CHEMIJA. 2012. Vol. 23. No. 3. P. 180–186

Corrosion study of stainless steel incubated in solutions consisting of biocide (*Oxonia-Active*®) and *Aspergillus niger* suspension

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Stainless steel exposed to synergetic action of biocide *Oxonia-Active*® (*OA*) and microbial spores was investigated. The corrosion effect of AISI 316L stainless steel treated by *OA* (consisting of dihydrogen dioxide and ethaneperoxoic acid solution) and in a mixture consisting of *OA* and *Aspergillus niger* spore suspension (*Bio-OA*) was explored. The stainless steel shows higher corrosion behavior in *Bio-OA* than in *OA* immersion. Taking into account the electrochemical results and the AFM measurements, we can assert that *Bio-OA* acts more destructively on the metallic surface than pure *OA*. This effect should be taken into account because it can affect the quality of the food and beverages produced in equipments made of AISI 316L stainless steel.

Key words: biocorrosion, stainless steel, Oxonia-Active, fungi, polarization

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INTRODUCTION

Fungi, including yeasts and molds, are unicellular or multicellular eukaryotic microorganisms [1–4]. Some strains are important for food biotechnology as starter cultures having the ability to modify food characteristics [5] and also for industrial biotechnology to produce antibiotics [6] and other beneficial by-products such as enzymes [7], vitamins [8], organic acids [9, 10] and others. The food industry is highly affected by food spoilage with fungi [11]. It is estimated that 5% to 10% of the world's food production is annually lost due to fungal biodeterioration [12]. Mycotoxins produced as secondary metabolites by fungi during the stationary phase of growth in some specific physico-chemical conditions can induce the risk of health problems [13]. The control of fungal spoilage on this surface is therefore essential and decisive to prevent different biological risks. The bioprocessing applications can have even more stringent requirements due to the high degree of cleanliness required to convey sterile and non-sterile products or solutions. The metallic surfaces of bioprocessing equipments inevitably interact with the electrolytic environment by exposing to washing, disinfecting solutions and to microorganisms present in food products [14, 15]. Electrochemical behavior of stainless steels for bioprocessing industry is studied intensively [16, 17]. Despite the well-established bioelectrochemical behavior of some microorganisms and even their applicability in biofuel cells [18], the synergic effect of microorganisms present in complex mixtures on the stainless steel is still a challenging research area.

The aim of our present work is to extend the study of stainless steel electrochemical behavior in mixtures consisting of *Oxonia-Active®* (*OA*) and fungi and at the same time to investigate possible synergic effects of these mixtures on the corrosion of the metallic surface. The corrosion effect of stainless steel AISI 316L (*SS*) immersed in *OA* (consisting of dihydrogen dioxide 27.5% and ethaneperoxoic acid 8%) and in the mixture consisting of *OA* and *Aspergillus niger* suspension (*Bio-OA*) was investigated. The behavior on the stainless steel was evaluated by electrochemical and AFM measurements. The electrochemical measurements are interesting due to their high sensitivity, straightforward procedures and low cost. The rate of the corrosion process was registered by linear polarization resistance (LPR) technique, which enables to register the rate of the corrosion process. Atomic force microscopy (AFM) techniques can be used in order to determine the roughness of stainless steel surface due to its high spatial resolution at nanometer scale [10, 19, 20].

EXPERIMENTAL

AISI 316L stainless steel. Investigations were performed on AISI 316L stainless steel. Steel coupons of 40 × 10 × 2 mm were used in the experiments after being sequentially polished with a series of grit abrasive papers from 800 to 2 000 μm to obtain a smooth surface. Finally, the coupons were polished to a glossy surface using 0.3 μm aluminum oxide powders. The polished coupons were first for five times rinsed with ultrapure water (UPW), pH 5.6, conductivity 4 μS/cm, concentration of total dissolved solids (TDS) was 2 mg/L; then it was followed by degreasing with acetone [21] to eliminate any fat-based substances. The *SS* coupons, which served as a working electrode, were covered with a tetrafluoroethylene-perfluoroalkylvinylether copolymer foil except an exposed test area of 1 cm^2. The working electrode was embedded horizontally in the electrochemical cell for polarization measurements and the reference electrode (SCE) was connected with the electrolyte using a Luggin capillary tube [22]. The prepared samples were immediately exposed to the test medium for all corrosion experiments described herein.

