Corrosion of Aluminum Alloy 2024 Caused by *Aspergillus niger*

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Abstract:

Corrosion of aluminum alloy 2024 (AA 2024-T3) exposed to fungus *Aspergillus niger* cultured on glucose containing potato dextrose agar was studied in a high humidity and sterile atmospheric environment. The biofilm evaluation, weight loss measurement, quantitative pit analysis and corrosion product characterization were carried out. Results showed that, under this condition, the presence of *A. niger* accelerated corrosion process. The corrosion rate of AA 2024-T3 by *A. niger* was more than 4 times of that exposed to NaCl. Also, severe pitting corrosion, comparable to AA 2024-T3 exposed to NaCl, and aluminum depletion accompanied with copper enrichment were observed. Furthermore, the corrosion morphology was found to be related to the biofilm thickness. The dominant metabolite of *A. niger* under our experimental condition was identified to be oxalic acid, an aggressive corrodent that causes similar corrosion rate and corroded pit morphology as those observed in in this study. Therefore, oxalic acid produced by *A. niger* under our specific experimental condition is believe to be the main cause for the corrosion of AA 2024-T3.

Keywords: aluminum alloy; microbiologically influenced corrosion; weight loss; surface roughness

1. Introduction

The annual costs of corrosion are in trillions and account ~ 6% of the Gross World Product (Jackson, 2013). Microbiologically influenced corrosion (MIC) is due to the result of the presence or activities of microorganisms such as, but not limited to, bacteria, microalgae, lichens and fungi (Little et al., 2007). MIC could pose serious concerns in industries including water distribution systems (Teng et al., 2008), underground storage tanks (Sowards and Mansfield, 2014a), and oil storage, transportation (Rajasekar and Ting, 2010; Giacobone et al., 2011; AlAbbas et al., 2013; Sowards et al., 2014b), and nuclear waste storage facilities (Giacobone et al., 2011).

A great deal of research has been dedicated to the causative bacteria on metal corrosion (Sheng et al., 2007; Moradi et al., 2011; Chen et al., 2014; Wang et al., 2014; Chen et al., 2015a; Lou et al., 2016), such as sulfate-reducing bacteria (SRB), iron-oxidizing bacteria (FeOB), iron-reducing bacteria (FeRB) and acid-producing bacteria (APB). However, relatively few studies have focused on the involvement of fungi. In fact, a wide variety of fungi commonly existing in nature are capable of influencing metal corrosion (Salvarezza et al., 1983; Ayllon and Rosales, 1994; Hagenauer et al., 1994; Little et al., 1997; Videla et al., 1988; Galarde et al., 1999; Little and Ray, 2002; McNamara et al., 2005; Araya et al., 2007; Belov et al., 2008; Rosales and Iannuzzi, 2008; Smirnov et al., 2008; Qu et al., 2015; Ching et al., 2016). For example, fungus *Hormoconis (Cladosporium) resinae*, a main microorganism found in the aviation fuel system, could affect the corrosion of aluminum fuel tanks with the corrosive acid metabolites or the corrosion inhibitor produced by the microorganism (Salvarezza et al., 1983; Videla et al., 1988; Ayllon and Rosales, 1994). Other fungal species, such as filamentous species *Aspergillus niger* and *Penicillium frequentans*, isolated from different corroded sites of aircraft, were reported to cause aggressive corrosions toward AA 7075 (Smirnov et al., 2008).

While accelerated corrosion due to fungi has been documented, several studies have reported that these fungal species could also act as corrosion inhibitors. For

instance, *A. niger* and *P. frequentans* had been reported to protect pure aluminum from corrosion during two years of exposure, mainly by developing a passivating layer of aluminum oxides at localized corroded sites (Juzeliunas et al., 2005; Juzeliunas et al., 2007; Miecinaskas et al., 2007). It seems fungal influenced corrosion depends on many factors including metal types, fungal species and exposure time (Juzeliunas et al., 2005; Araya et al., 2007; Juzeliunas et al., 2007; Miecinaskas et al., 2007; Belov et al., 2008; Rosales and Iannuzzi, 2008; Smirnov et al., 2008; Qu et al., 2015). Also, few studies have generated enough quantitative information on pitting morphology to permit detailed investigation on the corrosion mechanism of aluminum or its alloys by fungi.

