminium is also of interest because it plays an important role in the metabolism processes of *A. niger*. 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 inhibition of fungal growth took place [19].

Experimental

Microorganisms were isolated from metal samples exposed to atmosphere at outdoor stations arranged according to the standard ISO 9223, 8565 in the places covering different environmental conditions in Lithuania. Climatic parameters, air composition and water adsorption on metal samples were measured continuously at these sites.

Microorganisms were isolated from the metal samples after 6, 9 and 12 months of exposure. The isolation was performed in two ways: (1) directly from corroding samples using a sterile metal loop and (2) preparing suspensions of different dilution from the rainwater, which rinsed the samples. The microorganisms were inoculated on two media: (1) solid malt, supplemented with antibiotics to suppress bacteria growth, for isolation of microscopic fungi, (2) beef-extract agar for bacteria isolation. More details on the isolation, identification, growing and obtaining pure culture are given elsewhere [7–9].

The At-cut quartz discs ("plano–convex") of the fundamental frequency of 2.4 MHz were used to prepare the QCM sensors. The "convex" side of the specimen was sputtered by a 3-μm layer of gold. The plain side was cleaned with acetone and glued with Al foil. Commercially available Al foil, with a purity of 99.5% and thickness of 15 μm, has been used. The foil was glued with epoxy resin to the quartz surface, mounted in a special holder and kept under 16 kg cm^{-2} pressure for 24 h under ambient conditions. According to Sauerbrey's [20] equation, the mass to frequency factor for the 2.4 MHz resonator was C=78 ng cm^{-2} Hz^{-1}.

The quartz specimens were mounted between two silicone rings (the exposed area—0.5 cm^2) and fixed in a special chemically resistant plastic holder. The metal surface was cleaned by acetone, dried and sprayed with a 3% glucose solution containing *A. niger* conidia (about 10^6 cfu ml^{-1}, cfu—colony forming units). The reference sample was sprayed with the glucose solution free of microorganisms. Then, the holders were mounted into the wholes in the cover of the exposure vessel, and it was closed. The exposure vessels contained some of a saturated potassium sulphate solution, which maintained relative humidity at approximately 97%. The exposure vessel was kept in a glass container with a thermostat, which maintained the temperature at 26±2 °C. Two wires provided connection of the quartz oscillator in the exposure vessel to the frequency-counting equipment outside the exposure area.

After 3 months of exposure, the samples were checked for vitality of the microorganisms by taking replicas from its surface on different agar media: malt extract, maize and Czapek's agar. The replica samples were exposed at 26±2 °C temperature for 5 days and, then, inspected by microscopy. The colonies of *A. niger* were grown from the taken replicas, which indicated vitality of the inoculated microorganisms.

Results and discussion

Different evolution stages of *A. niger* on Al are shown on the scanning electron microscope (SEM) micrographs

(Fig. 1): the initial colonisation with preferential localisation at microcracks (Fig. 1a), fungi growth (Fig. 1b), formation of dense mycelium and conidia colony (Fig. 1c) and numerous conidia of a new generation. The preferential colonisation of microcracks observed in Fig. 1a was discussed in more detail previously [7, 8].

Figure 2 shows the frequency change of the Al sample during subjection to influence of microorganisms. The analogous data for the abiotic sample are given for comparison. Obviously, the frequency decrease (mass gain) for the colonised sample is much higher than that for the abiotic one.

Slow synchronic oscillations for both samples are caused by the changes in atmospheric pressure due to variation in climatic conditions in the course of the experiment. The pressure change related to the initial pressure value is given in Fig. 2. It is well known that decrease in atmospheric pressure favours adsorption of water on metals [21]. Thus, an increase in mass should be observed on the QCM curve when the pressure decreases and vice versa. Such an effect is clearly demonstrated on the inert (Pt) abiotic electrode (Fig. 3).

