

Electrochemical study of stainless steel characteristic modification on correlative effect of fungal cell suspension and *ActiSEPT* used as biocide for equipment disinfection in bioprocessing of food

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The electrochemical behavior of AISI 304 stainless steel used as material of biotechnological equipment, in a simulated study on singular effect of *ActiSEPT*, a biocide consisting in sodium dichloroisocyanurate and synergic effect of *ActiSEPT* and two fungal suspensions of *Aspergillus niger* and *Geotrichum candidum* were investigated by linear polarization of metallic surface. This aspect plays an important role in the stainless steel surface corrosion with implications in the bioproduct quality and influences the safety of life.

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1. Introduction

Fungi are the most important microorganisms used in biotechnology for different biotechnology processes, such as: enzymes, antibiotics organic acids, vitamins production, etc. [1–4]. Some of the fungal species, *Aspergillus niger* and *Geotrichum candidum*, extensively used in biotechnology fields are impressive producers of hydrolytic enzymes already applied in a variety of industrial processes [5–8]. These processes are going on in bioreactors which are the most used systems for the microorganism cultivation. The bioreactors are usually made from 316L stainless steel, but some of them are made from less expensive type stainless steel as AISI 304 [9]. In majority of the bioprocesses, the reactors are operated in batch mode, under sterile conditions [10]. The cleaning and sterilizing are one of the most important activities carried out in the bioprocesses of food [11]. The industrial bioreactors should be sterilized before inoculation, because the contamination with small quantities of undesirable microorganisms is a common cause of process failure [12]. The control of microorganisms is essential to ensure proliferation of the desired species at the expense of the non-desired ones which may have the potential to influence of the process in all stages. The most used biocides used for disinfection of equipments in bioprocessing are based on chloride solutions. In this study the electrochemical behavior of AISI 304 stainless steel upon correlative effect of *ActiSEPT* biocide (sodium dichloroisocyanurate) and fungal suspensions, such as *Aspergillus niger* and *Geotrichum candidum* was investigated by linear

polarization (LP) of the stainless steel, this technique offering faster measurements of metallic surface corrosion processes.

2. Experimental

2.1 Materials

AISI 304 Stainless Steel (SS). The prepared samples in rectangular samples (40×10×2) mm were pretreated by mechanically polishing with abrasive paper of increasingly finer grit (from 800 to 2000 μm) and finally by chemical cleaning [13–15]. The *SS* samples as working electrode were covered with a tetrafluoroethylene-perfluoroalkylvinylether copolymer foil excepting an exposed test area of 1 cm².

Biocide. *ActiSEPT* containing a salt of sodium dichloroisocyanurate (NaDCC) is a commercially disinfectant manufactured in Great Britain for Medicarom/Romania. It is effective against to all types of microorganisms used in biotechnology. *ActiSEPT* release hypochlorous acid only when it is dissolved in water having biocidal activity against fungi. The fresh biocide solutions were prepared by adding of the commercially tablets of *ActiSEPT* in sterile water to obtain a concentration of 140 ppm active chlorine when the disinfectant effectiveness lasts of 600 s.

Fungal strains. Two fungal suspensions were prepared as following: *Aspergillus niger* (1.2×10⁶ spores/mL) and *Geotrichum candidum* (1.3×10⁶ spores/mL). The spore concentration was measured using

a Thoma cytometer [13]. An aliquot volume of each fungal suspension (20 mL) was used in the electrochemical experiments for 100 mL as total volume [16]. The final concentrations of fungal suspensions in experiments were smaller (*A. niger*- 2.5×10^5 spores/mL and *G. candidum*- 2.6×10^5 spores/mL).