In this study, we observed and characterized the corrosion of one aluminum-copper alloy, AA 2024-T3, corroded by a common fungal species *A. niger* in a humid and sterile atmospheric environment. We placed AA 2024-T3 coupons on potato dextrose agar (PDA) surfaces, and then dispersed an *A. niger* spore suspension onto the entire agar and coupon surfaces, and allowed the biofilm to develop and AA 2024-T3 to corrode. Parallel experiments with specimens exposed to 3.5 wt% NaCl solution were conducted for comparison. The fungal biofilm examination, quantitative pit analysis, corrosion rate determination, and electrochemical measurement were carried out.

2. Experimental Section

2.1. Materials and Methods

Aluminum alloy 2024 (AA 2024-T3) sheet was purchased from McMaster-Carr. It contained Si 0.50 wt%, Fe 0.50 wt%, Cu 3.8 ~ 4.9 wt%, Mn 0.3 ~ 0.9 wt%, Mg 1.2 ~ 1.8 wt%, Cr 0.1 wt%, Zn 0.25 wt%, Ti 0.15 wt% and Al to 100 wt%, as per ASTM standard B 209-96, (1996). The alloy had an ultimate tensile strength of 400–430 MPa, a yield strength of 270–280 MPa or greater, and an elongation of 10-15%. Potato dextrose broth, agar, acetone, ethanol, sodium chloride, and Tween-80 were from Sigma-Aldrich. Hexane (98.5 wt%) and nitric acid were from Fisher Scientific. FilmTracer Live/Dead biofilm viability kit fluorescent staining solutions were purchased from Invitrogen. Deionized (DI) water with a conductivity of 1 $\mu\text{S cm}^{-1}$ or less was prepared in-house.

2.2. Preparation of AA 2024-T3 coupons

Rectangular test specimens (i.e., coupons) of 20 mm $\times$ 10 mm $\times$ 0.4 mm were cut from an AA 2024-T3 sheet, which was pre-polished by the manufacturer. Coupons were sequentially cleaned in a sonicating bath with hexane, acetone, ethanol, and deionized water for 2 - 4 minutes each and then air dried. After weighing, coupons were soaked, in ambient condition, in a 75 vol% ethanol solution for 24 hours for sterilization.

2.3. Preparation of spore suspension

The experimental procedure is illustrated in Fig. 1. The spore suspension of *A. niger* strain NRRL 13201 was prepared according to ASTM standard (ASTM G 21-90, 1990). Specifically, *A. niger* was cultured on potato dextrose agar (2.4 g potato dextrose, i.e., D-glucose, broth and 1.5 g agar per 100 ml DI water) at 23 °C and more than 90% relative humidity for 1 - 3 weeks, and then the spores were collected and dispersed in a sterile Tween-80 solution (0.5% v/v) in a centrifuge tube. After the suspension was vigorously vortexed for breaking the spore clumps, a counting chamber (Bright-Line

Hemocytometer, Hausser Scientific, PA, U.S.A.) was used for quantifying the spore concentration with the aid of a light microscope (Inverted microscope IX 70, Olympus American Inc., U.S.A.) coupled with a digital camera (MT 300, Amscope, CA, U.S.A.). The suspension was diluted with sterilized DI water to the standard concentration of 1×10^6 spore ml^{-1} for use in the corrosion studies.

2.4. Corrosion studies

Corrosion studies [see Fig. 1] were conducted in sterile six-well cell culture plates (CytoOne, USA Scientific Inc., USA) in a similar fashion for six systems marked as control, NaCl, agar, NaCl + agar, *A. niger*, and *A. niger* + NaCl [Table 1]. PDA agars in the 6-well plates used were approximately 7 mm thick, corresponding to ~ 6.3 ml of agar gel that contained ~ 96 wt.% of water. Coupons taken from the ethanol solution were immediately rinsed with sterilized DI water to remove ethanol prior to being placed on the agar surfaces. For the cases with NaCl, the ethanol sterilized AA2024-T3 coupons were dipped in a 3.5 wt.% NaCl solution for ~ 30 s, and then removed and quickly placed either inside the well (NaCl) or on top of the agar gel (NaCl+agar). These coupons normally retained ~ 0.1 ml of residual NaCl solution on each of them. For the coupons to be exposed to *A. niger*, after placing the AA2024-T3 coupon on the agar, 1 ml aliquot of the prepared spore suspension was separately introduced into each well to provide a sufficient amount of *A. niger* spores in the system. For the coupon placed on the agar surface (four of the systems except control and NaCl), the entire back surface was in intimate contact with the agar gel, and most of the liquid/solution on the front surface of the coupon drained into the agar gel.