To eliminate the influence of pressure, QCM experiments need to be performed with two sensors: an indicator (biotic) and a reference one (abiotic). When plotting the data, those for reference sample have to be subtracted from the data measured on the indicator sample, and the resultant curve shows the effect due entirely to the microbiological activity (Fig. 2b). The reference sample has to be stable enough in order not to increase its mass due to corrosion in the course of exposure. A reasonably high stability of aluminium has previously been proven by QCM measurements in aqueous environments [22]. (Note that the final value set for the abiotic sample in Fig. 2a is greater when compared to that observed at the initial stages. However, a primary reason of such mass growth is the discussed above influence of atmospheric pressure, which after 3 months, is lower when compared to the initial exposure stage.)

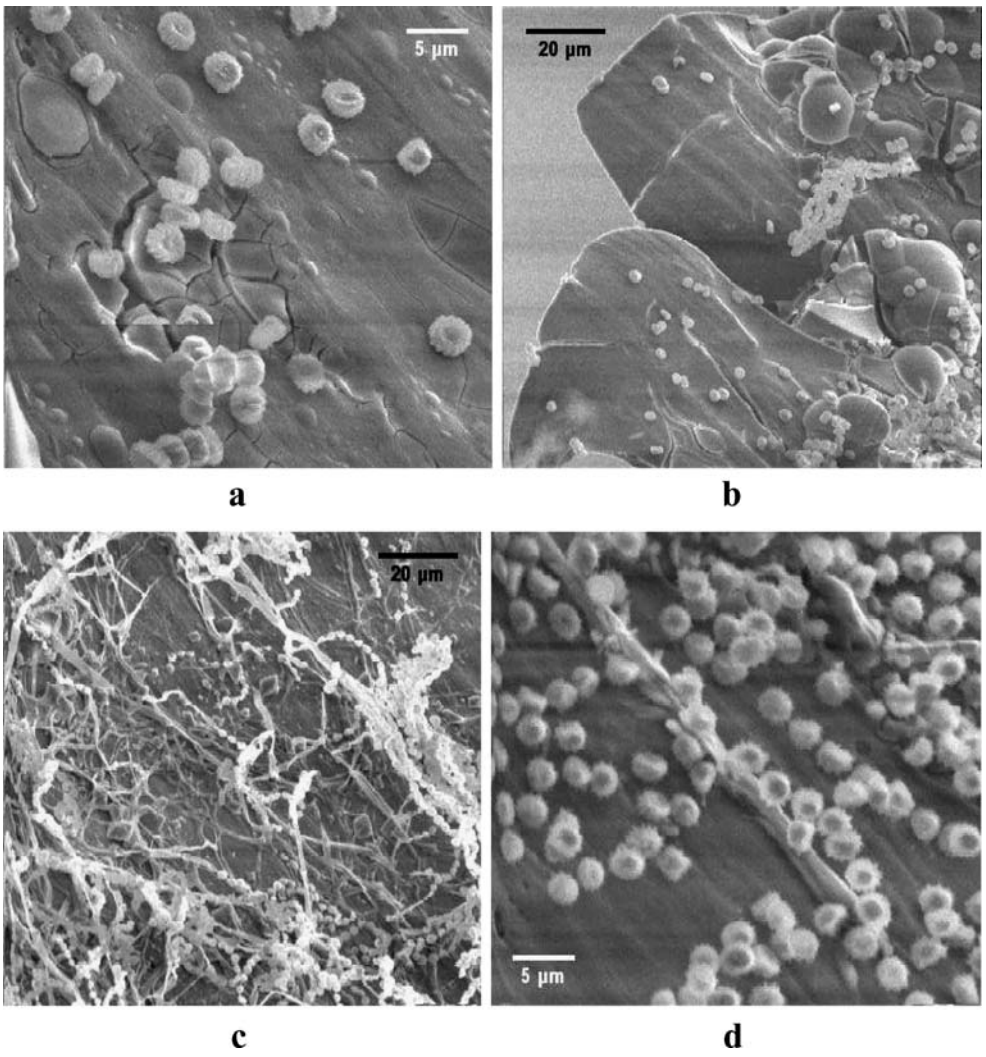

Fig. 1 SEM micrographs of Al after 1.5-year exposure under influence of *A. niger*: **a** a site of initial colonisation with preferential localisation at microcracks; **b** fungi growth; **c** formation of dense mycelium and conidia colony; **d** numerous conidia of new generation