2.2 Electrochemical Measurements

The electrochemical behavior of SS samples was performed in the *ActiSEPT* biocide solution with and without fungal suspensions. The electrochemical measurements were carried out in a glass electrochemical-cell equipped with three electrodes, at (15 ± 1) °C [16]. The working electrode (WE) was AISI 304 stainless steel, the counter electrode (CE) was a platinum foil (2 cm²) and the saturated calomel electrode (SCE) was as reference electrode (RE). The entire three-electrode assembly was placed in a Faraday cage to limit the noise disturbance and then it was connected to Bio-Logic SP-150 potentiostat – galvanostat (France), using EC-Lab® Express v 9.46 software. The electrochemical investigations were carried out through linear polarization (LP) technique. The potential range was between -1 V to +1 V (vs. SCE) with a scan rate of 50 mV·s⁻¹. The triplicate polarization measurements were initiated for each sample, after 40 s and 600 s from immersion of SS in the solution. All samples, one by immersion of SS in biocide and the others by immersion of SS in fungal cell suspensions, were analyzed at the same working conditions. The electrochemical parameters as corrosion potential (E_{corr}), current density (j_{corr}) and corrosion rate (V_{corr}) were calculate by the Tafel fit analysis tools. The R_p of the metallic surfaces was calculated through j_{corr} and β_a and β_c [17].

3. Results and discussions

The electrochemical behavior of AISI 304 Stainless Steel immersed in fungal spore suspensions, in biocide solution and those in mixture solutions consisting in biocide and fungal spore suspensions were investigated by LP technique, at temperature of (15 ± 1) °C, during 40 s and 600 s from immersion. The results are presented in the Figs. 1-5 and Table 1-4.

The SS samples immersed only in *Aspergillus niger* spore suspension present the more negative values of E_{corr} -275 mV (SCE), respectively j_{corr} equal to 0.12 $\mu\text{A}/\text{cm}^2$, at 40 s (Table 1). The E_{corr} potential was shifted in the more positive direction when the samples were immersed in *Aspergillus niger* suspension, after 600 s (Fig. 1, curve 1-2). The E_{corr} potential is of -66 mV (SCE) and j_{corr} increases to 0.17 $\mu\text{A}/\text{cm}^2$, as result of fungal effect in the corrosion modification on metallic surface (Table 2). The SS surfaces immersed only in *Geotrichum candidum* spore suspension indicate a E_{corr} potential of -190 mV (SCE) and j_{corr} of 0.10 $\mu\text{A}/\text{cm}^2$ (lowest values recorded), after a contact time of 40 s (Table 3). The E_{corr} potential on SS surfaces was of -195 mV (SCE) and j_{corr} of 0.14 $\mu\text{A}/\text{cm}^2$, after 600 s from immersion (Fig. 1, curve 3-4). These data

confirm a lower electrons density, in the electrochemical system which contains only fungal suspensions.

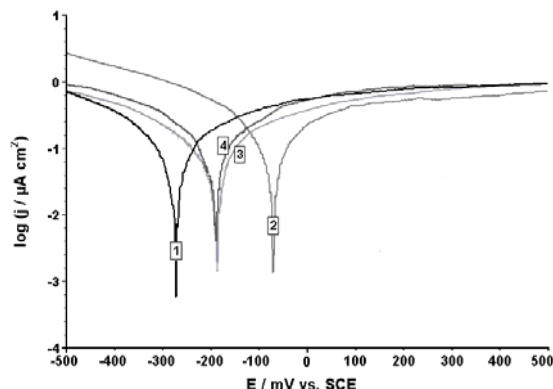


Fig. 1. Polarization curves of SS by immersion in different fungal spore suspensions: *Aspergillus niger* at 40 s (1) and 600 s (2) and *Geotrichum candidum* at 40 s (3) and 600 s (4)

3.1 The correlative effect of biocide and fungal cell suspensions on SS surface modification

The polarization curves of SS immersed only in biocide *ActiSEPT* solution (curves number 1 and 2) with the polarization curves of SS immersed in the mixture consisting in biocide and different fungal suspensions (curves number 3 and 4) were compared. Figure 2 presents the polarization curves of SS by immersion in *ActiSEPT* biocide solution, at 40 s and 600 s, respectively in mixture consisting in biocide and *Aspergillus niger* spore suspension, at 40 s and 600 s.

