EIS Study on Biocorrosion of Some Steels and Copper in Czapek Dox Medium Containing Aspergillus niger Fungus

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The paper reports a study about biocorrosion of S235J2G3 low carbon steel, AISI 304 stainless steel and pure copper in Czapek-Dox media inoculated with Aspergillus niger fungus. A comparison of corrosion behavior in media without/sucose (30 g L⁻¹) was made using electrochemical impedance spectroscopy (EIS) technique. The influences of sucrose addition together with the immersion time on Nyquist and Bode diagrams for all three metallic materials were investigated. From the comparative EIS experiments it has resulted that the corrosion behavior is different but more significant growth of fungus was recorded in colonisate solution with sucrose. Both Nyquist and Bode spectra indicated the formation of a thicker biofilm owing to the presence of sucrose as a carbon source, leading to much faster biocorrosion especially in the case of copper. Values of circuit parameters for fitting EIS data were determined.

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

In all processes involving the use of metallic materials in the presence of microorganisms like bacteria, the microbial corrosion and degradation take place. This is why in the last decades biocorrosion has become a significant area of scientific research. The electrostatic charge accumulation on the surface of the metal and the surface roughness favor the formation of colonies as a biofilm containing bacterial cells [1-5]. The attachment of bacteria, release of metabolites and formation of biofilms, which are corrosive, cause damage to the metal structure which is termed ‘microbiologically influenced corrosion’ (MIC) [6-9]. There are no official documents for the cost of MIC, but studies have shown that at least 20% of the total corrosion costs are due to biocorrosion [10,11] and the damages magnitude is influenced by the presence and activities of microorganisms and their metabolites.

The detection, monitoring and prevention of biocorrosion have gained significance in recent years. The problem is complex because different microbial population (aerobic and anaerobic bacteria) in a system interacts during the corrosion of metal in different ways. In general, MIC does not involve a new mechanism of corrosion. The role of microorganisms on the metal surface is to carry out specific biochemical reactions that alter the physical/chemical conditions and favor the maintenance of cathodic and/or anodic half-reactions at the metal/solution interface [12-16]. However, few papers reported the methods to control the biocorrosion of various metals [17-21].

The biocorrosion of carbon steel and stainless steel is the main cause of problems in the pipes of oil industry [22], affecting the costs of production and storage by various repairs and replacements (i.e., replacing pitted pipes, water heaters and other appliances affected by corrosion products). Biocorrosion of mild carbon steel as well as iron was extensively studied in the presence of different species of sulphate-reducing bacteria [13,22-24]. Owing to activity of Thiobacilli, sulfuric acid is produced from the oxidation of various inorganic sulphur sources such as thiosulphates. This acid causes corrosion of mild steel and iron with formation of sulphaes, which are nutrients for sulfate reducing bacteria [25,26].

Biocorrosion of low carbon steel due to various species of mitosporic fungi was studied by Lugauskas et al. in organic media [27]. It was shown that micromycetes as Arthrinium phaeospernum, Aspergillus niger and Chrysosporium merdarium affected steel surface most actively, whereas the products of the activity of both Penicillium cyclopium and Cladosporium herbarum are veiled by the formed layers of Fe oxides and hydroxides. The biocorrosion of mild carbon steel have been studied by us in conditions of synergistic action of both stray current (during polarization in a.c. voltage) and Aspergillus niger filamentous fungus [28]. Such corrosion type is common in buried steel structures [29,30]. Also, the presence of Aspergillus niger in Czapek Dox medium led to citric acid as metabolite on S235J2G3 carbon steel surface [31]. Electrochemical behavior of stainless steels for bioprocessing industry, especially food industry, was studied intensively in recent years. The existence of Pseudomonas spp. or its biofilm on the stainless steel causes formation of oxygen concentration cells, because on low oxygen concentration zones the metal acts as anode and the oxygen-rich zones become the cathode [16]. In general, the pitting corrosion of metals by sulfate reducing bacteria is a result of the electron transfer from metal surface to the bacterial sulphate reduction pathway. In a series of works, Stoica et al. [32-37] have investigated the biocorrosion of AISI 304 stainless steel immersed in fungal suspensions (Aspergillus niger, Geotrichum candidum) during the exposure at the disinfectant solutions of ActiSEPT or Oxonia-Active.

