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Electrochemical Behaviour of AISI 304 Stainless Steel Immersed in Mixtures Consisting by Biocide and Fungal Suspensions

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1. Introduction

Chemical disinfection of industrial facilities in food bioprocessing is a major consumer of biocides and represents an essential technological issue. Due to this requirement, the metallic surfaces of the equipments used in bioprocessing inevitably interact with electrolyte environments that are exposed to washing and disinfecting solutions with or without microorganisms through on electrochemical mechanism (Landoulsi et al., 2008; Osarolube et al., 2008). Recently the electrochemical behaviour of stainless steel surfaces have become interested to the many researchers (Hiromoto & Hanawa, 2006; Stoica et al. 2010a). However, only a few research studies were devoted to the electrochemical behaviour of stainless steels used in bioprocesses having a synergic effect on biocides and microorganism (Stoica et al., 2009; Stoica et al., 2010b). The electroanalytical techniques used in previous studies through discharge of an electric field can generate some chemical and physical processes, reversible or irreversible because of the fungi present in the environments (Shen et al., 2008 ; Yang et al., 2008) and on metallic surfaces. These processes are strongly influenced by many factors, such as: biological factors (microorganisms type; cell wall; size and shape of the cell; cells density, arrangement and cell position, fluid medium properties in medium conductivity, electric field waveforms and the number of electric pulses (Yang et al., 2008). The complex phenomena occurring in the electrochemical biocide-fungi-metallic surface system are studied as electrochemical interface processes occurring at the limit between molecules of aqueous solution (biocide solution) coming into contact with the metallic electrode 'live' (fungal cell membranes) and the metallic surface (AISI 304 Stainless Steel). The electron transfer in these electrochemical systems respects the general laws of charge transfer, but also presents the specific properties based on the dynamic environment in which the electron transfer occurs, at the processes of adsorption/desorption and at the surface reactions. These can occur between molecules of biocide and biological surfaces as well as between biocide penetration through these surfaces endowed with distinct architecture, composition and characteristics. The study of these complex processes requires a multidisciplinary approach regarding the metallic surfaces, fungi, biocides and electrochemical processes interface. The aim of this chapter is to systematically present the relevant aspects about the interface electrochemical processes on metallic surfaces with fungi and biocides. A relevant study on the electrochemical behaviour of the AISI 304

Stainless Steel in mixtures consisting by biocide and fungal suspensions is presented. The results show that there is a synergic effect between the active substances from the disinfectant and fungal suspensions and the applied electric potential during tests, thus this effect can be taken into consideration in the food bioprocessing safety.

2. Theoretical aspects regarding the stainless steel, some factors that influence the hygiene of food contact surfaces and metallic surface corrosion

A variety of materials are used in the construction and fabrication of different food equipments. Various metals as well as non metals (*e.g.* plastics, rubber) are used as materials depending on the applications and because of the high demands imposed on the corrosion resistance and the hygiene the food processing industry. These materials vary in their properties regarding their workability, compatibility with type of food product, processing conditions and sanitary design features, depending the applications. The stainless steel is the obvious choice of better material for equipments used on food contact surfaces (Holah & Thorpe, 1990; Saikhwan et al., 2006). This choice is due to the corrosion resistance of stainless steels coupled with their strength and durability, their ability to be readily cleaned and sterilized without deterioration using a wide range of cleaning/sterilizing systems, and the fact that they impart neither color nor flavor to foodstuffs and beverages.

2.1 Stainless steel categories

There are more than 70 standard types of stainless steel and many special alloys, fabricated by multiple processing steps which modify their properties. These steels are produced in the rough form (AISI - American Iron and Steel Institute - types). Generally, stainless steels are mainly iron based with 12% to 30% chromium, up to 22% nickel and minor amounts of carbon, copper, molybdenum, selenium and titanium. The AISI designation for these materials is well known with the number series 300 referring to austenitic stainless steels and the 400 series covering the ferritic and martensitic stainless steels. These stainless steels can be classified into distinct categories including ferritic, martensitic, austenitic and duplex stainless steels (SS) (Figure 1).
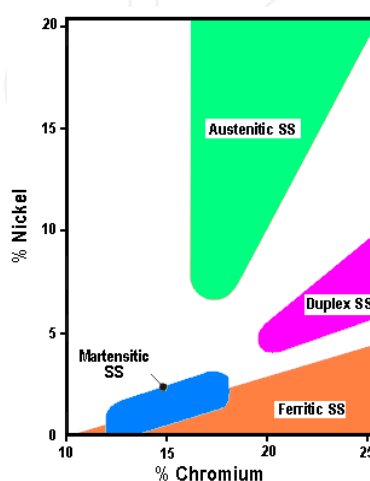

Fig. 1. The stainless steels categories

2.1.1 Ferritic stainless steels

The ferritic Stainless Steel is a magnetic type possessing a microstructure which is primarily ferritic. There are more economical from a cost point of view, but they have limited corrosion resistance compared to the more used austenitics. Similarly the ferritics have limited toughness, formability and weldability in comparison to the austenitics. An example of ferritic grades is AISI 430 used widely for applications in cutlery, kitchen sinks and catering/gastronomy industry and enclosured equipments, where the corrosion resistance requirements are not so demanding due to material economic advantages (Carvalho et al., 2006).