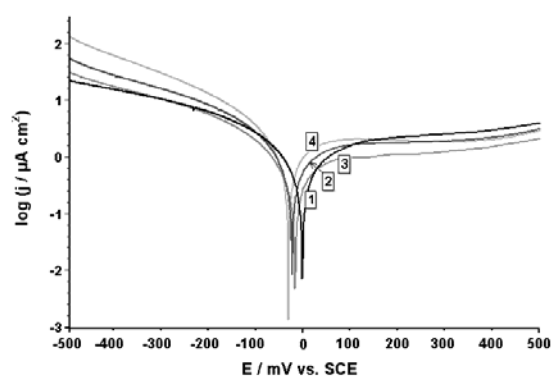


Fig. 2. Polarization curves of SS immersed in *ActiSEPT* biocide solution, at 40 s (1) and 600 s (2), respectively in mixture consisting in biocide and *Aspergillus niger* spore suspension, at 40 s (3) and 600 s (4).

It can be observed that the E_{corr} potential for SS was shifted to negative direction when the samples were immersed in *ActiSEPT* biocide with *Aspergillus niger* suspension, after 40 s (Fig. 2, curve 3). Also, it can be observed a shift of E_{corr} potential with the immersion time to the more negative direction (after 600 s) in case of

ActiSEPT biocide solution (Fig. 2, curve 2), respectively for mixture with biocide and *Aspergillus niger* suspension (Fig. 2, curve 4). The absence of the parallelism of cathodic and anodic branches between the curves 1 and 2 (biocide) and curves 3 and 4 (mixture), suggests that the initiated corrosion processes on the SS surfaces immersed in this mixture are different from that on SS immersed only in *ActiSEPT* biocide. Table 1 shows the electrochemical parameters on the AISI 304 stainless steel surfaces immersed in the *ActiSEPT* biocide solution with and without *Aspergillus niger* spore suspension, after 40 s from immersion.

Table 1. Electrochemical parameters on SS surfaces immersed in *Aspergillus niger* suspension (a), in *ActiSEPT* biocide (b) and mixture of biocide with *Aspergillus niger* suspension (c), after 40 s from immersion

	E_{corr} (mV)	j_{corr} ($\mu\text{A}/\text{cm}^2$)	β_c (mV)	β_a (mV)	R_p (Ωcm^2)
a	-275	0.12	279	356	56×10^4
b	+2	1.54	330	620	60×10^3
c	-12	0.85	328	635	11×10^4

As it can be observe from data, the E_{corr} is of +2 mV (SCE) and j_{corr} is of $1.54 \mu\text{A}/\text{cm}^2$ for the SS samples immersed only in *ActiSEPT* biocide. In case of use the mixture consisting from *ActiSEPT* biocide and *Aspergillus niger* spore suspension the E_{corr} potential is sifted of -12 mV (SCE), at 40 s from the immersion and it observe a decreasing of current density from $1.54 \mu\text{A}/\text{cm}^2$ to $0.85 \mu\text{A}/\text{cm}^2$. Table 2 presents the electrochemical parameters on AISI 304 stainless steel surfaces immersed in the *ActiSEPT* biocide solutions with and without *Aspergillus niger* spore suspension, at 600 s from immersion.

Table 2: Electrochemical parameters on SS surfaces immersed in *Aspergillus niger* suspension (a), in the *ActiSEPT* biocide (b) and mixture of biocide with *Aspergillus niger* suspension (c), after 600 s from immersion

	E_{corr} (mV)	j_{corr} ($\mu\text{A}/\text{cm}^2$)	β_c (mV)	β_a (mV)	R_p (Ωcm^2)
a	-66	0.17	284	434	44×10^4
b	-10	1.36	300	670	66×10^3
c	-30	1.74	205	690	40×10^3