Copper is a common material for tanks or pipes used in many industries because of its robustness and malleability. Also, in various industrial and domestic cases, Cu pipes are used for the transport of cold or hot water. Although often assumed to have inherent anti-microbial properties, Cu metal undergoes however degradation by biocorrosion.

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The corrosion of copper by microorganisms such as *Arthrobacter luteolus* bacterium causes blue-green water (BGW) problem [15,38-43]. Recently, Lugauskas et al. [44,45] have investigated the influence of microorganisms on the corrosion behaviour of Cu in environments polluted with organic substances.

With this in view, the objective of this study is to evaluate the formation in Czapek Dox broth of *Aspergillus niger* biofilms and their effects for three metallic materials: carbon steel, AISI-304 austenitic steel and pure copper. Previously, we have investigated the biocorrosion process of S235J2G3 carbon steel in the same immersion conditions, the process being initiated by *Aspergillus niger* filamentous fungi [31]. In another paper [46] the research was focussed on polarization curves technique and microscopy in order to prove biocorrosion of AISI 304 stainless steel and pure copper. In that work two Czapek-Dox media, without and with sucrose as carbon source for the fungus, have been inoculated with *Aspergillus niger* and the corrosion rate and microographies were observed during 14 days. The present paper extends these investigations by considering the possibility of using electrochemical impedance spectroscopy (EIS) technique to better understand the action of fungus during corrosion of all three metallic materials, carbon steel, stainless steel and copper.

**Experimental part**

All three types of metallic samples consisted in sheets with 0.4 mm thickness. The surface of coupons was first sequentially polished with grit abrasive paper (from 800 to 2000µm) and with aluminum oxide suspension to obtain a glossy surface, then was cleaned with distilled water and dried before every measurement. Chemical compositions of S235J2G3 carbon steel for general use [47], AISI 304 austenitic steel with low carbon content [48] and copper with a purity for electrical purposes [49] were according to European standards. The used Czapek-Dox culture medium has prepared according to recommended chemical composition [50] by introducing inorganic salts (Merck) in water as solvent and adding excess agar-agar to form a gel. Prior to inoculation with spores of *Aspergillus niger* the colloidal solutions were sterilized by autoclaving for more than 30 min at 110°C. Tests were performed using *Aspergillus niger* strain (approx. 10⁶ spores/mL) [51]. In order to evidence the influence of sucrose on the growth of *Aspergillus niger* we used and denoted two different Czapeck-Dox culture media as: A medium (without any carbon source) and B medium with 30 g/L sucrose (Merck) as easily assimilated carbon source for fungus.

Electrochemical impedance spectroscopy (EIS) measurements were carried out with an ac voltage amplitude of ±10 mV in the 10 mHz < f < 20 kHz frequency range. The impedances of metallic sheets at open-circuit potential were represented as Nyquist (imaginary component of impedance vs. real component) and Bode (both impedance modulus and phase angle vs. frequency) spectra. A VOLTALAB 40 type PGE 301 potentiostat/galvanostat provided with a frequency response analyzer was used. The EIS experimental data were processed with a VoltaMaster 4 graphical interface. Equivalent electrical circuits as electrochemical models of the metal/Czapek-Dox solution interface were established using the specialized software Zview 2.90c (Scribner Assoc.) and used for data fitting.

EIS studies were performed in an electrochemical cell with one single compartment having the investigated metallic sheet as working electrode, a platinum sheet as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The experiments have been carried out in stationary conditions at room temperature (23±2°C). All samples and electrochemical cell prior to the determination were sterilized by autoclaving for half an hour to 110°C and 5 bar pressure.