2.1.2 Martensitic stainless steels

Martensitic stainless steels are similar in structure to the ferritics, but by the addition of more carbon, they can be hardened and strengthened by heat treatment in the same way to carbon steels. They are classified as a "hard" ferro-magnetic group. Their corrosion resistance is inferior to that of austenitic stainless steels, therefore they are generally used in mildly corrosive environments. The martensitic grades commonly used are AISI 403, AISI 410 and AISI 420 and they are widely used for cutting and grinding applications, especially for knives.

2.1.3 Austenitic stainless steels

Austenitic stainless steel is non-magnetic (*i.e.*, has a low "permeability") and has excellent ductility, formability and toughness, even at cryogenic temperatures. Depending on the nickel content the austenitics respond to cold working by increases in strength, which can surprisingly be useful in severe forming operations, avoiding premature tearing and cracking. The most representative austenitic grades are AISI 304 and AISI 316. Most stainless steel containers, pipeworks and food contact equipments are manufactured from the most representative austenitic grades either 304 or 316 type austenitic stainless steels. They are widely used in food processing, beverage industry (Mai et al., 2006) and others: bulk storage and transportation and many other applications. For example the sugar, starch and wine industry requires equipments with good corrosion resistance and thus have adopted the AISI 304 and AISI 316 stainless steel. Beer is produce using raw materials like water, barley, hops, malt by fermentation, filtration, canning and sterilization process. Beer, wort and mashed grain is generally not corrosive to stainless steel such as 304, even though the process vessels and pipe systems during brewing operate from low temperatures up to the boiling point. In sections with temperatures above 60°C, there is a risk of chloride-induced stress corrosion cracking, often from the outside, in case the insulation material gets wet.

2.1.4 Duplex stainless steels

The duplex stainless steels have a balanced or mixed structure of austenite and ferrite and as a result have characteristics of both "basic" types. Just like the ferritics, they are ferro-magnetic with a good formability and weldability as the austenitics. In adition, the duplex stainless steels have the general corrosion resistance similar to or better than that of AISI 304 and 316 (Tavares et al., 2010). Examples of duplex grades are AISI 2304, AISI 2205, AISI 2507.

2.2 Factors that influence the hygiene of food contact surfaces

Food contact surfaces include any item that comes in direct contact or could potentially be in contact with the exposed food. The surfaces must be cleaned and disinfected before beginning each day's work, after each use and before changing to a different type of food, such as raw meats to vegetables. The cleaning and disinfection in food industries is an issue of utmost importance since high hygienic standards assure the safety and quality of end products and therefore the consumer's health.` This subchapter will shortly describe the passive film, the finishing, roughness and cleanability on the surfaces.

2.2.1 Passive film

The stainless steel corrosion resistance arises from a protective layer formed on metallic surfaces. The protective layer, sometimes only a few nm in thickness is called a passive film and it is formed instantaneously in an oxidizing atmosphere such as air, water or other fluids that contain oxygen (Olsson et Landolt, 2003). Once the layer is formed, the metallic surface becomes *passivated* and the oxidation or *rusting* rate will slow down to less than 0.05 mm/year. The passive film has normally considerable practical importance also in food production quality since the thin film protects the underlying metal from corrosion. The protective film is strongly adherent on the metallic surfaces and presents longer stability in time having some impact on food safety.

2.2.2 Surfaces finishing and cleanability

The surfaces finishing grade renders the quality of the surfaces described by roughness. The surfaces roughness is characterized in two main directions: one perpendicular to the surface, described as height deviation and the second one in the plane of the surface, described by spatial parameters and identified as texture (Round et al., 2001). The surfaces status was defined by the following characteristic parameters: R_a known as the arithmetic average height parameter, R_q known as the root mean square and R_{max} is the difference in height between the highest and lowest points on the surface relative to the mean plane. The R_a is the most universally roughness parameter used (Buchalla et al., 2000; Turssi et al., 2001) to with a general quality surface control (Whitehead & Verran, 2006). The variation of the average roughness can be useful to express the corrosion process on the surfaces (Sánchez et al., 2008). At the same time the roughness is important for surfaces cleanability (Leclerq-Perlat & Lalande,1994; Boulange-Petermann et al., 2004). A lower roughness allows for easy cleaning intended to eliminate bacteria, which considerably reduces service cost of materials. Surfaces in contact with the food product should have an standard roughness (R_a) value. For surfaces coming in contact with a product having a large area, the R_a should be less than 0.8 µm (Holah and Thorpe, 1990). Under special conditions a roughness higher than 0.8 µm can be accepted in case test results prove that the required cleaning capacity is reached.

2.3 The metallic surfaces corrosion

The corrosion is the destruction or the deterioration of metallic materials by direct chemical, electrochemical or biochemical reactions with different environments (Landoulsi et al., 2008; Osarolube et al., 2008). The corrosion processes are a very broad and complex phenomenon which can be uniform (*e.g.* general corrosion), located (*e.g.* crevice and pitting corrosion) and or in any other way.