The E_{corr} potential is of -10 mV (SCE) and j_{corr} is of $1.36 \mu\text{A}/\text{cm}^2$ for the system with biocide. In case of mixture consisting from *ActiSEPT* biocide with *Aspergillus niger* spore suspension the E_{corr} potential is shifted to -30 mV (SCE) and an increasing of current density with about $0.38 \mu\text{A}/\text{cm}^2$ was observed as a synergic effect of the biocide - fungal corrosion. The shifts of E_{corr} potential suggest that the *Aspergillus niger* suspension

added in the biocide is a depolarization agent for the AISI 304 stainless steel surfaces, confirmed by the shifts in the cathodic (β_c) and anodic (β_a) Tafel slopes [18-20]. These shifts affect predominantly anodic processes (Table 1). After 600 s from immersion the cathodic (β_c) and anodic slope (β_a) were shifted also (Table 2). These results suggest that the effect of mixture consisting from biocide with *Aspergillus niger* spore suspension is controlling predominantly the anodic reactions, the fungal suspension inducing a corrosion processes on the metallic surfaces [21, 22]. The shifts of E_{corr} and the increasing of j_{corr} indicated a synergic effect of *Aspergillus niger* spore and *ActiSEPT* biocide on chemical characteristics of stainless steel surface. This fact can substantially accelerate the corrosion process on the metallic surfaces. Figure 3 shows the polarization curves of SS immersed in biocide solution and respectively in mixture consisting in biocide and *Geotrichum candidum* spore suspension, after 40 s and 600 s.

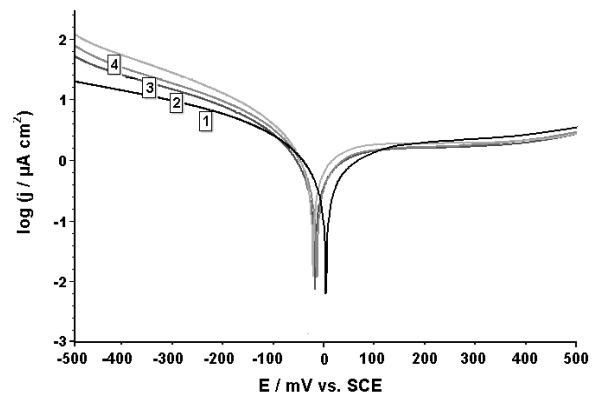


Fig. 3. Polarization curves of SS immersed in *ActiSEPT* biocide solution, after 40 s (1) and 600 s (2) from immersion and respectively in mixture consisting in biocide and *Geotrichum candidum* spore suspension, after 40 s (3) and 600 s (4)

The polarization curves, for SS immersed in *ActiSEPT* biocide solution in combination with *Geotrichum candidum* spore suspension indicate a shift of E_{corr} potential to the negative direction, after 40 s from immersion (Fig. 3, curve 3). Also, it can be observed a shift of E_{corr} potential to more negative direction with increasing of immersion time, in the case of *ActiSEPT* biocide (Fig. 3, curve 2), as well for the mixture with biocide and *Geotrichum candidum* spore suspension, after 600 s from immersion (Fig. 3, curve 4). The absence of the parallelism of cathodic and anodic branches between the curves 1 and 2 (biocide) and curves 3 and 4 (mixture) suggests that the corrosion processes on the SS metallic surfaces immersed in the mixture are different from that on samples immersed only in biocide. Table 3 presents the electrochemical parameters on the AISI 304 Stainless Steel surfaces immersed in *ActiSEPT* biocide solution, with and without *Geotrichum candidum* spore suspension, after 40 s.

Table 3: Electrochemical parameters on SS surfaces immersed in *Geotrichum candidum* suspension (a), in ActiSEPT biocide (b) and in mixture of biocide with *Geotrichum candidum* suspension (c), after 40 s from immersion

	E_{corr} (mV)	j_{corr} ($\mu\text{A}/\text{cm}^2$)	β_c (mV)	β_a (mV)	R_p (Ωcm^2)
a	-190	0.10	260	395	68×10^4
b	+2	1.54	330	620	60×10^3
c	-10	1.47	305	690	62×10^3

In case of the SS immersion in mixture consisting in biocide and *Geotrichum candidum* spore suspension the E_{corr} potential is of -10 mV (SCE), after 40 s from immersion and a smaller decreasing of current density with $0.07 \mu\text{A}/\text{cm}^2$ was observed as a slight effect of *Geotrichum candidum* on the metallic surface. Table 4 shows the electrochemical parameters on the AISI 304 stainless steel surfaces after immersion in ActiSEPT biocide solution, with and without *Geotrichum candidum* spore suspension, at 600 s.