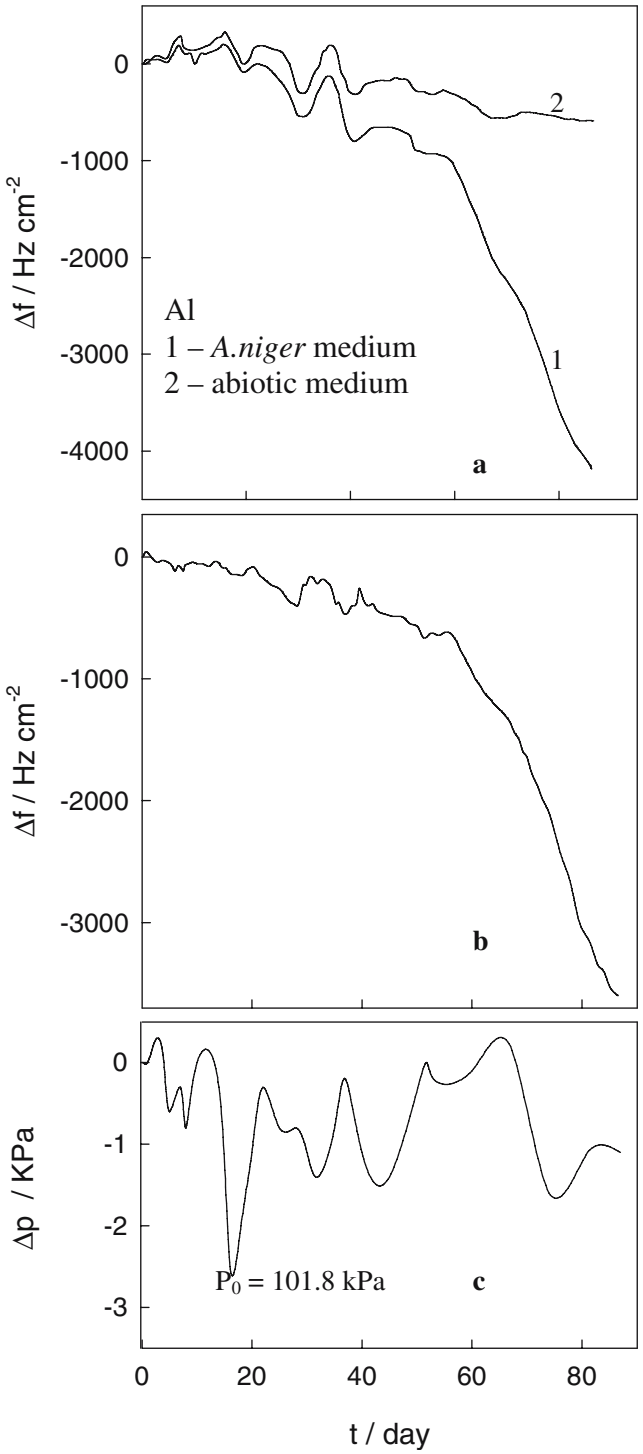

Fig. 2 **a** Frequency change of Al sample determined by QCM when subjected to influence of *A. niger* (*1*) and for abiotic sample (*2*); **b** microbiologically induced mass change obtained subtracting the reference data of sterile sample; **c** atmosphere pressure change relatively to the initial pressure

The microbiological activity increases exponentially after some incubation period (Fig. 2b). The increase in mass may be attributed to several phenomena, i.e. development of biomass, secretion of metabolites, the uptake of water by the colony and accumulation of corrosion products