2.3.1 Electrochemical corrosion

In food industry due to specific requirements (moisture, aqueous solutions, significant concentrations of organic/inorganic acids, sulfiting agents used to treat foods, high in salinity content, highly concentrated disinfecting solutions, *etc.*) the stainless steel surface is corroded through exposure in different environments. Most corrosion processes are electrochemical (corrosion of metals involves electrons transfer) (see equations: 1 - 4).

$$Me \rightarrow Me^{n+} + ne^- \text{ (anodic reaction; metal dissolution)} \tag{1}$$

$$e.g. \text{ Fe} \rightarrow Fe^{+2} + 2e^- \tag{2}$$

$$O_2 + H_2O + 4e^- \rightarrow 4\,OH^- \text{ (catodic reaction; consume the electrons)} \tag{3}$$

$$Me + nH_2O \rightarrow Me(OH)n + nH^+ + ne^- \quad \text{(overall reaction)} \tag{4}$$

The excess of electrons resulting from the reaction loads the metal negatively and the anodic process can not continue within measurable intensity, wheareas on the metallic surface there is a cathodic process of depolarizing agent reduction (*e.g.* hydrogen ions). The reduction of hydrogen ions is the most common reaction that accompanies aqueous corrosion of metals. During corrosion processes the hydrogen ions can be absorbed on the metallic surface and diffuses inside, while the rest of hydrogen forms gas molecules and escapes. The hydrogen dissolved in metals significantly affects their mechanical properties, composition and structure of passive films formed on the surface. The electrochemical corrosion initiation at sub-microscopic level involves several phenomena starting from breakdown of passive film in a stochastic and sporadic way to localized dissolution of oxide covered metal and mass transport of atoms across the surface to support the continuing dissolution process (Marcus et al., 2008).

2.3.2 Corrosion severity

The surface corrosion is an issue in many industries and it is even a greater challenge on the food processing industry, where it can cause direct (equipments failure) or indirect damage through the loss of production time for maintenance of equipments, the risk of food products contamination by corrosion products or endanger workers' safety and operational security (Holah & Thorpe, 1990; Ofoegbu et al., 2011). When the equipments begin the long walk down the dark road of corrosion, small amounts of metallic elements in the alloy may migrate into foodstuffs from equipment leading to human ingestion and can cause adverse health effects. The severity of the corrosion can be estimated easier through corrosion rate (V_{corr}). A corrosion rate for food equipments surfaces which exceeds 0.02 mm/y reduces the equipments lifetime (Fontana, 1987). The corrosion on metallic surfaces can lead to the formation and expansion of cavities and grooves. This in turn provides breeding sites for microorganisms, thereby compromising the efficacy of cleaning and disinfection procedures and encourages more biofilm adhesion and biofilm resistance to detachment. The biofilms probably do not participate directly in the corrosion process, but they can lead to some changes of the interfacial environment by the increase of cells concentration that facilitates the corrosion process on the surface (Yuan & Pehkonen, 2007).

3. The fungi relevance on food industry

The living world is classified in three domains such as the *Bacteria*, the *Archaea* and the *Eukaryota* (Woese, 1990). Some authors argued that these species represent separate lines of

descent that diverged early from an ancestral colony of organisms (Woese & Gupta, 1981; Woese, 1998). The Eukaryota domain include three major multicellular groups: animals, plants and fungi. Fungi including yeasts and molds are unicellular or multicellular eukaryotic microrganisms (Tournas et al., 2006; Pommerville, 2010). The fungal cells are larger than bacteria and structurally more complex than other microorganisms (Hugo et al., 2004). Some strains are important in food biotechnology as starter cultures with the ability to modify food characteristics and in industrial biotechnology to produce antibiotics and other beneficial by-products, such as enzymes, vitamins, organic acids (Deacon, 1997; Maier et al., 2009; Gautam et al., 2011) and others. *Saccharomyces cerevisiae* plays an important role in various fermentation processes to produce enzymes, antioxidants and vitamins. *Candida mycoderma* and *Aspergillus niger* have a major role to forming the citric acid (Tisnadjaja et al., 1996; Papagianni, 2007). Moreover, *Aspergillus niger* is important as a producer of proteins and a great variety of enzymes such as catalase, celullase, endoglucanase, glucosoxidase, invertase and pectinase (Lu et al., 2010). On the other hand, the food spoilage by fungi raises an economic issues and it is estimated that annually between 5% and 10% of the world's food production is lost due to fungal biodeterioration (Pitt & Hocking, 1997). The risk of health problems can appear due to mycotoxins produced as secondary metabolites by fungi during the stationary phase of growth in some specific physico-chemical conditions. The control of fungal spoilage of food products is therefore an essential and decisive matter to prevent different biological safety risks.

4. Biocides

Biocides represent a very diversified group of chemical substances with a crucial role in pharmaceutical and food industry and this role is becoming increasingly significant (Zani et al., 1997; Bridier et al., 2011). In food industry biocides are commonly used to disinfect the processing areas, equipments, containers, surfaces or pipes associated with the production, transport and storage of food or drink, including drinking water.

4.1 Biocides effectiveness
Biocides act in different ways (sometimes several biocides are combined in a product to increase the overall effectiveness) to destroy, render harmless, prevent the action, or otherwise exert a controlling effect on any harmful organism by chemical or biological means (Table 1). The biocides effectiveness is limited and much dependent on application conditions (Bessems 1998). The efficacy of biocides greatly depend on contact time, concentration temperature, *p*H and microorganisms type (Russell and McDonnell, 2000; Russel, 2003; Kitis 2004).

