STUDIES ON BIOCORROSION OF STAINLESS STEEL AND COPPER IN CZAPEK DOX MEDIUM WITH *ASPERGILLUS NIGER* FILAMENTOUS FUNGUS

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The paper reports studies about corrosion of AISI 304 stainless steel and of copper in two Czapek-Dox media inoculated with Aspergillus niger fungus: without sucrose ("A" medium) and with 30 g L^{-1} sucrose ("B" medium). From the comparative polarization experiments it has resulted that in "A" solutions the corrosion rate for austenitic steel increases more than 3 times in the first 14 days after inoculation, while for copper is more than 100 times faster. In "B" solutions the formation of a thicker biofilm is expected for both electrodes owing to the presence of sucrose as a carbon source for the fungus. It is responsible for much faster biocorrosion, especially in the case of copper. Micrographs of the metallic surfaces confirm the formation of biofilm.

Keywords: biocorrosion, Czapek Dox culture medium, *Aspergillus niger*, AISI 304 stainless steel, copper

1. Introduction

Rolled metallic products are widely used in both machine building and civil engineering (carbon steel, austenitic stainless steel, etc.) as well as for domestic appliances and electrical equipment (copper and its alloys, aluminum). In a first stage, microorganisms as bacterial cells are fixed on metal surfaces forming colonies that usually produce metal corrosion [1]. Then, microbiological colonies (usually bacteria and fungi) grow rapidly, forming biofilms which cover the attacked surface [2]. Corrosion due to biofilm formation and its growth is defined as microbiologically induced corrosion (MIC) [3].

Metal structures, in particular those operating in liquid environments that contain organic products are exposed to microbiological corrosion [4]. Such

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corrosion, under the action of *Aspergillus niger* or *Penicillium spp*., has been observed, for instance, on steel greased wire ropes; in both cases citric acid was identified in the products of the metabolism [5, 6]. Also, it has been reported the occurrence of corrosion cracks at tanks with oil and its products, owing to the mold induced corrosion [7].

Filamentous fungus *Aspergillus niger* is virtually ubiquitous in nature [8, 9], therefore its influence on the corrosion of various metals (Cu [4], Zn [4, 10] or Al [10-12]) has been much studied. Accelerated corrosion of copper owing to microorganisms in aqueous solutions containing some complex ions and bioligands was also reported [13]. Laboratory studies aimed to investigate the behavior of rolled carbon steel at corrosion due to the synergistic action of stray currents (during polarization in a.c. voltage) and of *Aspergillus niger* have confirmed that the presence of filamentous fungi substantially accelerates the corrosion of the metal [14-16]. Corrosion of AISI 304 stainless steel during the exposure at the disinfectant solutions with fungal suspension was reported recently [17,18]. By our long-term (five years) investigation we have shown that filamentous fungi, especially *Aspergillus niger* and *Penicillium funiculosum* existing in the soil have an important role in the deterioration of underground power cables, being active by degrading outer protective polymer layer and by accelerating the corrosion of the metallic screen made of copper [19-22]. With this in view, the objective of this article is to present some experimental results dealing with the corrosion of a stainless steel (AISI 304 austenitic steel) and copper, both commonly used in practice. We made a comparative investigation of their behavior in sterile Czapek Dox electrolyte medium and in the same medium inoculated with *Aspergillus niger*. Attention was paid to the presence of sucrose as a carbon source for the fungus.

2. Experimental

In order to evidence the corrosion of stainless steel and copper in the presence of *Aspergillus niger* fungus (shortly *A. niger*), some electrochemical determinations were comparatively performed in Czapek Dox culture media. Chemical compositions of the used AISI 304 austenitic steel samples and copper samples are presented in Tables 1 and 2.