Biocide. *OA* is approved by the Romanian National Register of Biocide Products to be used in the food processing industries, as well as for sterilization of aseptic processing and packaging equipment [23]. *OA* is very fast-acting, especially at low concentrations and room temperature. Some authors used *OA* in the concentration range of 0.2–1% (v/v) [24, 25]. *OA* is very suitable to protect processing equipment, which is based on non-corrosive AISI 304 and AISI 316 stainless steel, when it is used at recommended concentrations of 0.2–0.28% (v/v) [26]. *OA* was manufactured by Ecolab (GmbH & Co), it is based on 27.5 wt% of H_2O_2 and 8 wt% of $C_2H_4O_3$ (ethaneperoxoic acid). The tests were performed with *OA* of 0.2% prepared in UPW; solution pH was 3.6, its conductivity was 226 μS/cm, TDS was 112 mg/L, the experiments were performed at 15 °C and they lasted for 1 800 s.

Fungal strain. Tests were performed using *Aspergillus niger* strain No ATCC 16404, it was provided by the Ion Cantacuzino Institute (Bucharest, Romania) with the spore's concentration of 1.2×10^6 spores/mL in UPW (pH 6.4; conductivity 78 μS/cm; TDS 47 mg/L). This suspension includes only spores of fungi (*Aspergillus niger*) and was prepared in UPW; the preparation was performed in the same way as for biocide. The spore concentration was measured using a Thoma cytometer [16]. In order to prepare *Aspergillus niger* suspension (*Bio-OA*) 20 mL aliquot of initial fungal suspension was used to compose 100 mL of suspension having 0.2% of *OA*. The final concentration of *Aspergillus niger* spores in *Bio-OA* suspension was $2.5 \cdot 10^5$ spores/mL. The *Bio-OA* suspension was as follows: pH 3.55, conductivity 1 600 μS/cm and TDS 900 mg/L.

Electrochemical study. The corrosion behavior of *SS* samples was tested by incubation in *OA* and *Bio-OA*. The electrochemical measurements were carried out in a glass-based electrochemical-cell equipped with three electrodes at temperature of 15 ± 1 °C. The AISI 316L stainless steel coupons were switched as working electrodes, a platinum foil of 2 cm^2 was used as a counter electrode (CE) and a saturated calomel electrode (SCE) was used as a reference electrode (RE). The entire three-electrode assembly was placed in a Faraday cage to reduce electromagnetic noise disturbance and then it was connected to potentiostat-

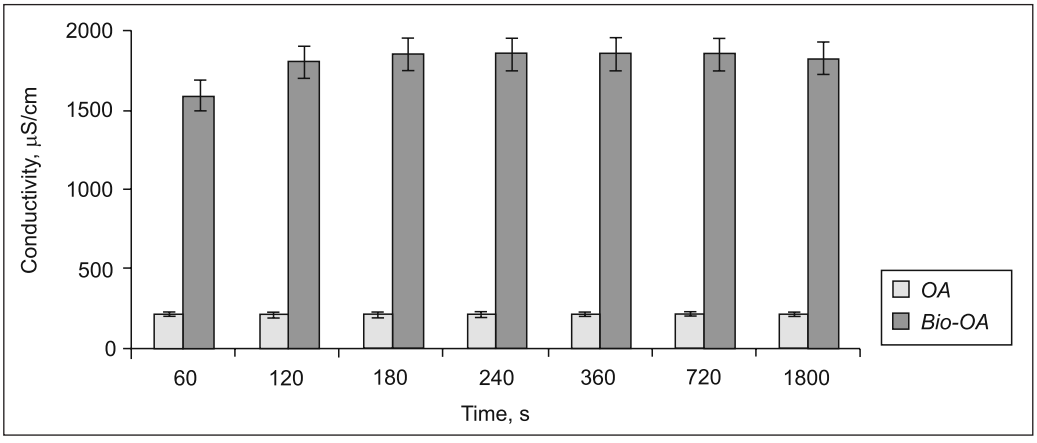

Fig. 1. Average conductivity variation in time of *OA* and *Bio-OA* solutions

galvanostat – Bio-Logic SP-150 (France). The investigations were carried out using EC-Lab® Express v 9.46 software. The electrochemical measurements were based on determination of the open circuit potential (OCP) and application of LPR techniques; these measurements were performed after 40 s and 1 800 s incubation in a solution. In LPR applied potential was within –0.5 V – +1 V (SCE), with potential sweep rate of 50 mV/s [22]. Measurements were performed at least for three times for each sample.