All biocides show varying degrees of activity against bacteria, bacterial spores, fungi, viruses and protozoa and at least some have algicidal activity (Russell, 2003). The biocides efficacy depends on a large of intrinsic and extrinsic factors. The intrinsic factors are characterized by the biocide type and its concentration, contact time and application (Russell & McDonnell, 2000). Furthermore, the relationship between contact time and concentration biocides determines the microbial reduction. The stability of the active biocide compounds in the environment also has an influence on the microbial action. The temperature is important, as most substances have a lower efficiency at low temperature. The contact mode also influences the biocides efficacy, as well as the contact time (mechanical effects) and the *p*H which plays an important role. Extrinsic factors consist in

the presence of organic matters, the concentration and the age of the microorganisms, the microorganisms ability to transform the biocide into inactive forms (McDonnell & Russell, 1999). The biocides have a broad spectrum of antimicrobial activity and generally act to several targets sites in microbial cells (Figure 2) and damage them as a results of the biocidal effect (Russell, 2003).

Biocides	Action mode	References
Alcohols	Membrane damage, cell lysis	Larson & Morton, 1991
Chlorine compounds and halogens	Oxidising agents	McDonnell & Russell, 1999
Peroxydes		
Quaternary Ammonium Compounds	Membrane destabiliser, cell lysis	
Biguanides	Chlorhexidine specifically inhibits membrane – bound ATPase	Breslin & Tharp, 2001

Table 1. Biocides types in food processing and action mode
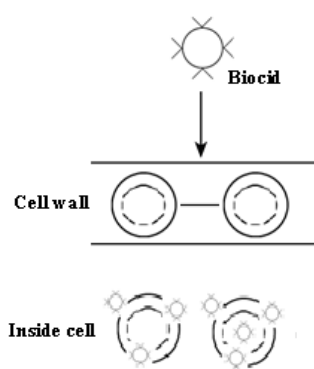
Fig. 2. Action mode of biocide entry into a microorganism type (adapted from Russell, 2003).

4.2 Legislative framework of using biocide

During the past decades, the food production has become more complex; the production volume is larger, the operations are more mechanical, the food is more processed and the time and distance between production and consumption are longer. The new trends in food production and consumption lead to an increased need for efficient safety practices in the food processing industry. Currently, the biocides industry as a whole is governed by end-industry growth, technological developments, regulatory changes and the growing use of biocides as an aid in the hygiene processes. Science 2009 the world biocide demand is estimated to grow to 6,880 million dollars (5.4% annual growth from 2004) and by 2014 to 9,050 million dollars (5.6% annual growth from 2009). Accordingly, emerging EU (European Union) regulations such as Biocidal Products Directive 98/8/EC (BPD) (from May 14th 2000) and Registration, Evaluation and Authorisation and Restriction of Chemicals (REACH), strive to increase the safety and the eco-efficiency of chemical products and production processes (BPD, 1998; REACH, 2006). This directive has as objectives: (i) to harmonise the

European market for biocidal products and their active substances such that product authorisation content in one Member State can be recognized in other Member States; (ii) to provide a high level of protection for people, animals and the environment (from the use of biocidal products) through risk assessment. These objectives requires the submission and evaluation of data relating to substances' chemistry, toxicity to humans, and toxicity and fate in the environment and (iii) to ensure products are sufficiently effective against the target species. The Registration REACH - a new chemical regulation was implemented by the EU on June 1st 2007 and is being implemented in stages to be completed by 1st June 2018. The main objective of REACH is a high level of protection for human health and the environment, while maintaining the competitiveness and innovation of EU chemicals industry. REACH provides a single regulatory framework for the control of chemicals, replacing the previous patchwork of controls, and ensures that information on the properties of chemicals is transmitted down the supply chain, thus enabling them to be safely handled.

5. Study of electrochemical behaviour of AISI 304 stainless steel immersed in mixtures consisting of *Neoseptal* and fungal suspensions

In this study the corrosion behaviour of AISI 304 Stainless Steel (*SS*) treated by biocide *Neoseptal* solution (noted as *Neo*) and in mixtures consisting of *Neo* with fungal suspension (*Neo - Aspergillus niger*, *Neo - Candida mycoderma* and *Neo - Saccharomyces cerevisiae*) was investigated.

5.1 Materials and methods
5.1.1 AISI 304 stainless steel
Tests were performed using AISI 304 Stainless Steel in rectangular samples and pretreated by mechanically polishing with abrasive paper of increasingly finer grit between 800 and 2000 μm and finally chemical cleaning. The samples as working electrode were mounted in a tetrafluoroethylene-perfluoroalkylvinylether copolymer (PFA), sleeve sample holder except for an exposed test area of 12 cm^2 (Stoica et al., 2010b).

5.1.2 Biocide solution
The biocide (*Neoseptal*) is a commercial disinfectant, effective against all types of microorganisms in food industry manufactured by Dr.Weigert (Germany) based on H_2O_2 (dihydrogen dioxide – 30% wt.). Fresh solutions of *Neoseptal* were prepared by dilution of commercially *Neoseptal* biocide. The concentration of *Neoseptal* was 0.2% performed at 20±2°C and acted for at least 30 min.