**Results and discussions**

**Corrosion of carbon steel for general use**

Figures 1 a-d show the experimental impedance data obtained for the S235J2G3 steel samples in Czapek Dox A medium after different immersion times. The first Nyquist curve (fig. 1a) refers to an immersion of steel in a sterile A medium, before introduction of *Aspergillus niger*, and therefore it may be considered an initial time of immersion. In this figure the first capacitive semicircle at the highest frequency, with very small diameter, may be neglected being probably a short disturbance of electrical double layer due to occurrence of Fe²⁺ ions by corrosion. As a consequence, we have considered as the main effect only the large semicircle (inductive loop) having a diameter around 800 Ω cm². It is seen that Nyquist semicircles after 3 days (fig. 1b) and 14 days (fig. 1c) of immersion in inoculated A solution differ significantly from data of sterilized medium. The diameter value (which is in fact the polarization resistance, Rp) decreases drastically to 10 Ω cm² for the steel coupon immersed after 3 days, and to 60 Ω cm² after 14 days. Such changes indicate the intensification in time of corrosion process and the pit propagation onto metal surface in the biotic medium. Taking into account lower values of imaginary part of impedance (Zm) than of real part (Zr), all Nyquist plots present depressed semicircles, with the centre of semicircle under the real axis at the higher frequencies. This fact is due to roughness, meaning inhomogeneity of the metal surface. Additionally, the straight line with angle of 45° at low frequency noticed in figure 1c is attributed to the diffusion resistance offered by the biofilm of the electroactive species, *Aspergillus niger*, from bulk solution to the metal surface. This biofilm does not affect the corrosion rate, such features being due to the formation of porous and non protective films on the surface.

The formation of biofilm is confirmed by Bode diagrams (fig. 1d), where the impedance modulus decreases gradually in time. Evolution in time of maximum phase angle is as follows: it starts from -10° which is characteristic for an electrical conduction of metallic material (steel), then has a value of -30° corresponding to a thin porous film, and finally reaches to -35°, this decrease of angle being due to a thicker porous film. Biofilm formation of fungi on the surface may be responsible for reduced capacity of electrochemical system, as will be explained further by simulating the electrical equivalent circuit.

Experimental impedance data obtained for the S235J2G3 carbon steel samples in Czapek Dox B medium after different immersion times were represented in figures 2 a-d. Immersion of steel in the sterile B medium, which contains an addition of 30 g L⁻¹ sucrose before introduction of *Aspergillus niger* (fig. 2a), has led to a Nyquist semicircle with a quite similar diameter of approx. 600 Ω cm² as compared with immersion in A medium (fig. 1a). Nevertheless, *Nyquist not-fully* semicircle recorded after 3 days (fig. 2b) has significant higher diameter, of approx. 1200 Ω cm², suggesting the beginning of a passivation phenomenon with diffusion limiting. Similarly, a portion of Nyquist not-fully semicircle was recorded again after 14 days.
of immersion (fig. 2c), but now it returns to a lower diameter, of approx. 600 Ω cm². All Nyquist plots in figures 2 a-c. Also, on the basis of lower values of imaginary part of impedance \( Z_{\text{Im}} \) than of real part \( Z_{\text{Re}} \), it can say that all Nyquist plots present depressed semicircles with the centre under the real axis, which is due certainly to roughness and adsorbed cells of fungus. Moreover, figure 2c shows that the capacitive semicircle is continued with a straight line proving the formation of a dense biofilm.

On the whole, the rate of growth for Aspergillus niger fungus in Czapek Dox B medium, in the presence of sucrose, which is easily assimilated carbon source, is greater than in A medium, as expected. Bode diagrams for immersion in sterile B medium (fig. 2d) can also give an indication about the passivation produced by biofilm. Thus, the impedance modulus increases gradually in time. The maximum phase angle starts from -10° in sterile B medium that corresponds to a very conductive non-covered steel, then increases to -27° after 3 days in inoculated solution and to -35° after two weeks in inoculated solution; this final value suggests the formation of a thicker porous film during prolonged time.