AFM study. An AFM study was performed in contact mode with a BioScope II from Veeco (Santa Barbara, USA). A silicon nitride tip was used to image the metallic surface. The atomic force microscope was placed on an active vibration isolation table set inside a box to eliminate external noise. *OA* and *Bio-OA* were directly drop-wise deposited (100 µL) on *SS* sample for 30 minutes. After this treatment, the metallic sample was washed with distilled water and then it was dried. The samples were visualized by AFM in air at a resolution of 512 × 512 pixels and a scan rate of 0.5 Hz. All of the scanned images were of 50 µm × 50 µm in size. Only by the end of the experiment, this area was increased to verify the general view of the sample. All other AFM investigations and evaluations were performed according to previously described protocols and setups [27, 28].

RESULTS AND DISCUSSION

Conductivity variations of solutions. Conductivity is the ability of a liquid to conduct electricity, which is related to the concentration of ions in the liquid. Figure 1 represents the average conductivity variation in time of *OA* and *Bio-OA* solutions.

The presence of the *Aspergillus niger* spores in *Bio-OA* (Fig. 1, series *Bio-OA*) showed a significant effect on the conductivity in comparison with that observed in *OA* solution, which does not contain any fungal spores (Fig. 1, series *OA*). This fact could suggest that the electrolyte composition in *Bio-OA* is varying within time. These changes can be explained by the synergic action between biocide and the fungal spores, e. g. some Na^+, K^+, Ca^{2+}, Mg^{2+} and some other ions could be extracted from spores by the biocide solution, although it is difficult to elucidate the exact composition of such electrolyte because it is varying within time [29]. As a result, *Bio-OA* is relatively conductive and it is one of the reasons why it accelerates the corrosion of AISI 316L stainless steel.

Electrochemical study. Electrochemical behavior of AISI 316L stainless steel immersed in *OA* and respectively in *Bio-OA* was investigated at 15 ± 1 °C by OCP and LPR techniques. OCP is used as a criterion for the characterization of corrosion behavior. Figure 2 shows the OCP curves for the samples immersed in *OA* and *Bio-OA*.

Fig. 2 indicates that OCP becomes almost stationary in both media after few seconds. Therefore, potential curves were registered after 40 s from the immersion. The polarization curves were registered after two different incubation periods: after 40 s and after 1 800 s (Figs. 3, 4 and Tables 1, 2).

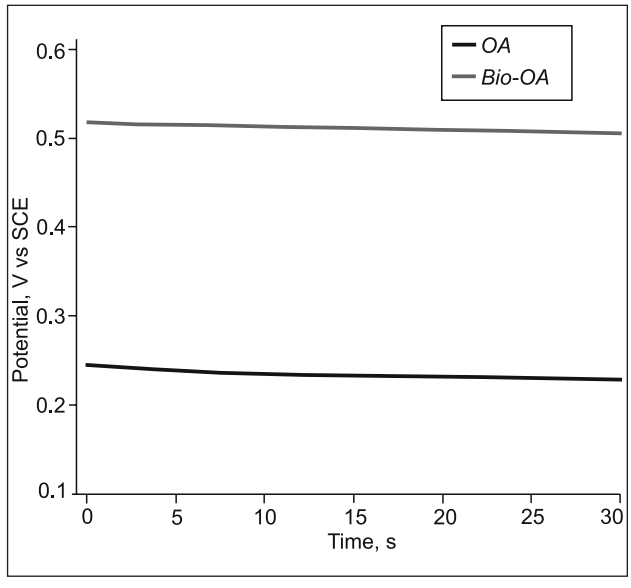

Fig. 2. Averaged values of OCP determined by evaluation of the current density vs. potential plots