5.1.3 Fungi strains
Three fungal types were tested: a) *Aspergillus niger* ATCC 16404 (provided by the "Ion Cantacuzino" Institute (Bucharest-Romania), with the spores concentration of 1.41x10^{7}spores/mL; b) *Candida mycoderma* isolated from spoiled wine, of suspension containing 1.38x10^7 cells/mL; c) *Saccharomyces cerevisiae* (Pakmaya, Rompak), with a cell number in the suspensions of 1.40x10^7 cells/mL. The cells concentrations were measured using a Thoma cytometer. An aliquot volume of fungal suspension (5 mL) was used in the electrochemical experiments (50 mL as total volume).

5.1.4 Electrochemical measurements

Tests were performed on the corrosion behaviour of samples of *SS* in *Neo* with and without fungal suspensions. All electrochemical measurements were carried out in a glass electrochemical-cell (Metrohm, Switzerland) equipped with three electrodes, at room temperature (22±1°C).The working electrode was AISI 304 Stainless Steel type, the counter electrode (CE) was a Platinum foil and as the reference electrode (RE) was a saturated calomel electrode (SCE). The entire three-electrode assembly was placed in a Faraday cage to limit the noise disturbance and then connected to potentiostat-galvanostat Bio-Logic SP-150 (France). The investigations are carried out using EC-Lab® Express v 9.46 software. The electrochemical measurements were carried out using the Linear Polarization Technique (LP). The polarization measurements were initiated after 60 seconds immersions to access an equilibrium open potential on the sample surface. In the preliminary study the potential was tested for different ranges, as ±2V; ±1.0 V (SCE) a variation of scan rate between 100÷10 mV/s. There were no large modifications of the results in the polarization curves tested. The following measurements recorded for a potential of ±1.5V (SCE) at the 20 mVs^{-1} were take into consideration because of larger scan rate appeared in the distorsion of the curves also for higher potential range. At the same time all solutions were examined for conductivity measurements, using the Metrohm 712 Conductometer.

5.2 Results and discussions
5.2.1 Electroactivity of working solutions

The electrochemical behaviour of *SS* samples in *Neo* with and without fungal suspensions was investigated at room temperature. Figure 3 shows the polarization curves obtained for *SS* immersed in *Neo* and also in different fungal suspensions.
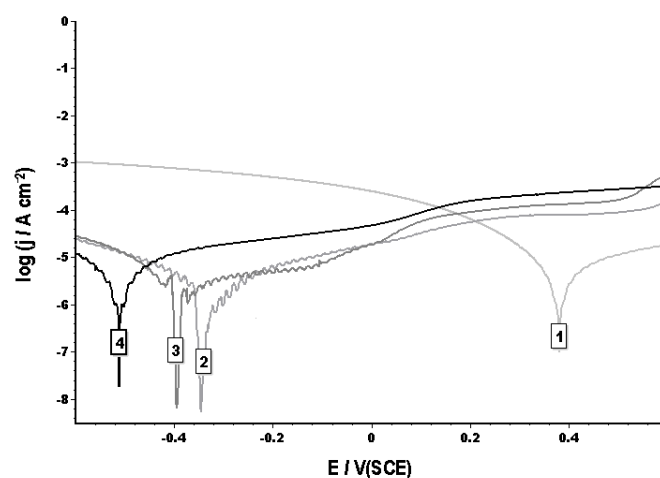

Fig. 3. Polarization curves of SS in *Neo* (1) and different fungal suspensions: *Aspergillus niger* (2), *Candida mycoderma* (3) and *Saccharomyces cerevisiae* (4).

A larger range passivation of the processes could be observed in both electrochemical reactions (anodic and cathodic). From the polarization curves (Fig. 3, curves 2-4), that the potential E_{corr} values show a more negative range for all fungal suspensions tested, whereas for *Neo* E_{corr} the value is situated at a positive potential range (Fig. 3, curve 1). Electrochemical parameters for *Neo* (Fig. 3, curve 1) are E_{corr} +390 mV and corrosion current

density, i_{corr} 24 μA/cm². The potential E_{corr} and i_{corr} values are -316 mV respectively 30.27 μA/cm² for *Aspergillus niger* suspension and -338 mV, respectively 37 μA/cm² for *Candida mycoderma* suspension. In case of *Saccharomyces cerevisiae* suspension a – 511 mV value for E_{corr} respectively 70.40 μA/cm² for i_{corr} were obtained. These data confirm on the one hand the electroactivity behaviour of the tested biocide solutions and on the other hand a high electrons density, in the electrochemical system which contains only fungal suspensions. Just like electrons in wires, these ions contribute to the transport charge in the electric field and thus to the current flow. In this situation the system responds through a current predominant anodic and it can speed up the metal dissolution. A more current density is produced by *Saccharomyces cerevisiae* suspension (Fig. 3, curve 4). This fact could explain a more intense sensibility of these cells for the applied potential, due to their size. These cells appear bigger in comparison to *Aspergillus niger* spores and *Candida mycoderma* cells (Bui et al., 2008). Some authors consider that the larger cells are more sensitive to lower electric potential field, thus stronger than the smaller cells (Teissie et al., 1999; Kotnik et al., 2001). The applied potential for the acceleration of corrosion tests and biocide can produce a permeabilization of the plasma membrane and appear an electrochemical contamination in mixtures, due to the intracellular ions transit out of cells.