**Corrosion of AISI 304 stainless steel**

Figures 3 a,b show the experimental impedance data obtained for AISI 304 austenitic steel samples in Czapek Dox B medium after different immersion times. It is worth to mention that all impedance values are with one order of magnitude higher than the impedances for S235J2G3 carbon steel. Our obtained impedances with values of tens kΩ cm² are in very good agreement with values (40-440 kΩ cm²) reported earlier for AISI 304 steel in suspensions media with Aspergillus niger content [34]. In figure 3a there are represented Nyquist spectra for three cases: immersion...
in sterile A medium, in inoculated with *Aspergillus niger* after 3 days and after 14 days. The non-depressed capacitive semicircle occurred at high frequencies has the smallest diameter for sample immersed in sterile medium indicating an intense corrosion. In the following two cases almost identical values of diameter are observed, suggesting a relatively constant corrosion rate in time. However, the formation of biofilm is evidenced even at initial time (case of sterile A medium) by the onset of a straight line at low frequency. This tendency is amplified after 3 and 14 days of immersion, where the length of linear portion is prolonged and it maintains the same slope of 45°, meaning a diffusion-controlled process through porous biofilm.

Bode spectra (fig. 3b) illustrate the corresponding changes on the stainless steel surface. The impedance modulus decreases gradually with immersion time. The maximum phase angle also decreases in time, changing from -80° (electric insulator surface) to -70° after 3 days and to -35° after 14 days, the last being a characteristic value for porous layer which is placed at low frequency. It may be noticed that in the case of AISI 304 stainless steel the biofilm formed by *Aspergillus niger* cells may also include iron and nickel oxides (or hydroxides) resulted by corrosion of sample material.

In figures 4 a,b there are represented Nyquist and Bode spectra for AISI 304 stainless steel in three cases of immersion in Czapek Dox A medium after different immersion times are presented in figures 5 a,b. It may be observed in figure 5a that Nyquist capacitive semicircles are extremely flat compared to stainless steel and even carbon steel. This very depressed shape means a high degree of inhomogeneity of copper surface in given experimental conditions with both high roughness and adsorbed fungus species. It is known that the interception of the real impedance axis with the Nyquist semicircle at high frequency is exactly the uncompensated ohmic resistance of the bulk solution [52]. As figure 5a shows, the start for each semicircle is gradually moved toward high ZRe value showing an increase of solution resistance during immersion time, whereas the semicircle diameter increases in time, too. Hence, the corrosion process of copper is intensified, correspondingly. The order of magnitude for impedance values obtained is of tens Ω cm², correlated very well with the previous results of corrosion rate of copper determined by potentiodynamic polarization experiments in the same system [46] as well as by weight loss experiments [53]. It may observe for the
third Nyquist semicircle (two weeks immersion) a
continuation with a long straight line, inclined at 45°
attributed to biofilm formation.

Bode diagrams (fig. 5b) show a gradual increase of
impedance modulus in time. Interestingly, in the same
figure we noticed very low values of maximum phase angle
which first increases from -5° (in sterile medium) to -9°
(after 3 days in inoculated medium) and then decreases
again to almost -60°. The order of magnitude of these values
suggest a very electrically conductive metal/medium
interface which is probably correlated to occurrence of a
non-adherent biofilm, meaning that the copper surface
almost remains free and corrodes continuously during
exposure to the aggressive medium.

Figures 6 a,b show the experimental impedance data
obtained for pure copper samples in Czapek Dox B medium
after different immersion times. One can see in the Nyquist
representation (fig. 6a) that the variation of solution
resistance during immersion time increases excessive,
from initial value of 40 Ω cm² reaching more than 350 Ω
cm² after two weeks of immersion and suggesting a
significant gelification of B medium compared to A
medium. Figure 6a shows only capacitive semicircles for
all experiments and a gradually increase of diameter
(polariation resistance, Rp) from initial time up to 14 days,
i.e. a diminution of corrosion rate of immersed copper
coupons. The last semicircle (curve 3) has a final part like
a snail in low frequency range that suggests a strong
adsorption process. figure 6b presents only the variation
with ac frequency of phase angle as a part of Bode diagram;
regarding impedance modulus no changes during exposure
of copper surface to Czapek Dox B medium were
registered. One can see in figure 6b a quite similar behavior
of phase angle, with maximum values in the range of small
values, from -7° to -10°. We suppose that in Czapek Dox B
medium the attached biofilm includes predominantly in the
first 3 days some conductive corrosion products consisting
in copper oxide or hydroxide species, making on the whole
an electrical conductive interface between electrode
surface and electrolyte. After this initial period the
accumulation of fungus metabolism products is
predominatly and these non-conductive products adsorb
on copper surface, thus explaining the increase of
maximum phase angle.