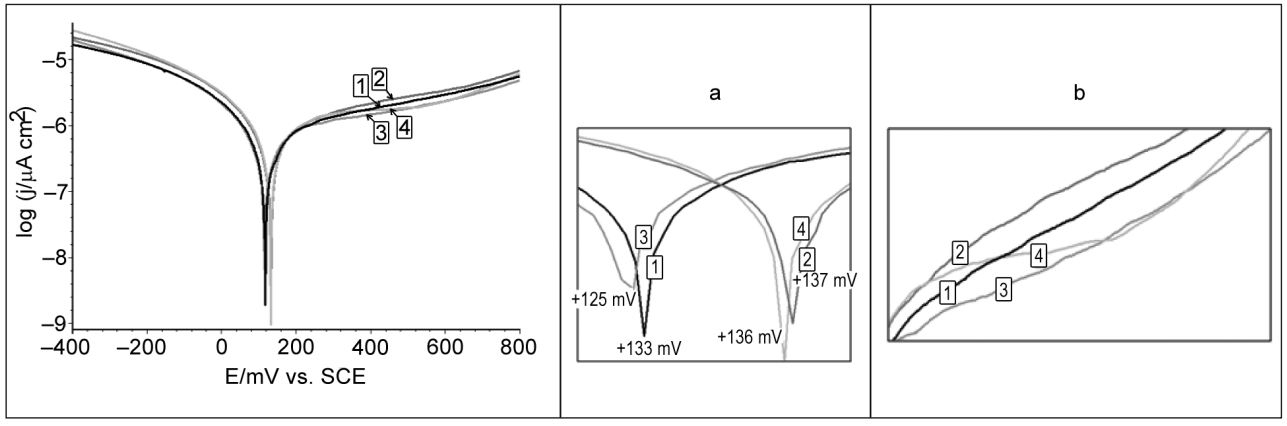

Fig. 3. Polarization curves of *SS* incubated in *OA* after 40 s (1) and 1 800 s (2) and in *Bio-OA*, after 40 s (3) and 1 800 s (4); the shift of E_{corr} (inset a) and the evaluation of slopes of curves (inset b)

Fig. 3 represents the polarization curves of *SS* incubated in *OA* and in *Bio-OA*, after 40 s and 1 800 s. Polarization curves of *SS* incubated in *OA* (Fig. 3, curves No. 1 and 2) and those of *SS* incubated in *Bio-OA* (Fig. 3., curves No. 3 and 4) were evaluated.

It was observed that the E_{corr} potential for *SS* has shifted to negative potential values when the samples were incubated in *Bio-OA* for 40 s (Fig. 3, inset a, curve 3). After 1 800 s incubation, a shift of E_{corr} towards more positive potentials was observed (Fig. 3, inset a, curve 2); this shift was dependent on the duration of incubation in *OA*. If *SS* electrodes were incubated in *Bio-OA*, even a clearer shift towards more positive potentials was observed (Fig. 3, inset a, curve 4). More detailed evaluation of Tafel plots (Fig. 3, inset b) illustrates that the lines 1 vs. 2 and 3 vs. 4 are not parallel and are related to different in nature corrosion within different time frames.

Figure 4 provides the averaged values of the corrosion current density for *SS* incubated in *OA* and *Bio-OA*.

After 40 s incubation in *Bio-OA* solution, the j_{corr} values were lower in comparison with those registered after 1 800 s incubation. This effect is related to the fact that at the initial 40 s period biocide did not completely damage the *Aspergillus niger* spore wall and therefore the composition of the solution was not significantly changed and for this reason the *SS* surface contamination was not very intensive. After much longer (1 800 s) incubation in biocide a large amount of spores were damaged and the intra-cellular substance was released into the solution. Therefore the increase of j_{corr} was observed. It can be predicted that due to this kind of contamination the induced corrosion could at some extent damage *SS*-based equipment used in bioprocessing.

Table 1 represents the electrochemical parameters of AISI 316L stainless steel surface, which is incubated in *OA*, in the *Aspergillus niger* spore suspension and in *Bio-OA*, after 40 s period from the immersion *of SS*.

As it can be observed from the presented data, E_{corr} is (i) + 133 mV vs. SCE for the *SS* samples incubated in *OA*; –218 mV vs. SCE in *Aspergillus niger* spore suspension for 40 s (Table 1). In the case of incubation in *Bio-OA*, E_{corr} is shifted to +125 mV vs. SCE. Table 2 presents the electrochemical parameters of AISI 316L stainless steel surfaces immersed in *OA*, in the *Aspergillus niger* spore suspension and in *Bio-OA* after incubation for 1 800 s.