Triplicates were used for each system. All six-well plates were covered by lids and kept in a glove box at 23 °C and more than 90 % relative humidity for 21 days. To maintain this high humidity, ~ 1 l of sterile water was sprayed into the glove box every day. Corrosion studies were also repeated three times to ensure the reproducibility.

2.5. Biofilm visualization and evaluation

Biofilms were evaluated based on confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). CLSM scanning was used for visualizing the morphology of biofilm and the coupon surfaces. It was carried out using a confocal microscope (BX61W1 plus FV1000, Olympus) with its software FV10-ASW (Version 3.0, Olympus) after staining the biofilm with Live/Dead fluorescent stains (FilmTracer™ Live/Dead Biofilm Viability Kit, Cata# L10316, Invitrogen) for 20 minutes. The green/red fluorescent light (for live/dead cells, respectively) was detected using excitation/emission wavelengths of 480/500 nm and 490/635 nm, respectively. SEM characterization on a scanning electron microscope coupled with energy dispersive X-ray (SEM/EDX) (TM-3000 Tabletop SEM, Hitachi High Technologies America, Dallas, TX) was carried out for assessing the morphology of biofilms on the front surface of each coupon after following fixation and dehydration procedures. Biofilms were fixed by immersion in a glutaraldehyde solution (2 wt%) at 4 °C for 1 day, dehydrated in a series of ethanol solutions of increasing concentrations (0, 25, 50, 75 and 100 vol%) and then in a desiccator for 1 day at room temperature.

2.6. Weight loss measurement

Corrosion rates via weight loss were computed according to the equation in ASTM D 2688-05 (2005), after following the chemical cleaning procedures in the ASTM G1-03 standard (C.1.2 in Table A1.1) (2003). Coupons removed from the corrosion study systems were washed with a 75 vol% solution of ethanol in water, and then brushed under flowing tap water to remove biofilms and corrosion products. Subsequently, dry coupons were immersed in a 69.2 wt% nitric acid solution for around 3 minutes to completely remove corrosion products, rinsed with DI water to remove the acid, and finally were dried under flowing air. The coupons before and after experiments were weighed using an analytical balance (E1RR80, Ohaus) with an accuracy of 0.1 mg. Then corrosion rates were calculated by using Equation 1 (ASTM D2688-05, 2005).

$$\text{Corrosion rate} = W / (d a t) \quad \text{Eq. 1}$$

where W is the weight loss; d is the density of the metal (for aluminum, d is 2.78 g cm^{-3}), t is the exposure time, and a is the initial surface area of both sides (edge surfaces, accounting only ~ 6% of the total surface area, were ignored). Results from all replicate experiments were averaged.

2.7. Observations and analysis of pitting morphology

The coupon surfaces and corrosion products were examined by SEM/EDX. A 3D optical microscope (infinite focus microscopy, IFM, Alicona Corporation, Austria) was used to obtain 3D images and surface roughness information. Roughness parameters were averaged or compared by scanning different locations (i.e. avoiding scratches and excluding edges) accounting for at least 50% of the entire surface. Areal surface roughness parameters were calculated according to EN ISO 4287 (1997), DIN 4776-1990 (1990), and the study by Stout et al. (2000). Parameters, such as S_k (the height of core material or “kernel”, representing the surface roughness), S_{vk} (the depth of valleys, i.e. pits), V_{vv} (the volume of valley voids or pits), S_v (the maximum valley depth of the selected area), and S_{dr} (the developed interfacial area ratio or $S_{dr} = (\text{actual surface area} - \text{projected surface area}) / (\text{projected surface area}) \times 100\%$). Schematic diagrams for such parameters could be found in an earlier study (Chen and Ju, 2015b). The average pit depth could be estimated from the combination of S_k + S_{vk} . The maximum pit depth and pitted area percentage could be estimated based on the method proposed by Chen et. al. (2015b; 2016) as follows:

$$\text{Pitted area percentage} = 6.62 \times (S_{vk} \times V_{vv} / S_{dr}) \quad \text{Eq. 2}$$

$$\text{Maximum pit depth} = 0.96 \times (S_v + S_k) \quad \text{Eq. 3}$$

The pitted area percentage could provide valuable information on both pit quantity and size, and would be useful for identifying coalesced pits.

2.8. Metabolite analysis

After the coupon was removed from each well, an agar disc was extracted by inserting a glass tube, and the exact mass was weighed. Three agar discs from three different wells were collected; each agar disc was placed in 2 ml DI water inside a glass vial, and then vigorously vortexed. Each solution was filtered using a $0.32 \mu\text{m}$ PVDF filter, and the solution was analyzed by a Shimadzu LC-10A high-performance liquid chromatography (HPLC) system (Shimadzu Corp., Columbia, MD) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories Inc., USA), a UV-Vis detector (SPD-10A), and a refractive index detector. A $0.008 \text{ N H}_2\text{SO}_4$ solution was used as the mobile phase at 0.6 ml min^{-1} and the column temperature was set at 35°C . Standard solutions

of organic acid were used to generate calibrations for determining their presence and concentrations.

3. Results and discussion

3.1. Biofilm morphology and development

After 2 days, the agar and coupon surfaces turned slight yellowish and showed initial mycelial growth from the germinated spores [Fig. 2(A)]. After 21 days, a transparent and gel-like layer, which extended from the edges into the central region of the coupon (with a clear boundary), appeared on all inoculated coupons [Fig. 2(B)]. CLSM analysis revealed the presence of un-uniformly distributed biofilm with elongated and branched mycelial networks. The mycelial networks in the site (b), as shown in CLSM images, were much denser and thicker [with a thickness of $\sim 168 \mu\text{m}$, Fig. 2(C)] than in site (a), which had a thickness of $\sim 54 \mu\text{m}$ [Fig. 2(B)]. The SEM image on the central part [site (a)] of the coupon [Fig. 2(E)] showed, as indicated by arrows, several visible micro pits and trenching around white spots, most likely intermetallic particles in AA 2024-T3, formed under thinner hyphae layers. Along the edges of the coupon (site (b)), the mycelium networks were interconnected with some corrosion products [Fig. 2(F)], more details of these corrosion products are described in section 3.4.

3.2. Corrosion of AA 2024-T3 in the presence of *A. niger*

Corrosion rates in the control and agar systems were relatively small and not statistically different (p-value = 0.78), with values of $2.5 \pm 1.1 \mu\text{m y}^{-1}$ and $2.7 \pm 1.7 \mu\text{m y}^{-1}$, respectively [Fig. 3]. The NaCl and NaCl+agar systems also had no statistically different corrosion rates (p-value = 0.33), but the values were higher, at $33.3 \pm 2.3 \mu\text{m y}^{-1}$ and $37.2 \pm 3.5 \mu\text{m y}^{-1}$, respectively, as compared to the control and agar systems. The results indicated that the presence of agar did not affect the corrosion rate of AA 2024-T3 under our experimental environment. Corrosion rates for coupons exposed to *A. niger* without and with NaCl were $127.3 \pm 9.3 \mu\text{m y}^{-1}$ and $149.0 \pm 7.8 \mu\text{m y}^{-1}$, respectively, while insignificantly different (p-value = 0.17) from each other, they were significantly greater ($\sim 4\times$) than the rates caused by NaCl alone. These results showed the presence of *A. niger* accelerated the corrosion process of AA 2024-T3.