Table 1

Chemical composition of AISI 304 stainless steel [23]

Element	Fe	C	Cr	Ni	Mn	P	S	Si
Content [wt.%]	66.3–74	max. 0.08	18-20	8-10.5	max. 2	max. 0.045	max. 0.03	max. 1

Table 2

Chemical composition of electrical household copper [24]

Element	Cu	P	Pb	Bi	Ag	Other
Content [wt.%]	min. 99.95	0.002-0.007	max. 0.005	max. 0.0005	max. 0.015	max. 0.03

The Czapek Dox medium is used for the general cultivation of fungi consisting essentially of a balanced and buffered mixture of inorganic salts and water and being used as a solution or, with excess added agar-agar, as a gel. The culture medium was prepared from the following amounts of p.a. Merck reagents: 2 g $NaNO_3$, 0.7 g KH_2PO_4, 0.3 g K_2HPO_4, 0.5 g KCl, 0,5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4$, 10 g agar-agar, all dissolved in 1000 mL distilled water. During experiments we tried to evidence the influence of easily assimilated sucrose (Merck) on the growth of *Aspergillus niger* (approx. 10^6 spores/mL) [25]; therefore we used and denoted two different Czapeck Dox culture media as: A medium (without any carbon source) and B medium with 30 gL^{-1} sucrose as carbon source. Prior to inoculation with spores of *A. niger* the solutions were sterilized by autoclaving for 30 minutes at 110^0C.

Potentiodynamic polarization measurements were performed in the potential range starting from open-circuit potential (OCP) to -1400 mV cathodic limit and in anodic direction from -1400 mV to +2600 mV, with a 0.5 mVs^{-1} scan rate. A Voltalab 40 PGZ 301 potentiostat having VoltaMaster 4 interface driven by PC was used as equipment. For computation of corrosion rate of steel and copper (expressed in mm/year) we used z=2 for the number of transferred electrons and the metal density values of 7.86 g/cm^3 (for steel) or 8.96 g/cm^3 (for copper), respectively.

The electrochemical cell contained a working electrode (AISI 304 or Cu, 0.4 mm thick sheets), a Pt plate as auxiliary electrode and a saturated calomel reference electrode (SCE). All experiments have been carried out in stationary conditions at room temperature (23±5^{o}C). The surface of working electrodes was cleaned with abrasive slurry of alumina grains, then rinsed with distilled water and dried. Afterwards, all the tools (electrochemical cell, the electrodes, etc.) were sterilized by autoclaving 0.5 hours at 110^0C prior to the corrosion test.

The morphology of electrode surface and defects induced by corrosion of metallic specimens in Czapek Dox media with *Aspergillus niger* inoculation were evidenced by an optical microscope type AD7013MT Dino-Lite Premier.

3. Results and discussions

Corrosion potential and current density are commonly utilized as important parameters to evaluate the kinetics of corrosion. The Tafel polarization curves were firstly registered in sterile media and then after inoculation with *A. niger*. Czapek Dox electrolyte consists in mineral salts dissolved in concentrations up to 0.4 wt.% and approx. 1 wt.% agar-agar. Sucrose acts as an excellent food for fungus being easily assimilated, so we have made a comparison of corrosion behavior in a medium without carbon source (A) and a medium with 30 gL^{-1} sucrose (B). We expect for the disaccharide (sucrose) to significantly promote the

colonization process thus increasing irregularities onto the metallic surface and corrosion rate.

3.1. Investigation of biocorrosion of stainless steel

In Figs. 1 and 2 the semi-logarithmic polarization curves of AISI 304 stainless steel in Czapek Dox A and B media are presented, illustrating the evolution in time of corrosion behavior. Curves 1 were recorded as reference of this evolution and correspond to stainless steel immersed in sterile media. Curves 2 and 3 from both Figs. show the changes after 3 or 14 days in the presence of *Aspergillus niger*.