The E_{corr} potential of +137 mV, –190 mV, +133 mV vs. SCE was registered if *SS* was incubated in *OA, Aspergillus niger* suspension and *Bio-OA*, correspondingly (Table 2). The shifts of the E_{corr} potential suggest that the *Aspergillus niger* suspension if added in *OA* acts as a depolarization agent for the AISI 316L stainless steel surface. This fact was confirmed by shifts in the cathodic (β_c) and anodic (β_a) Tafel slopes [30–2]. These shifts affect predominantly anodic processes (Table 1). After 1 800 s from immersion, the cathodic (β_c) and anodic slope (β_a) were shifted more significantly (Table 2). These results suggest that the fungal suspension and *Bio-OA* affect predominantly anodic reactions and they significantly

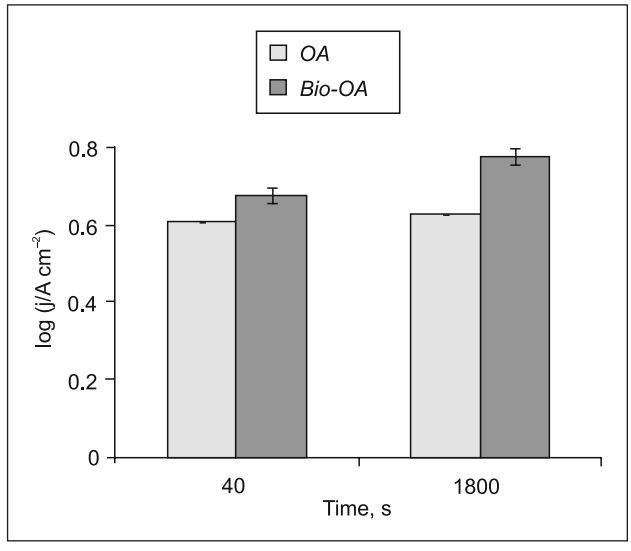

Fig. 4. Average values of the corrosion current density for *SS* incubated in *OA* and *Bio-OA*

Table 1. **Electrochemical parameters of SS surfaces immersed in *OA* (a), in *Aspergillus niger* suspension (b) and *Bio-OA* (c), after 40 s from the immersion of *SS***

Electrolyte	Time, s	E_{corr}, mV vs SCE	*Bc*, mV/dec.	*Ba*, mV/dec.
Oxonia-Active® (0.2% sln.) (*OA*)	40	+133 ± 4.24	230 ± 7.78	453 ± 41.50
Aspergillus niger		–218 ± 0.00	311 ± 0.00	427 ± 0.00
Oxonia-Active® with *Aspergillus niger (Bio-OA)*		+125 ± 6.62	230 ± 2.75	570 ± 77.50

Table 2. **Electrochemical parameters of SS surfaces immersed in *OA* (a), in *Aspergillus niger* suspension (b) and *Bio-OA* (c), after 1 800 s incubation**

Electrolyte	Time, s	E_{corr}, mV vs SCE	*Bc*, mV/dec.	*Ba*, mV/dec.
Oxonia-Active® (0.2% sln.) (*OA*)	1 800	+137 ± 7.78	221 ± 9.90	493 ± 42.00
Aspergillus niger		–190 ± 0.00	295 ± 0.00	360 ± 0.00
Oxonia-Active® with *Aspergillus niger (Bio-OA)*		+133 ± 3.73	223 ± 13.29	562 ± 33.16

facilitate corrosion processes of metallic surfaces [33]. The shifts of E_{corr} and the increasing of j_{corr} indicate a synergic effect of *Aspergillus niger* spores and *OA* on the facilitation of *SS* corrosion. The observed predominant anodic process is usual in the corrosion of metallic surfaces and it is in good agreement with previously reported experimental results.

AFM Study. The characterization of surface topography is an important methodology in the evaluation of biocorrosion [34]. Therefore the AFM is performed during the quantitative analysis of surfaces in order to detect nano-scale irregularities [35]. The topography of any surface will take the form of a series of fells and valleys, which may vary both in height and spacing. Surface roughness could be evaluated in two principal planes [36]. One plane is perpendicular to the surface and it is described as height deviation, and the second plane of the surface is described by spatial parameters and identified as texture [35]. There are a number of amplitude parameters such as R_a known as the arithmetic average height parameter, R_q known as the root mean square and R_{max} known as the difference in height between the highest and lowest points on the surface relative to the mean plane. R_a is the most universally used roughness parameter for general surface-quality control [37]. The AFM images of the *SS* surfaces before and after the incubation in *OA* and *Bio-OA* are presented in Fig. 5. The two roughness parameters R_a and R_{max} were used to evaluate quantitatively the surface topography of each sample. Results are presented in Table 3 and Fig. 6.