The optical microscopy examination showed that coupons exposed to *A. niger* were severely corroded, forming many dark visible pits, had very corroded edges, and a layer of multi-colored corrosion products on the coupon (as shown in some of the images in the Supporting Information section). While the front surface, where *A. niger* biofilm was in direct contact with, had a few shallow and round micro-pits [Fig. 4(A)], the entire back surfaces were corroded with deep irregular pit clusters [Fig. 4(B)] and micro pits. It is worth noting that there was no fungal growth on the back surface. These results showed that, in the presence of *A. niger*, *A. niger* biofilm formed on the front surface of the coupon and produced metabolites, which likely distributed on the front surface and migrated into the agar gel, where the back surface of the coupon was in direct contact with, and caused the enhanced corrosion, including pitting corrosion, on both surfaces of the AA 2024-T3 coupon.

Several studies reported the effect of *A. niger* on corrosion of aluminum and its alloys. Some demonstrated that this fungus caused corrosion damages (Hagenauer et al., 1994; Belov et al., 2008), while the others claimed that it protected pure aluminum from corrosion due to the formation of the passivating layer of aluminum oxides (Juzeliunas et al., 2005; Juzeliunas et al., 2007; Miecinaskas et al., 2007). The acceleration or inhibition of aluminum alloy corrosion depends on factors such as culturing medium, metabolites produced by fungi, and alloy types (Juzeliunas et al., 2005; Juzeliunas et al., 2007; Miecinaskas et al., 2007; Smirnov et al., 2008; Belov et al., 2008). In our short-term study, the results revealed that AA 2024-T3, an aluminum-copper alloy, was susceptible to pitting corrosion in the presence of *A. niger*.

A. niger is known for excreting many kinds of organic acids including citric acid, oxalic acid, succinic acid, malic acid, gluconic acid and fumaric acid (Sazanova et al., 2014). HPLC analysis of the aqueous solutions with samples taken from the agar gels after exposure to *A. niger*, showed that the dominant organic acid excreted by this *A. niger* strain was oxalic acid [Fig. 5], and its concentration increased with the exposure time to *A. niger* as the glucose was being consumed. It should be noted that HPLC analysis of the medium and the agar gel extracted at the beginning of the corrosion study (time 0) showed no obvious peaks for oxalic acid. We would also like to point out that oxalic acid is highly soluble in water (the solubility of oxalic acid in water is 143 g l^{-1} at 25°C), and due to the intimate contact of the coupon with the agar gel, the oxalic acid produced by *A. niger* dissolved in the thin liquid aqueous layer on the coupon, which drained into the agar gel and distributed throughout the gel. Since the gel contained ~ 96 wt.% of water and had relative large pores (~ 400 nm for a 2 % agar gel) (Pernodet et al., 1997), the distribution of oxalic acid within the gel was expected to be relatively uniform. In fact, when samples were taken from different locations, such as near the top and the bottom of the gel, within the agar gel, the oxalic acid concentrations were found to be close to each other. The concentration of oxalic acid within the agar gel was found to be ~ 0.068 M, and approximately accounted for 96% of the total organic acids detected after 21 days of exposure. The pH value in the gel, measured directly using a micro pH electrode, was ~ 2.3, at day 21.

Oxalic acid excreting from *A. niger* was speculated to be the main cause of inducing severe corrosion of AA 2024-T3. Separate experiments were conducted by immersing AA 2024-T3 coupons in a 0.068 M of the oxalic acid solution with a pH of 2.3 (i.e. pH was adjusted by NaOH, since the 0.068M of oxalic acid solution had a pH ~ 1.5) and utilizing electrochemical impedance spectroscopy (EIS) to monitor the corrosion. The corrosion rate calculated from the weight loss for 5 days exposure in the immersion experiment was ~ $200 \text{ } \mu\text{m y}^{-1}$, and the value calculated from EIS spectra was ~ $180 \text{ } \mu\text{m y}^{-1}$ (more details on estimating the corrosion rate using EIS spectra can be found in the Supporting Information section). These values were in relatively good agreement with the corrosion rate of AA2024-T3 in the presence of *A. niger* for 21 days (~ $127 \text{ } \mu\text{m y}^{-1}$). It indicated that the main reason of corrosion in the fungal system was likely due to oxalic acid.