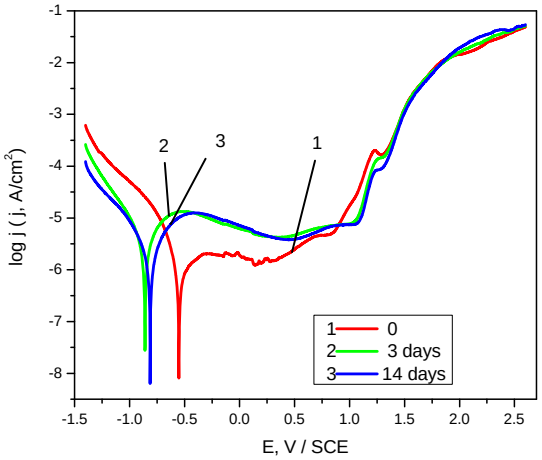

Fig. 1 Potentiodynamic polarization curves of AISI 304 steel in sterile Czapek Dox A medium (1) and in the presence of *Aspergillus niger* after 3 days (2) and 14 days (3).
Scan rate: 0.5 mVs^{-1}.

It has been found that all cathodic branches of curves have a quite similar shape because the current in the cathodic process can be assigned to the reduction of the H^+ protons (hydrogen evolution) but, most probably, to the reduction of dissolved oxygen (with OH^- formation), both processes being kinetically controlled. From the semi-logarithmic curves of AISI 304 austenitic steel we calculated the Tafel slope values (b_c) of around -62 mV per decade for sterile media; b_c values in the presence of *Aspergillus niger* were within -96 ÷ -117 mV per decade range.

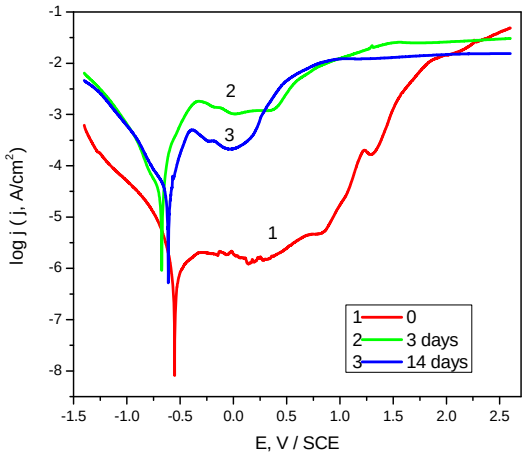

Fig. 2 Potentiodynamic polarization curves of AISI 304 steel in sterile Czapek Dox B medium (1) and in the presence of *Aspergillus niger* after 3 days (2) and 14 days (3). Scan rate: 0.5 mVs^{-1}.

In the anodic direction of Tafel curves for all investigated electrolytes there are three main potential domains, where processes in stagnant conditions are controlled by charge transfer kinetics. The first portion lies from stationary (open-circuit) potential to approx. -0.35 V (vs. SCE) and corresponds to the active behavior leading to uniform corrosion of metallic electrode, where the iron dissolution occurs:

$$Fe \; - 2\, e^{-} \rightarrow Fe^{2+} \tag{1}$$

In Table 3 were also listed two well-known corrosion parameters: the polarization resistance, R_p, usually determined by linear polarization in a potential region near the open-circuit (stationary) potential, and the corrosion rate, v_{corr} – expressed in mm/year. As Table 3 shows, in both A and B solutions the E_{corr} potential for AISI 304 steel has shifted to negative potential values when the samples were immersed in fungus inoculated media. The corrosion rate (J_{corr}) for austenitic steel in A solution increases more than 3 times in the first 14 days after inoculation; in B solution (with sucrose) the amplitude of corrosion is more significant.

After 3 days of immersion in solution with *Aspergillus niger* the anodic processes are depolarized. As a consequence of metabolic products resulted from fungus growth, usually carboxilic acids (primarily citric acid [5,6]), an increase in corrosion current density (J_{corr}) and also in the corrosion rate (v_{corr}) is observed. However, in both A and B media, after longer immersion (14 days) J_{corr} maintains approx. the same value as in 3 day immersion and this may suggest a blockage of metallic surface with a biofilm formed. The anodic Tafel slopes have values of

180 mV per decade for sterile medium and they increase significantly (b_a = 255-273 mV per decade) for media with *Aspergillus niger* whether or not they contain sucrose.