5.2.2 The corrosion effect of mixtures solutions

The electrochemical behaviour of the *SS* in the *Neo* with fungal suspension (without nitrogen) was evaluated at room temperature at different contact times after adding the fungal suspensions. The polarization curves are presented in Figures 4-6. Using Tafel fit. and R_p fit. analysis tool, made it possible to obtain the electrochemical parameters like corrosion potential (E_{corr}), current density (j_{corr}), polarization resistance (R_p), corrosion rate (V_{corr}) . The results are presented in Tables 2-4. Figure 4 presents the variation of E_{corr} potential values during polarization curves of *SS* immersed in *Neo* and in *Neo - Aspergillus niger* at different contact time from the immersion of samples.
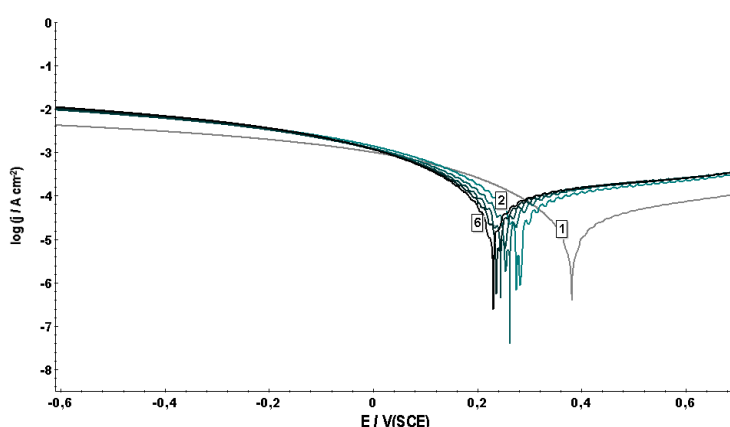

Fig. 4. Polarization curves of *SS* immersed in *Neo* (1) and *Neo - Aspergillus niger* at 3 min. (2); 6 min.(3); 9 min.(4); 12 min. (5) and 30 min. (6).

The potential E_{corr} values are in a negative range for the *Neo - Aspergillus niger* (Fig. 4, curves 2-6), whereas E_{corr} value is situated in the positive range for *Neo* (Fig. 4, curve 1). The absence of the parallelism of cathodic and anodic branches between the set of curves 2-6

corresponding to the *Neo - Aspergillus niger* and curve 1 for the only *Neo* biocide suggests that the processes that occur on *SS* surface are not similar.

Table 2 shows the electrochemical parameters of *SS* immersed in *Neo* and in *Neo - Aspergillus niger*.

Solution	Time (min.)	E_{corr} (mV)	j_{corr} (µA/cm²)	βc (mV/dec.)	βa (mV/dec.)	R_p (Ω·cm²)	V_{corr} (mm/y)
Neo	3	390	24	182	405	1722	0.0207
Neo - Aspergillus niger	3	296	70	203	478	595	0.0608
	6	263	80	198	536	580	0.0691
	9	257	77	201	497	562	0.0668
	12	247	77	198	509	553	0.0666
	30	239	76	189	519	573	0.0657

Table 2. Tafel parameters of *SS* immersed in *Neo* and *Neo - Aspergillus niger*.

As it can be observed from the Table 2 the potential E_{corr} value of *SS* immersed in *Neo* is +390 mV(SCE). By adding the *Aspergillus niger* suspension in *Neo*, the spores are exposed initially to a chemical attack. The spores wall due to its affinity to the active molecules oxidizing – H_2O_2, may restrict acces to the plasma cells membrane and to the cytoplasm. The oxidizing molecules have a damaging action to the spores wall, more or less crossed, then act at a cytoplasmic level where it causes the first serious damage. The spores wall penetration is physically a charge transfer between the wall (regarded as electrode) and the spores cytoplasm (regarded as electrolyte) and has as effect the charge modification of the double layer near the spores surface and also shift in potential. Under these circumstances, the wall can be crossed by a net current which raises with increasing the concentration of ionic species participating in the process. The application of electrical potential exposes the *Aspergillus niger* spores (chemically attacked) to the aggression of the electric field. The potential effects manifest through dielectric breakdown and migration of the cytoplasma, followed by electrolytic contamination of the *Neo* biocide. At the same time, the electrical applied potential leads to the occurrence of some oxidations and reductions followed by a potential E_{corr} shift in the negative range with an amplitude of about 94 mV (*i.e.* from +390 mV (SCE) at +296 mV) (SCE). During the experiment the E_{corr} values increases slightly, through a shift in positive range with an amplitude of about 8 mV to the end of the exposure. The shift of potential E_{corr}, suggests that the *Aspergillus niger* suspension added in *Neo* biocide is a depolarization agent for *SS* surfaces.

The electrochemical system *Neo - Aspergillus niger* indicate a current flow which generating the orderly movement between the surface and biocide solution whithout an interference. Morever ther is a current resulting after oxidation of some biomolecules in solution (not attacked intracytoplasmic) and a current after oxidation of intracytoplasmic electrolysis products, but go into solution because of the dielectric breakdown. This fact shown that in *Neo - Aspergillus niger* the current which flows through working electrode has a higher intensity than the current which flows through working electrode in *Neo* only. The addition of *Aspergillus niger* suspension is characterized by an increase of current density with 46 µA/cm², *i.e.* from 24 µA/cm² to 70 µA/cm². During the experiment, a slight breakthrough

corrosion current density was observed with an amplitude of about 10 µA/cm² at 6 minutes from exposure and after that it begins to decrease slightly up to the end of the exposure (Table 2). The decrease of current density suggests that the absorption of OH⁻ ions occurs and a possible surface passivation may appear. However, the current density does not return to the initial value and it maintains at levels three times higher than the initial value.