Fitting the impedance data by using equivalent electrical
circuits

The impedance spectra are analysed using equivalent
circuits, taking into account the contribution of each
phenomenon, such as the electrical double layer, electron
transfer during corrosion, film formation, and others. The
electrical circuits used to model the interfaces of AISI 304
stainless steel and pure copper are shown in figures 7 a,b.
To obtain more precise fitting results, the capacitance
elements (of electrical double layer and of biofilm) in the
equivalent circuits were both replaced with
constant phase elements (CPE). The components in
the equivalent circuit presented in figure 7a are the
following: Rs - the electrolyte solution ohmic resistance; CPE1 - constant
phase element for non-deal capacitance of electrical double layer;
Rp - polarization resistance; CPE2 - constant phase element for non-deal
capacitance of biofilm; Rf - ohmic resistance of biofilm.
the biofilm: non-ideal capacitance of biofilm (CPEf) and biofilm ohmic resistance (Rf).

The impedance equation of CPE is defined by the following equation:

\[ Z_{CPE} = \frac{1}{T(j\omega)^p} \]  

(1)

where T is the magnitude of capacitance (Ω⁻¹ cm⁻² s⁻¹ or approximately µF cm⁻²) which is the time constant component of CPE, \( \omega \) is the angular frequency of ac voltage (\( \omega = 2\pi f \), with frequency f in Hz), and j is the imaginary unit, \( j^2 = -1 \); p is the exponential part of the constant phase element. For p=1, the CPE can be considered to be an ideal capacitor.

The EIS spectra for sterile Czapek media (initial time of immersion) is represented by equivalent circuit from figure 7a. This result indicated that the corrosion at initial time in both sterile A and B media was under charge transfer control. EIS spectra for the inoculated A and B media can be better simulated with the equivalent circuit presented in figure 7b where the second sub-circuit accounts for biofilm characteristics. Numerical values for each parameter of circuit were calculated upon fitting the model circuit to the experimental data and the obtained values of fitted electrochemical parameters are summarised in tables 1 and 2.

It can be observed from both tables that the Rs values are similar for both sterile and biotic systems being of the order of tens Ω cm² for stainless steel and copper. Pseudo-capacitances of double layer (CPE1-T) have values of hundreds µF cm² for AISI 304 stainless steel and thousands µF cm² for copper, while the p exponents (CPE1-P) were 0.6-0.7 and 0.2-0.4, respectively; this means a more complex structure of the electric double layer in the copper/solution case which probably is due to adsorption and inclusion of inorganic corrosion products in biofilm. In general, the polarization resistance Rp decreases significantly for longer duration of immersion (14 days) being correlated with increase of corrosion current by the growth of fungus population and its intensified activity. The growth of the bacterial population may probably cause pitting corrosion on the metal surface and this is a subject for future research. The corrosion is also accompanied by changes in biofilm characteristics. CPEf-T values for cases of stainless steel in both A and B media are of usual order of magnitude (50-60 µF cm²) for the interface in aqueous solutions and the corresponding CPEf-P is close to 1 (near ideal behaviour of capacitance). On contrary, CPEf-T values for cases of copper in both A and B media have thousands of µF cm² and the corresponding CPEf-P is in the range of 0.4-0.9. This may be explained by the damage to the biofilm formed, resulting in an increase in the active area and a lot of irregularities available for the corrosion process. All these EIS results listed in tables 1 and 2 match well with the potentiodynamic polarization results regarding bio-