Table 3

Corrosion parameters for AISI 304 austenitic steel in Czapek Dox A and B media without/with *Aspergillus niger* fungus

Czapek Dox medium	E_{corr} [mV_{SCE}]	J_{corr} [μAcm^{-2}]	b_c [$mVdec^{-1}$]	b_a [$mVdec^{-1}$]	R_p [Ωcm^2]	v_{corr} [mm/year]
Sterile A medium	-550	0.82	-59	178	15218	0.051
A medium with *Aspergillus niger* after 3 day immersion	-860	3.34	-99	260	3817	0.201
A medium with *Aspergillus niger* after 14 day immersion	-815	2.82	-96	255	4521	0.175
Sterile B medium	-557	0.60	-65	180	21181	0.038
B medium with *Aspergillus niger* after 3 day immersion	-622	4.07	-117	271	3132	0.253
B medium with *Aspergillus niger* after 14 day immersion	-804	3.98	-113	283	3203	0.247

The second anodic domain corresponds to the passivation plateau up to 1-1.2 V (vs. SCE) potential value. This passive zone is a stationary state where the Fe dissolution is limited owing to the presence of passiving oxide film. The third anodic domain is a region where passivity was broken. It is known that microbiologically induced corrosion produces localized corrosion that exhibits pitting; thus, the increase of current density is mainly due to pitting corrosion. In general, we noticed that the beginning of pitting occurs at less positive potentials for B medium than for A medium, and therefore the range of passivation is shorter in medium containing sucrose. Also in this region a continuous anodic dissolution of Fe and Ni (from AISI 304 steel) takes place together with removal of oxide corrosion products. At the end of polarization, the O_2 evolution is observed with oxygen bubbles occurring onto sample surface; the anodic current tends to limit owing to a high anodic overpotential. This new slow rate step is considered the rate-determining step and consists in the molecular oxygen formation and its evolution.

A faster growth of *Aspergillus niger* film and the microbiological induced corrosion are proved by optical micrographs (Figs. 3). The optical images show the formation of a biofilm by adhesion of fungus to a metal surface. During

biofilm development the metabolic activity of fungus causes corrosion of metal, as well as dissolution of protective oxide films.

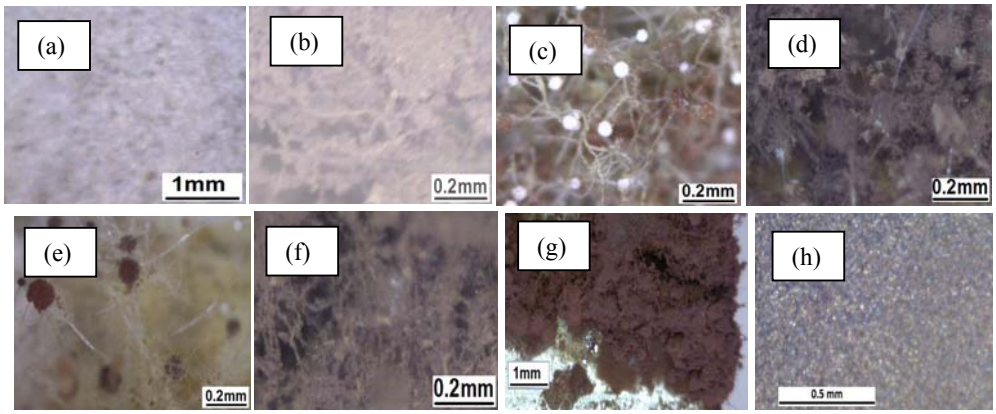

Fig. 3 Representative images regarding biocorrosion of AISI 304 samples in the investigated Czapek Dox media: - after 8 day immersion in sterile A medium (a) or in B medium (b); - after 3 days (c) and 8 days (d) in A medium inoculated with *Aspergillus niger*; - after 1 day (e), 3 days (f) and 8 days (g) in B medium inoculated with *Aspergillus niger*; -after 14 days in "B" medium inoculated and removal of colonies or adsorbed corrosion products from the sample surface (h).