An increasing content of hydrogen and oxygen from oxidation of biomolecules by biocide substance could appear in the electrochemical system which contains *Neo - Aspergillus niger*. A large flow of electrons have as effect the cell current increase an electrode polarization. This polarization is confirmed by the shift in the cathodic (β_c) and anodic (β_a) Tafel slopes (Stoica et al., 2010b). As it can be observed from the Table 2, the *Neo - Aspergillus niger* move the cathodic (β_c) slope from 182 mV/dec. to 203 mV/dec. and the anodic (β_a) slope from 405 mV/dec. to 478 mV/dec. The shift of the Tafel slopes observed throughout the experiment (Table 2) reveals that the *Aspergillus niger* fungal suspension induces a corrosion mechanism on metallic surface (Stoica et al., 2010b). These results suggest that the *Neo - Aspergillus niger* controlling predominantly the anodic reactions.

There is a shift of the potential E_{corr} and the increase of j_{corr} and the decrease of polarization resistance, during experiment from the Figure's 4 curves and from the Table's 2 data. These results indicate a synergic effect between H_2O_2, *Aspergillus niger* spores and applied electric potential. This fact can substantially accelerate the corrosion process of metallic surfaces immersed in the mixture consisting of biocide and fungal suspension. The predominant anodic parameters could be an answer of the degradation metallic surfaces and this phenomenon is in good agreement with data previously reported in literature (Stoica et al, 2010b).

Figure 5 presents polarization curves of *SS* immersed in *Neo* and *Neo - Candida mycoderma* at different contact time.
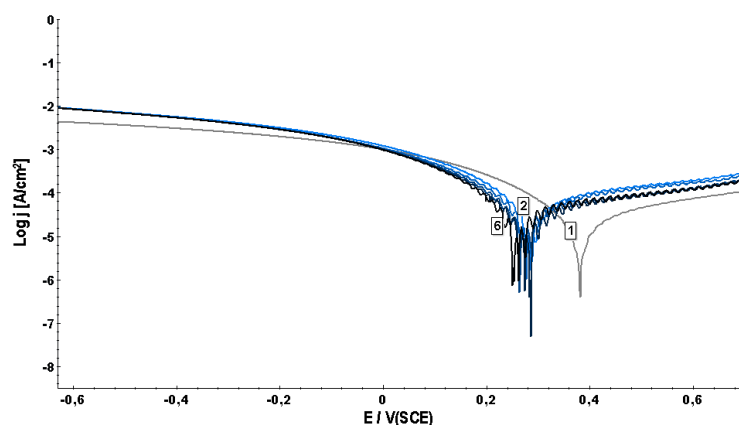

Fig. 5. Polarization curves of *SS* immersed in *Neo* (1) and *Neo - Candida mycoderma* at 3 min. (2); 6 min.(3); 9 min.(4); 12 min. (5) and 30 min. (6).

In Figure 5, it can be observed that the potential E_{corr} values are in negative range for the *Neo - Candida mycoderma* (curves 2-6), whereas E_{corr} value is situated in the positive range for *Neo* (Fig. 5, curve 1). Absence the parallelism of cathodic and anodic branches between the set of curves 2-6 which corresponding the *Neo - Candida mycoderma* and curve 1 which corresponding the *Neo* suggests, that the processes that occur on *SS* immersed in *Neo -*

Candida mycoderma are not similar with the processes that occur on *SS* immersed in *Neo* in good agreement to *Neo - Aspergillus niger.*

Table 3 presents the electrochemical parameters of *SS* immersed in *Neo* and *Neo - Candida mycoderma.*

Solution	Time (min.)	E_{corr} (mV)	j_{corr} (µA/cm²)	βc (mV/dec.)	βa (mV/dec.)	R_p (Ω·cm²)	V_{corr} (mm/y)
Neo	3	390	24	182	405	1722	0.0207
Neo - Candida mycoderma	3	294	58	196	488	746	0.0503
	6	289	50	195	484	827	0.0437
	9	296	41	194	566	961	0.0354
	12	276	33	161	488	1222	0.0285
	30	281	88	185	546	949	0.0334

Table 3. Tafel parameters of *SS* immersed in *Neo* and *Neo - Candida mycoderma.*