3.3. Pit distribution and quantitation

Various pits were formed on the AA2024-T3 coupon surfaces under different environments investigated in this study. The presence of corrosive species, i.e., NaCl

and oxalic acid, was necessary for pitting, and pitting appeared to be associated with the high local concentration of these corrosive species. However, the severity of pitting was not necessarily well correlated with the weight loss values for AA2024-T3. For example, the corrosion rate based on weight loss was basically the same for the cases of NaCl and NaCl+agar, but the pit depths for the coupons associated with NaCl reached ~ 10 μm on the back surface [Fig. 4(D)] and ~ 25 μm on the front surface [Fig. S3(A)]. For the case of NaCl+agar, the pits were very heterogeneous. On the front surface of the coupon, pits as deep as ~ 130 μm in some locations (Fig. 4(E)) and ~ 8 μm in other locations [Fig. 4(F)] were observed, while on the rest of the coupon only shallow micro pits were noticed [Fig. S3(B)]. As a result, the average pit depth for this case was ~ 22 μm [(f) in Fig. 6(B)], and large standard deviations on the pitting parameters [case (f) in Fig. 6] were obtained. Pits were almost undetectable on the back surface of the coupons exposed to NaCl+agar [Fig. S3(C)].

For coupons exposed to *A. niger*, the central region of the front surface [site (a) in Fig. 2(B)] had a few shallow and round micro pits (a depth of ~2 μm) [Fig. 4(A)], while at the edge [site (b) in Fig. 2(B)], many deeper pits and pit clusters (a depth of ~ 15 μm) were observed [Fig. 4(B)]. As mentioned earlier, the biofilm located on site (a) was much thinner than that on site (b) [Fig. 2]. The coincidence suggests that the distribution of pits might have a spatial correlation with the biofilm thickness; in other words, more severe pitting occurred under a thicker biofilm [see the quantitative data, cases (b) and (c), in Fig. 6]. On the back surface, pits as deep as ~ 110 μm were observed, and the pit profile showed a “terraced” effect [Fig. 4(C)]. Therefore, the severe pitting in the presence of *A. niger*, due to the oxalic acid produced by this fungal strain, was comparable to that caused by NaCl for AA2024-T3 coupons.

In addition to IFM images presented in Fig. 4, SEM images were obtained to verify the presence of micro pits with wide bottoms (diameters of ~ 10 μm) [Fig. 7(A)]. There were some corroded remnants inside the micro pits and slight trenching around isolated intermetallic particles (e.g., Cu-Al-Mg and Al-Cu-Fe-Mn, as the white spots indicated by arrows in Fig. 7(A)) in the central region of the front surface. Many severe irregular pit coalescences, clusters, cavities and even cracks were observed near and along the edge of the front surface [Fig. 7(B)] and on the back surface [Fig. 7(C)]. It is worth noting that many large pits were composed of a greater number of regular smaller pits [Fig. 7(D)]. In a separated experiment with AA 2024-T3 immersed in an oxalic acid solution for 96 h, similar surface morphology with numerous micro-pits and pit clusters was observed [Fig. S5(A)].

Fig. 6 presents surface roughness parameters from the IFM scans (definitions of these parameters were provided in Section 2.7). The parameters used for pit quantification were: the average pit depth, the maximum pit depth and the pitted area percentage, which were estimated using Equations 2 and 3, and the pit volume (V_w). The control sample [case (a) in Fig. 6] and the central region of the front surface of the coupon [case (b) in Fig. 6] in the presence of *A. niger* were almost non-pitted or micro-pitted [Figs. 4 (A) and 7 (A)]. Their parameters did not differ significantly with the lowest values among all samples: the maximum pit depth of ~ 10 μm , the average pit depth of ~ 4 μm , the pitted area percentage of ~ 0.8%, and the pit volume of ~ 0.1 ml m^{-2} . The colonization of thin biofilm in the central region of the front surface initiated micro pitting, but these micro pits did not obviously alter the surface roughness significantly. However,