By comparing the photographs from Figs. 3 (a,b) in sterile media with those from Figs. 3 (e-g) (inoculated media after 3 and 14 day immersion, respectively) we noticed the formation of *A. niger* colonies and their self-outspread over the electrode surface. The biofilm is thicker in B solutions (e-g images) due to a more intense metabolism. We explain the narrow passivation domain by fungi filament penetration in passivating oxide film. The biofilm limits the diffusion of ionic species of Fe, Ni, Cr, produced from steel corrosion.

3.2 Investigation of biocorrosion of copper

The potentiodynamic polarization curves of copper samples in A and B Czapek Dox media (remember that medium B has a supplementary 30 gL^{-1} sucrose content) are presented in Figs. 4 and 5. Similar to Figs. 1 and 2, curves 1 for sterile media were recorded as references. Curves 2 and 3 from both Figs. 4,5 show obviously the changes of corrosion behavior after 3 or 14 days in the presence of *Aspergillus niger*. All cathodic branches of polarization curves have almost similar shapes and they were assigned to either the reduction of the H^+ protons (with hydrogen evolution) or, most probably, the reduction of dissolved oxygen (with OH^- formation). The Tafel slope values (b_c) are smallest for sterile media and they gradually increase in the presence of *Aspergillus niger* being higher in inoculated B medium.

As for stainless steel, the following corrosion parameters of copper were obtained from the anodic branches: corrosion potential (E_{corr}), corrosion current density (J_{corr}), Tafel slopes (β_a), polarization resistance (Rp) and rate of corrosion (v_{corr}). Their values are listed in Table 4.

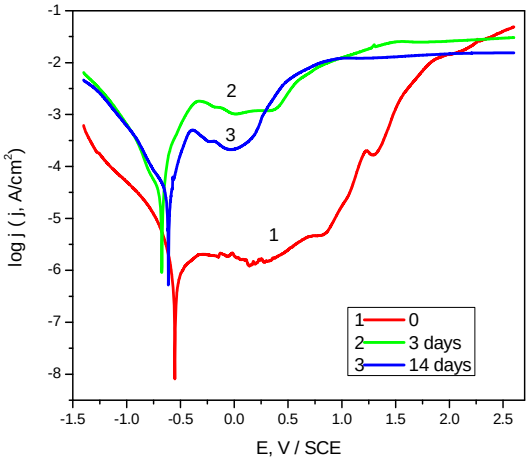

Fig. 4 Potentiodynamic polarization curves of copper in sterile Czapek Dox A medium (1) and in the presence of *Aspergillus niger* after 3 days (2) and 14 days (3). Scan rate: 0.5 mVs^{-1}.

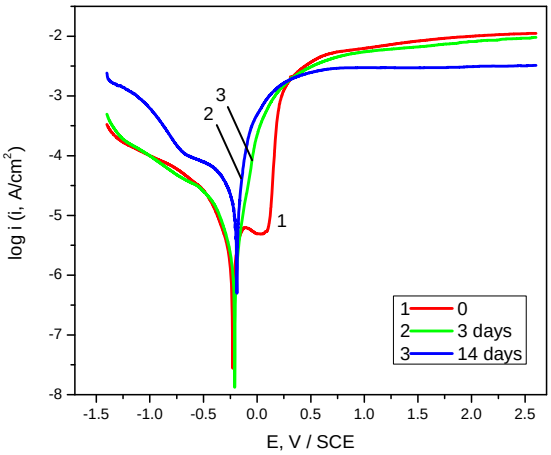

Fig. 5 Potentiodynamic polarization curves of copper in sterile Czapek Dox B medium (1) and in the presence of *Aspergillus niger* after 3 days (2) and 14 days (3). Scan rate: 0.5 mVs^{-1}.