Table 4: Electrochemical parameters on SS surfaces immersed in *Geotrichum candidum* suspension (a), in ActiSEPT biocide (b) and mixture of biocide with *Geotrichum candidum* suspension (c), after 600 s from immersion

	E_{corr} (mV)	j_{corr} ($\mu\text{A}/\text{cm}^2$)	β_c (mV)	β_a (mV)	R_p (Ωcm^2)
a	-195	0.14	302	315	48×10^4
b	-10	1.36	300	670	66×10^3
c	-30	1.74	200	790	40×10^3

The mixture consisting in ActiSEPT biocide with *Geotrichum candidum* spore suspension indicate the E_{corr} potential of -30 mV (SCE). The shifts of E_{corr} suggest that the *Geotrichum candidum* suspension added in the biocide is a depolarization agent on the AISI 304 stainless steel surfaces, affecting predominantly anodic processes. The presence of *Geotrichum candidum* spore suspension indicates also an increasing of current density ($0.38 \mu\text{A}/\text{cm}^2$). The mixture, after 40 s from immersion indicates the shifts in the cathodic (β_c) and anodic slope (β_a) (Table 3). After 600 s from immersion the cathodic (β_c) and anodic slope (β_a) are shifted, too (Table 4) and these shifts of the Tafel slopes reveal that the *Geotrichum candidum* suspension induces corrosion on the metallic surface [16, 21]. The shifts in the E_{corr} potential and increasing of the j_{corr} values indicate the synergic effect of mixture of the biocide with *Geotrichum candidum* spore suspension, manifested through distorted potential-current curves predominantly in anodic region, as in the case of use of *Aspergillus niger*, too. After 40 seconds contact time in the mixture of biocide with *Aspergillus niger* spore suspension the j_{corr} values indicate a less increasing, but after 600 s is observed a more increasing. This fact could explain that

the biocide molecule does not damage the *Aspergillus niger* spores in 40 s and an electrolytic contamination does not occur. Also, the mixture of biocide with *Geotrichum candidum* spore suspension has the same behavior, but the j_{corr} value is larger for the mixture with *Geotrichum candidum* ($1.47 \mu\text{A}/\text{cm}^2$), than that of the mixture with *Aspergillus niger* ($0.85 \mu\text{A}/\text{cm}^2$), at 40 s. The *Aspergillus niger* spores are more favorable to protect of chemical and electrical stressing on the metallic surface [7]. Thus, the mixture consisting of biocide with fungi can promote the corrosion process on the metallic surface and it affects the bioprocessing equipments.

3.2 The synergic effect of disinfectant and fungal spore on stainless steel corrosion

All electrochemical parameters indicate a shifting of E_{corr} potential and an increasing of j_{corr} values of the surfaces immersed in mixture consisting in biocide with fungal suspensions, in comparison with data obtained when the metallic surfaces were immersed only in biocide or fungal suspensions. The corrosion synergic effect on the AISI 304 stainless steel samples is reflected through R_p (polarization resistance) and V_{corr} (corrosion rate; mm/y). It can be observed that the R_p values are lower in the biocide and mixtures consisting in biocide with fungal suspensions than in fungal suspensions (Tables 1-4). The order of magnitude of R_p is the same for both mixtures of biocide with fungal suspensions comparing with the biocide, after 600 seconds from immersion. Nevertheless, the R_p values for SS immersed in mixtures are smaller than R_p values for SS immersed only in biocide. The AISI 304 stainless steel surfaces are more susceptible in mixtures containing biocide with fungal spore suspensions [13]. The V_{corr} was calculated based on EW (equivalent weight) of SS, density of SS and the exposed surface area included in the experimental setup for the EC-Lab® Express v 9.46 software. The data reported already in literature show that the SS surfaces present the increased corrosion rates by immersion in biocides with different fungal cell suspensions [13, 16]. The Figures 4 - 5 show the V_{corr} values of the SS surfaces immersed in ActiSEPT biocide, fungal spore suspensions and mixture consisting in biocide and fungal spore suspensions, at the contact time of 40 s and 600 s, respectively.