Fig. 5 reveals the differences between stainless steel surfaces before incubation and after incubation in *OA* and in *Bio-OA*. The differences between images show the differences in topography of differently treated surfaces [38]. Fig. 5a illustrates that a freshly prepared *SS* sample has a smooth and pit-free surface. No clear pits of corrosion can be observed on the *SS* surface after incubation in *OA* (Fig. 5b). The scratches that appeared after polishing with aluminum oxide are more clearly visible if *SS* was incubated in *Bio-OA* (Fig. 5c). The corrosion process can be expressed in different ways such as the intensity of corrosion estimated by evaluation of the average roughness variation [39]. The study of the AFM images in terms of surface roughness allows a more exact evaluation of the evolution of surface-degradation as well as the obtaining of some quantitative information [39]. As the dimensions of the scanned area were kept constant all along the experiment, it was possible to quantify the extent of the surface-degradation in terms of the average surface roughness.

Table 3 represents R_a parameter of the *SS* surface before and after the incubation in *OA*, and R_a parameter of the *SS* surfaces after incubation in *Bio-OA*.

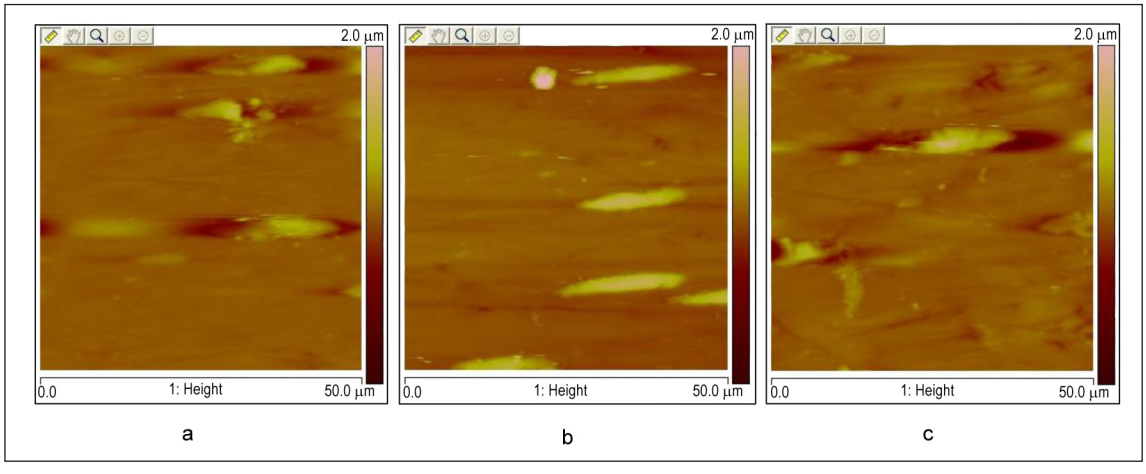

Fig. 5. AFM images of *SS* surfaces: before incubation in *OA* (a); after 1 800 s incubation in *OA* (b) and after 1 800 s incubation in *Bio-OA* (c)

Table 3. **R_a parameter of *SS* surfaces before and after immersion in *OA* and in *Bio-OA***

Corrosion media	R_a, nm
SS before incubation	31.13
SS incubated in *Oxonia-Active®* (*OA*)	32.40
SS incubated in *OA* with *Aspergillus niger* suspension (*Bio-OA*)	45.00

Tested *SS* samples are acceptable for food industry because the R_a values are lower than 0.8 μm [40]. Large areas of *SS* surfaces coming in contact with food products should have a surface polished / finished until the R_a values go below 0.8, although the cleaning-ability strongly depends on the applied surface finishing technology because it can affect the surface topography. But, from the data (Table 3), the R_a parameter of the *SS* surface before incubation was equal to 31.13 nm. After the incubation in *OA*, the R_a parameter increased to 32.4 nm. The R_a parameter of *SS* incubated in *Bio-OA* increased up to 45 nm (Table 3). No *Aspergillus niger* spores were detected by AFM; it means that the increase of surface roughness is not related to the absorption of *Aspergillus niger* spores on the surface of *SS* (Fig. 5c). Any increase in roughness could be attributed to the amount of material leaking from the surface [39]. The changes in the surface roughness are mainly attributed to the destruction of the protective layer [21]. Fig. 6 represents the R_{max} parameter of the *SS* surface before and after the incubation in *OA* and in *Bio-OA*.