<table>
<thead>
<tr>
<th>Circuit component</th>
<th>In sterile medium</th>
<th>After 3 days in inoculated medium</th>
<th>After 14 days in inoculated medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs, Ω cm²</td>
<td>22.3</td>
<td>51.3</td>
<td>16.5</td>
</tr>
<tr>
<td>CPE1-T, µF cm²</td>
<td>175</td>
<td>276</td>
<td>956</td>
</tr>
<tr>
<td>CPE1-P</td>
<td>0.77</td>
<td>0.64</td>
<td>0.58</td>
</tr>
<tr>
<td>Rs, Ω cm²</td>
<td>681300</td>
<td>81</td>
<td>157700</td>
</tr>
<tr>
<td>CPEf-T, µF cm²</td>
<td>-</td>
<td>60</td>
<td>101</td>
</tr>
<tr>
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</tr>
<tr>
<td>Rs, Ω cm²</td>
<td>-</td>
<td>1846</td>
<td>2295</td>
</tr>
</tbody>
</table>

Czapek Dox „A” medium (without sucrose)

| Rs, Ω cm² | 34.7 | 42.9 | 53.9 |
| CPE1-T, µF cm² | 100 | 528 | 524 |
| CPE1-P | 0.72 | 0.60 | 0.60 |
| Rs, Ω cm² | 286500 | 95 | 58 |
| CPEf-T, µF cm² | - | 49 | 50 |
| CPEf-P | - | 0.93 | 0.92 |
| Rs, Ω cm² | - | 142400 | 152900 |

Czapek Dox „B” medium (with sucrose)

| Rs, Ω cm² | 38.9 | 37.8 | 117.5 |
| CPE1-T, µF cm² | 32207 | 727 | 3215 |
| CPE1-P | 0.27 | 0.30 | 0.27 |
| Rs, Ω cm² | 331120 | 26 | 117 |
| CPEf-T, µF cm² | - | 13664 | 10 |
| CPEf-P | - | 0.40 | 0.90 |
| Rs, Ω cm² | - | 278000 | 20 |

Czapek Dox „A” medium (with sucrose)

| Rs, Ω cm² | 46.2 | 103.9 | 25.13 |
| CPE1-T, µF cm² | 10471 | 2 | 826 |
| CPE1-P | 0.43 | 1 | 0.16 |
| Rs, Ω cm² | 375500 | 2.527 | 344 |
| CPEf-T, µF cm² | - | 5933 | 2002 |
| CPEf-P | - | 0.74 | 0.85 |
| Rs, Ω cm² | - | 29.29 | 432.700 |

Table 1
VALUES OF CIRCUIT PARAMETERS FOR DIFFERENT IMMERSION PERIODS OF AISI 304 STAINLESS STEEL IN CZAPEK DOX MEDIA

<table>
<thead>
<tr>
<th>Circuit component</th>
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<th>After 3 days in inoculated medium</th>
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<td>2295</td>
</tr>
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Table 2
VALUES OF CIRCUIT PARAMETERS FOR DIFFERENT IMMERSION PERIODS OF COPPER FOR ELECTRICAL APPLICATIONS IN CZAPEK DOX MEDIA
corrosion of stainless steel and copper in the same biotic media, reported earlier by us [46, 53].

Conclusions

The results obtained using electrochemical impedance spectroscopy suggest that the growth of Aspergillus niger fungal in Czapek Dox solutions, especially in those with the addition of sucrose, significantly facilitates corrosion process of all investigated metallic surfaces: S235J2G3 low carbon steel, AISI 304 stainless steel and pure copper. For comparative Nyquist spectra the common feature is a depressed semicircle (at high frequency) followed by a straight line at lower frequency with a slope angle close to 45°, all indicate the formation of a thicker biofilm owing to the activity of Aspergillus niger colonies. The much more corroded is copper surface and the least corroded is AISI 304 stainless steel surface. We succeeded to modelize EIS results by equivalent electrical circuits and to obtain values of circuit parameters.

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