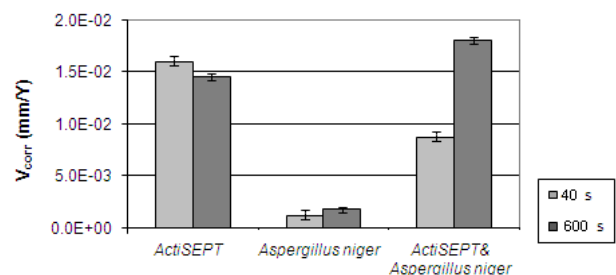


Fig. 4. V_{corr} data of the SS surfaces immersed in ActiSEPT biocide, *Aspergillus niger* spore suspension and mixture consisting in biocide and *Aspergillus niger* spore suspension, at the contact time of 40 s and 600 s.

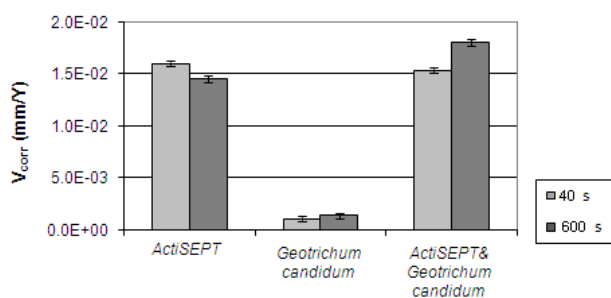


Fig. 5. V_{corr} data of the SS surfaces immersed in ActiSEPT biocide, *Geotrichum candidum* spore suspension and mixture consisting in biocide with *Geotrichum candidum* spore suspension, at the contact time of 40 s and 600 s

It can be observed that V_{corr} values increase in case of the mixtures consisting in biocide with fungal suspensions in comparison with the data when the metallic surfaces were exposed only to biocide solution. By combining the fungal suspensions with the biocide, the cells are stimulated to do chemical attack on the metallic surface. The oxidizing molecules provoke a damaging action to the cells wall, and then act at a cytoplasmic level where it causes the serious damage. The cells wall penetration is physically a charge transfer between the wall (regarded as electrode) and the cells cytoplasm (regarded as electrolyte) resulting in the charge modification of the double layer near the cell surfaces. Under these circumstances, the wall can be crossed by a net current which raises the concentration of ionic species participating in the corrosion processes on the SS surfaces. The chemical attack manifests through migration of the cytoplasm, followed by electrolytic contamination of the ActiSEPT biocide. An increasing content of hydrogen and oxygen from oxidation of biomolecules by biocide substance could appear in the electrochemical system which contains ActiSEPT - fungal suspensions. A large flow of electrons may increase the current density and corrosion rate values, respectively [13]. Thus, the AISI 304 stainless steel surfaces are more corrosive in the electrochemical system composed of ActiSEPT biocide combined with fungal cell suspensions. Even if the corrosion rate of AISI 304 Stainless steel does not exceed 0.02 mm/y [23] in mixtures of biocide with fungi, these mixtures alter the metallic surfaces and they can transform them into different surfaces which accelerate the corrosion. This fact suggests that the repeated bioprocessing line disinfections reduce the equipment lifetime. The SS surface chemical behavior is a considerable complex mechanism and requires further investigation for its understanding.

4. Conclusions

The electrochemical behaviors of the AISI 304 stainless steel surfaces in ActiSEPT biocide solution, fungal suspensions and their combination were studied by linear polarization technique. The mixtures consisting of 140 ppm ActiSEPT biocide and $2.5\text{--}2.6 \times 10^5$ spore/mL

from both fungal suspensions (very low concentration) indicate a synergic destructive effect upon metallic surface than only in biocide solution and the tested spore suspensions. The evaluated electrochemical data offer important clues for the equipment quality surface modification during cleaning.

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