After incubation of *SS* in *OA* and in *Bio-OA*, the R_a values varied not very significantly (Table 3), while the R_{max} value was significantly higher if compared to that of *SS* surface before incubation in *OA* and *Bio-OA* (Fig. 6). In the case if *SS* was incubated in *Bio-OA*, it can be observed that the R_{max} value increased more than in *OA* (Fig. 6). *Bio-OA* alters the metallic surface and decreases its corrosion resistance, abrasive resistance and it possibly increases the adhesion of biofilm [40–42]. Since no clear signs of biofilm adhesion were observed we can conclude that the biofilms probably do not participate in the corrosion process directly, but, according to data presented in some other researches, even very thin biofilms can lead to some changes of the interfacial environment increase cell concentration what facilitates the corrosion process on the surface [21].

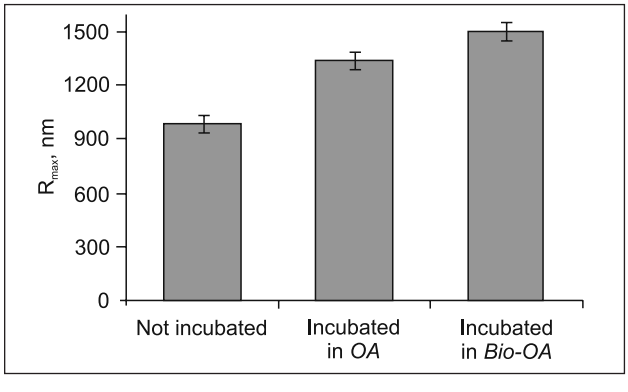

Fig. 6. R_{max} values of the SS surface before and after incubation in *OA* and in *Bio-OA* for 1 800 s

CONCLUSIONS

From the LPR and AFM results, it can be concluded that *Bio-OA* is more destructive for the *SS* surface than *OA*. The R_{max} values and j_{corr} values of *SS* incubated in *Bio-OA* are higher than those incubated in *OA*. At the macroscopic scale, the effect of *Bio-OA* can be ignored but investigations at the nanoscopic scale can negatively influence the quality of the products produced in biotechnological equipment, which is based on AISI 316L stainless steel.

ACKNOWLEDGEMENTS

This work has benefited from the financial support through the 2010 POSDRU/89/1.5/S/52432 Project "Organizing the National Interest Postdoctoral School of Applied Biotechnologies with Impact on Romanian Bioeconomy", Project cofinanced by the European Social Fund through the Sectoral Operational Programme "Human Resources Development 2007–2013".

Received 14 November 2011
Accepted 18 May 2012

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NERŪDIJANČIO PLIENO, INKUBUOTO BIOCIDO *OXONIA-ACTIVE* IR *ASPERGILLUS NIGER* SUSPENSIJOJE, KOROZIJOS TYRIMAS

S a n t r a u k a

Ištirtas sinergetinis biocido *Oxonia-Active*® (*OA*) ir *Aspergillus niger* sporų korozinis poveikis nerūdijančiam plienui. Korozijos efektas buvo stebimas nerūdijantį plieną AISI 316L veikiant *OA* (tirpalas pagamintas iš vandenilio peroksido ir peroksietano rūgšties) bei *OA* ir *Aspergillus niger* sporų suspensija (*Bio-OA*), didesnis korozijos poveikis buvo stebėtas veikiant plieną *Bio-OA* tirpalu. Korozijos poveikis buvo vertinamas elektrochemiškai bei atominių jėgų mikroskopu. Šio tyrimo rezultatai svarbūs maisto ir gėrimų pramonės gamintojams, kurių įranga yra iš nerūdijančio plieno AISI 316L.