Similar to the interpretation for stainless steel, the anodic branches of Tafel curves for copper in all investigated electrolytes may be divided in two main potential domains: active corrosion behavior and oxygen evolution. We suppose

that processes in stagnant conditions are also controlled by charge transfer kinetics.

Table 4

Corrosion parameters for copper in Czapek Dox A and B media without/with *Aspergillus niger* fungus

Czapek Dox medium	E_{corr} [mV_{SCE}]	J_{corr} [μAcm^{-2}]	b_c [$mVdec^{-1}$]	b_a [$mVdec^{-1}$]	R_p [Ωcm^2]	v_{corr} [mm/year]
Sterile A medium	-547	0.635	–88	95	20080	0.039
A medium with *Aspergillus niger* after 3 day immersion	-673	154.80	–111	102	82	9.616
A medium with *Aspergillus niger* after 14 day immersion	-618	83.17	–130	117	153	5.16
Sterile B medium	-220	1.84	–135	108	6929	0.114
B medium with *Aspergillus niger* after 3 day immersion	-212	1.82	–146	100	7006	0.113
B medium with *Aspergillus niger* after 14 day immersion	-185	13.80	–166	85	923	0.857

The first anodic portion assigned to uniform corrosion lies along approx. 250 mV (except for sterile B medium which is much shorter) and consists in the electrochemical dissolution of copper:

$$Cu \rightarrow Cu^{+} + e^{-} \tag{2}$$

As Table 4 shows, the E_{corr} potential for copper has a different variation in A and B solutions. It shifts to negative potentials when the samples were immersed in fungus inoculated A medium, owing to the increased acidity with metabolism products, especially the citric acid that forms complexes with copper species. On the contrary, E_{corr} potential shifts to more positive values in B solution due to the adsorption of sucrose molecules, more effective than acidification. In A solutions the corrosion rate (J_{corr}) for copper has a huge increase in the first 3 days of immersion and then it remains at high value, with only a smaller decrease after 14 days from inoculation. This decrease is explained by blocking the electrode surface with thicker biofilm. In B solutions (with sucrose) the amplitude of corrosion remains almost constant during 3 days of immersion, a fact explained by an initial period for accumulation of fungus metabolism products on copper

surface. Then, J_{corr} increases by an order of magnitude after 14 day immersion. This last increase is surely due to absorbed metabolic products.

The anodic Tafel slopes have values of around 100 mV per decade for both sterile media (A and B). However, their variation in inoculated media is also different: the b_a value increases slowly after 3 and 14 day immersion in A solution (from 95 to 117 mV per decade, due to adsorption of metabolism products), but it gradually decreases (from 108 to 85 mV per decade) for immersion in B solution due to sucrose desorption.

The second zone in sterile solutions shows a diffusive limited anodic current. This does not occur in the presence of *Aspergillus niger*, a fact explained by the formation of copper complexes. Thus, the polarization curves for 3 and 14 day immersion of copper in B medium exhibit a continuous corrosion with only a limitation of current at high overpotential values (oxygen evolution, over 1.2 V).

An increase of *Aspergillus niger* biofilm onto copper surface is also proved by optical micrographs. Figs. 6 (a-g) illustrate the representative optical images of surface modifications for copper electrode in the investigated Chapek Dox media.