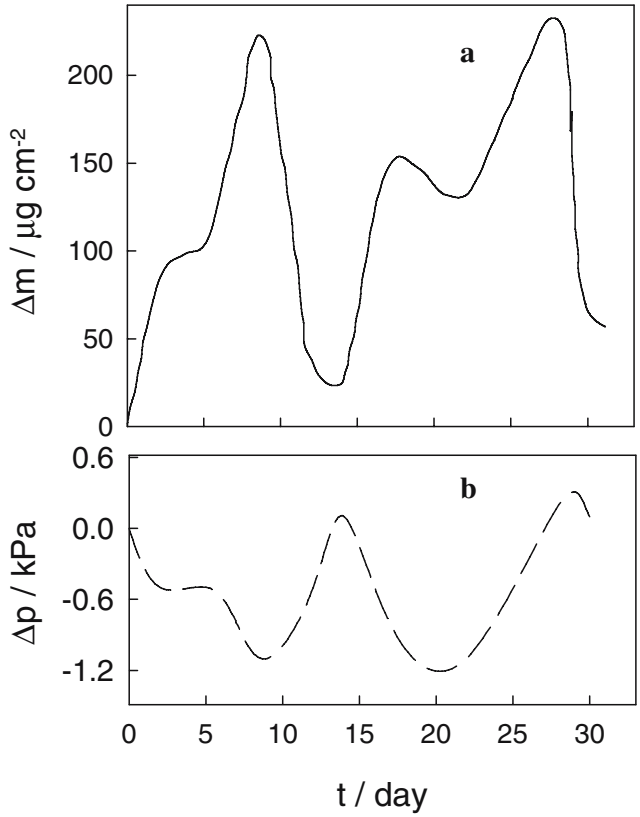

Fig. 3 The curve of mass change determined by QCM with Pt electrodes in humid atmosphere and synchronous change of atmosphere pressure

due to microbiological corrosion. It is difficult to discriminate between the contributions of each phenomenon from the data available. However, it may be assumed that the contribution of MIC to the mass gain is of little significance. Aluminium is known to be a metal with a thin but highly insulating passive layer, and corrosion on it takes place locally with development of corrosion pits. The mass of the corrosion products within the pits is much less than that developed due to other (biogenic) factors because the pits cover only a small part of the entire surface. Moreover, EIS data did not indicate an increase in the layer thickness during MIC of Al subjected to *A. niger* [7, 8].

The conversion of frequency (Fig. 2) into mass units may be performed according to Sauerbrey's [20] equation. This equation is appropriate for thin, rigid layers, which do not undergo shear deformations during oscillation. For instance, the mass of the corrosion products film and adsorption of the water layer may be calculated according to this equation [23–25]. However, some deviations from Sauerbrey's behaviour are probable for less rigid layers as those build up of microorganisms. A more complex theory in such a case is necessary, which accounts the acoustic impedance and, in turn, requires the data on density and shear modulus of the viscoelastic layer [26]. An attempt to evaluate the viscoelastic properties of biofilm in aqueous

environment was recently undertaken by reflectance measurements [16]. Little is known about viscoelastic effects under atmosphere conditions. Moreover, in the present study, evaluation of such effects is hardly feasible as the colony does not form a compact biofilm and changes its structural properties during long-term exposure (Fig. 1).

An approximate evaluation of mass change may be performed by using Sauerbrey's theory, as it has been done by Nivens et al. [14]. They estimated the mass of a single cell of *Escherichia coli* from QCM data to be 0.8 pg, which was comparable to the literature value of 1 pg.

In our experiments (Fig. 2), Sauerbrey's equation [20] gives the entire mass gain after 80 days exposure at about 300 μg cm^{-2} (the mass to frequency factor is C=78 ng cm^{-2} Hz^{-1}).

The presented data show that QCM may be successfully applied to study development of microbial populations on metal surfaces in situ under atmospheric conditions. The glued foil approach suggests some progress in the field of QCM application because the foils may be prepared from the bulk materials, which are of great technical importance, for instance, various alloys used in medicine, construction, computer technology, aircraft industry, etc.

Conclusions

The dynamics of aluminium colonisation by *A. niger* was clearly detected by sensitive mass measurements using QCM and comparison of the data for abiotic and biotic samples. The influence of atmospheric pressure on QCM data was demonstrated, and its elimination was suggested by using a reference sensor and subsequent subtracting of data. The QCM with glued foil electrodes shows possibility to expand the scope of QCM investigations from evaporated, sputtered or electroplated materials to those from which thin foils may be produced.

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