all parameters for the coupon edge [case (c) in Fig. 6], where a much thicker biofilm was present, were statistically significantly different from those in the central region (p -value < 0.05), and a maximum depth of $\sim 47 \mu\text{m}$ was observed. One thing worth noting is that, the most severely corroded regions of the edge, with metal mostly disintegrated, were not used in this pitting analysis. Only pits in the regions near the edge were quantified. The pit depth observed on the front surface was likely related to the biofilm thickness distribution. The back surface of coupons exposed to *A. niger* [case (d) in Fig. 6] displayed much more severe pitting corrosion with the maximum pit depth of $\sim 110 \mu\text{m}$, the average pit depth of $\sim 12 \mu\text{m}$, the pitted area percentage of $\sim 7.4\%$, and the pit volume of $\sim 0.5 \text{ ml m}^{-2}$. The pitting extent on the edge of the coupon exposed to *A. niger* and the back surface was comparable to that of coupons exposed to NaCl [back surface, case (e) in Fig. 6]. The coupon collected from the NaCl+agar system [case (f) in Fig. 6] also had high values of all these parameters, which could be the result of the local high NaCl concentration. The comparison of results [Figs. 3 and 6] among these systems showed that the weight loss values of AA 2024-T3 caused by *A. niger* was much higher than those of abiotic systems and the pitting corrosion was comparable to the coupons exposed to NaCl.

3.4. Copper enrichment in the presence of *A. niger*

After removing the biofilm, it was noticed that a few shiny particles with size ranging from a few to tens of microns were randomly distributed on the coupon. The particles were also observed on the back surface [Fig. 8A] where the biofilm was not colonized. It seemed that most of these particles (tens of microns) were inside the pits. As shown in Fig. 8(C), the elemental percentage of Al decreased while the copper content increased for both site (a) (i.e. smooth site) and site (b) in Fig. 8(B) (i.e. copper enriched particles), as compared to the original AA 2024-T3. It should be noted that severe pitting corrosion was observed on the back surface without directly contacting *A. niger*. The copper enrichment and severe pitting were probably induced by the metabolite, oxalic acid, which could accumulate underneath the coupon and diffuse through the agar. AA 2024-T3 contains $\sim 4\%$ Cu and $\sim 1.5\%$ Mg, and is susceptible to localized corrosion due to the presence of intermetallic particles. Copper enrichment could be attributed to the de-alloying of intermetallic particles of Al_2CuMg or dealumination of Al_2Cu (Chen et al., 1996; Buchheit et al., 1997). Similar results were observed when the AA 2024-T3 coupons were immersed in a 0.068 M oxalic acid solution for 96 h [Fig. 9]. These observations indicated that the corrosion of AA 2024-T3 due to oxalic acid produced by *A. niger* also exhibited the phenomenon of copper enrichment that generally occurred with corrosion of AA2024-T3.

4. Conclusion

The presence of *A. niger* enhanced the corrosion rate based on the weight loss to a value more than 4 times of those of NaCl or control systems. Results of pit morphology and quantitative pit parameters (e.g. maximum and average pit depths) showed that severe pitting corrosion was observed when AA 2024-T3 coupons were exposed to *A. niger*. The pitting was comparable to those AA 2024-T3 coupons exposed to NaCl. The colonization of biofilm on the front coupon surface induced micro-pitting at the center and severe pitting along the edge, which was roughly correlated to the spatial biofilm

distribution. The presence of *A. niger* caused depletion of aluminum and copper enrichment. The cause of corrosion was found to be related to the dominant acid metabolite, oxalic acid, produced by *A. niger* under our experimental conditions.

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Captions

Fig. 1. Schematic representation of the experimental procedure utilized to follow AA 2024 corrosion in the presence of *A. niger*.

Fig. 2. Representative images of the evolution of AA 2024 coupons on agar dispersed with *A. niger* spores after 2 days (A) and 21 days (B). A biofilm with a thinner (a) and a thicker (b) region was observed. Their corresponding CLSM top view images (C and D) are also shown, with the thicker and thinner regions having a thickness of $\sim 168 \mu\text{m}$ and $\sim 54 \mu\text{m}$, respectively. The SEM images of biofilm and corrosion products on the center region (site a) and the edge (site b) in (B) are shown in (E) and (F), respectively. (E) shows micro pits and trenching around intermetallic particles (indicated by arrows) under the thinner biofilm. Image (F) shows corrosion products of aluminum oxides and copper particles (as shown by arrows) under the thicker biofilm. The scale bars in (E) and (F) are $100 \mu\text{m}$ and $500 \mu\text{m}$, respectively.