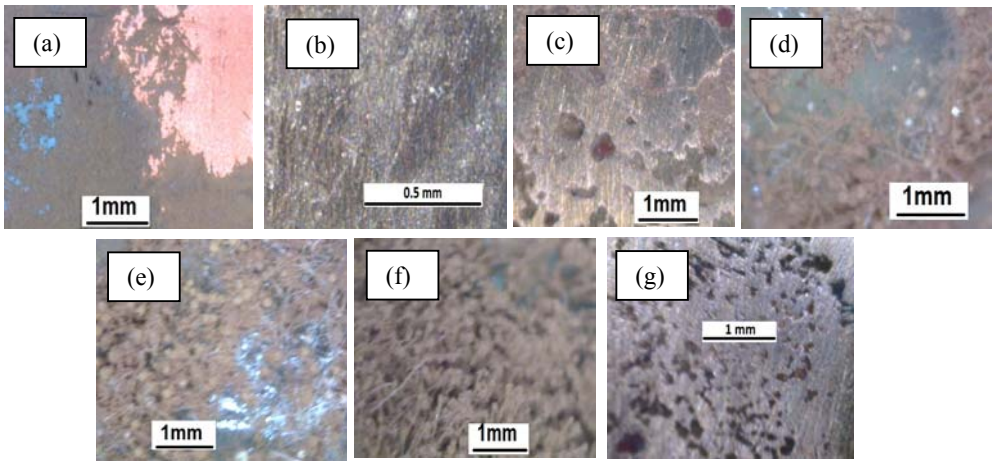

Fig. 6 Representative images regarding biocorrosion of copper in the investigated Czapek Dox media: - after 8 day immersion in sterile A medium (a) or in B medium (b); - after 3 days (c) and 8 days (d) in A medium inoculated with *Aspergillus niger*; - after 3 days (e) and 8 days (f) in B medium inoculated with *Aspergillus niger*; -after 3 days in B medium inoculated with *Aspergillus niger* and removal of colonies or adsorbed corrosion products from the sample surface (g).

The data in Table 4 and optical images (c,d) in Figs. 6 suggest that the immersion of Cu electrode in inoculated A solution led to the formation of a relatively compact biofilm. The metabolism products hinder the diffusion in the electrolyte bulk of copper ionic species occurred onto the metal surface by

corrosion. Conversely, at prolonged immersion in this medium the biofilm behaves as porous film and, as a consequence, E_{corr} tends to be more negative, reaching -0.618 V after 14 days of immersion.

The behaviour of copper sample is different in the inoculated B medium which contains sucrose as carbon source of fungus. It seems that the introduction of sucrose led to a dramatic change of corrosion potentials, because E_{corr} in sterile medium is with approx. 330 mV more positively than in medium without sucrose. In both cases, of 3 and 14 day immersion in inoculated B medium, the E_{corr} value shifts continuously towards a positive direction. This fact is obviously due to a significant increase of growth rate of *Aspergillus niger* fungus in the presence of easily assimilable sucrose, leading to a thicker biofilm. Indeed, Table 4 shows that both J_{corr} values in inoculated B medium after immersion of copper are with an order of magnitude lower than in inoculated A medium, with corresponding amplification of R_p and decrease of v_{corr} values. Nevertheless, we have noticed that the biofilm formed after 14 day immersion, although thicker, becomes much less compact / dense and does not limit the diffusion of corrosion products to solution volume.

It is interesting to note that corrosion degradations are not uniformly distributed on the surface of exposed metal. The massive losses were recorded in preferential areas, as seen in the (g) image of Fig. 6; even after an exposure of 3 days in B inoculated solution, deep craters occur onto copper surface.

4. Conclusions

The results of corrosion parameters suggest that the presence of *Aspergillus niger* fungal in Czapek Dox solutions, especially in those with the addition of sucrose, affects predominantly anodic reactions and it significantly facilitates corrosion process of metallic surfaces. After comparative evaluation of Aspergillus niger colonies, by optical micrographs, it can be stated that they are differently grown, and the copper surface is much more corroded than the AISI 304 stainless steel surface. It can be predicted that, due to this kind of contamination of solutions with Aspergillus niger, the induced corrosion could damage, at some extent, the electrical equipments or installations used in industrial bioprocesses.

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