Fig. 3. Corrosion rates calculated from weight loss of the coupons after 21 days exposure to *A. niger* are presented. The same letters on the corrosion rate bars indicate no significant statistical difference amongst coupons subjected to those conditions. The average and error bar for each data point presented were from the measurement of at least six samples.

Fig. 4. IFM images of the entire coupon surface ($10 \text{ mm} \times 20 \text{ mm}$), 3D view ($286 \mu\text{m} \times 217 \mu\text{m}$) and cross-sectional depth profiles of the center region (A) and the edge of the front (B) and the back (C) surface of coupons associated with *A. niger*, the back surface of coupons associated with NaCl (D) and the front surface associated with the most severely pitted area (E) and less severely pitted area (F) of NaCl+agar, respectively. The 3D view for each case is indicated by the red squares. White lines on 3D views show the cross sections selected for the profiles. The red boxes are used to indicate the location of the 3D view scans, and they are not drawn to scale.

Fig. 5. (A) HPLC spectra of the oxalic acid standard and the substances in the agar gel after 0, 3 and 21 days exposure to *A. niger*, and (B) the concentrations of oxalic acid and D-glucose in the agar gel as function of exposure time to *A. niger*.

Fig. 5. The maximum pit depth (A), average pit depth (B), pitted area percentage (C), and pit volume (D) obtained from IFM scans. The samples include control sample (a), the central region (b) and the edge of front surface exposed to *A. niger* (c), the back surface of coupons exposed to *A. niger* (d), the back surface of coupon exposed to NaCl (e), and the front surface of coupon exposed to NaCl+agar (f).

Fig. 7. SEM images of a coupon exposed to *A. niger* for 21 days: the center region (A) and edge (B) of the front surface, and the back surface (B and C). Trenching around intermetallic particles and micro-pitting are shown by arrows in image (A). Images C and D show cracks and pit clusters. The scale bars in (A-C) and (D) are $100 \mu\text{m}$ and $30 \mu\text{m}$, respectively.

Fig. 8. The representing SEM images (A and B) of the back surface of a coupon exposed to *A. niger* are presented. The element mapping of Al, Cu, O and C from EDX analysis for image (B) is shown beneath the images, the element percentages for selective sites (a and b) in (B) are summarized in (C) along with those of the original coupon from the EDX analysis. Copper enriched particles were observed on the back surfaces. The scale bars in (A) and (B) are $100 \mu\text{m}$ and $20 \mu\text{m}$, respectively.

576 **Fig.9.** A SEM image and the corresponding EDX element mapping for coupons exposed
577 to the oxalic acid solution (0.068 M and pH 2.3) for 96 hours. Corrosion products of
578 copper-enriched particles and aluminum oxides/oxalates are verified. The scale bar of
579 the SEM image is 20 μm .
580 **Table 1.** The corrosion study details for control and biotic samples

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

(C)

	Mass percentage (%)			
	Al	Cu	C	O
site (a) in (B)	48.9	7.1	29.7	13.3
site (b) in (B)	8.7	84.2	3.5	3.6
original AA 2024	88.9	3.2	4.3	1.7

Fig. 8

Fig. 9

Table 1.

Sample	experimental procedures
control	untreated coupons only exposed to the humid environment
NaCl	coupons immersed in sterile 3.5 wt% NaCl solution for ~ 30 s, and then removed from NaCl solution and placed in the wells
agar	untreated coupons placed on PDA surfaces
NaCl+agar	coupons immersed in sterile 3.5 wt% NaCl solution for ~ 30 s, and then removed from NaCl solution and placed on PDA
<i>A. niger</i>	coupons placed on PDA and inoculated with spore suspension
NaCl+ <i>A. niger</i>	coupons immersed in sterile 3.5 wt% NaCl solution for ~ 30 s prior, and then removed from NaCl solution and placed on PDA and inoculated with spore suspension

Note: Each coupon was placed inside a well of a six-well plate, which was enclosed with the lid.

Graphical abstract