Similar to the electrochemical system (which contains *Neo - Aspergillus niger)* the *Neo - Candida mycoderma* system random currents circulate, whose intensity is determined by different source. The applied electric potential exposes the *Candida mycoderma* cells to the aggression of the electric field. The experimental potential effects manifest through dielectric breakdown and migration of the cytoplasmic content, followed by an electrolytic contamination of the *Neo* biocide. At the same time, the potential leads to the occurrence of some oxidations and reductions, followed by a potential E_{corr} shift in negative range with an amplitude of about 96 mV *i.e.* from +390 mV (SCE) at +294 mV (SCE). During exposure was observed that the corrosion potential fluctuated suggesting alternative passivation and activation of the surface and conducting its the spontaneous reversible oxidations. The shift of E_{corr} suggests that the *Candida mycoderma* suspension added in *Neo* biocide is a depolarization agent for *SS* surface. The addition of *Candida mycoderma* fungal suspension is characterized by an increase of current density with 34 µA/cm², *i.e.* from 24 µA/cm² to 58 µA/cm². During the experiment, the current density showed a reduction value after at 6 minutes (Table 3). This suggests the initiation of a partial surface passivation process, but which is insignificantly in an amplitude of 47 µA/cm² at exposure end (Table 3). In electrochemical system of *Neo - Candida mycoderma*, the polarization is confirmed by the shift in the cathodic (β_c) and anodic (β_a) Tafel slopes (Stoica et al., 2010b). As it can be observed from the Table 3, *Neo - Candida mycoderma* moves the cathodic (β_c) slope from 182 mV/dec. to 196 mV/dec. and the anodic (β_a) slope from 405 mV/dec. to 488 mV/dec. The shift of the Tafel slopes observed throughout the experiment (Table 3) reveals that the *Candida mycoderma* fungal suspension induces a corrosion mechanism on metallic surface (Stoica et al., 2010b). These results suggest that the *Neo - Candida mycoderma* controlling predominantly the anodic reactions.

There is a shift of the potential E_{corr}, the increase of j_{corr} and the decrease of polarization rezistance, during experiment (Figure 5 curves and Table 3). The results indicate a synergic effect between H_2O_2 *Candida mycoderma* cells and apllied electric potential, which manifests through a distorted current predominantly anodic. The predominant anodic mechanism observed in this case, is in good agreement with experimental results previously reported in case of H_2O_2 – *Aspergillus niger* – applied electric potential.

Figure 6 presents the polarization curves of *SS* immersed in *Neo* and *Neo - Saccharomyces cerevisiae* at different contact time.
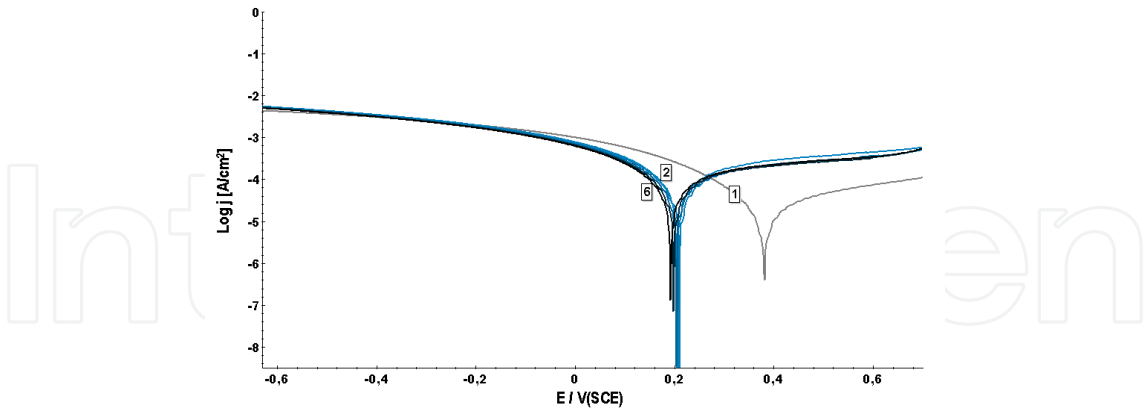

Fig. 6. Polarization curves of *SS* immersed in *Neo* (1) and *Neo - Saccharomyces cerevisiae* at 3 min. (2); 6 min.(3); 9 min.(4); 12 min. (5) and 30 min. (6).

The potential E_{corr} values are in negative range for the *Neo - Saccharomyces cerevisiae* (Fig. 6, curves 2-6), whereas E_{corr} value is situated in the positive range for *Neo* (Fig. 5, curve 1). The absence of the parallelism of cathodic and anodic branches between the curves 2-6 corresponding the *Neo - Saccharomyces cerevisiae* and curve 1 corresponding the *Neo* suggests that the processes are not similar with the processes that occur on *SS* immersed in *Neo - Aspergillus niger* and *Neo - Candida mycoderma* system.

Table 4 reveals the electrochemical parameters of *SS* immersed in *Neo* and *Neo - Saccharomyces cerevisiae*.

Solution	Time (min.)	E_{corr} (mV)	j_{corr} (µA/cm²)	βc (mV/dec.)	βa (mV/dec.)	R_p (Ω·cm²)	V_{corr} (mm/y)
Neo	3	390	24	182	405	1722	0.0207
Neo- Saccharomyces cerevisiae	3	213	114	247	449	418	0.0993
	6	216	98	238	492	475	0.0852
	9	210	95	236	482	507	0.0824
	12	205	93	236	514	507	0.0802
	30	198	94	236	495	499	0.0816

Table 4. Tafel parameters of *SS* immersed in *Neo* and *Neo-Saccharomyces cerevisiae*.

Similar to electrochemical systems (*Neo - Aspergillus niger* and *Neo - Candida mycoderma*), through electrochemical system *Neo - Saccharomyces cerevisiae*, the random currents circulate, by their source of origin. The applicated potential exposes the *Saccharomyces cerevisiae* cells to the aggression of the electric field. The experimental potential effects manifest through dielectric breakdown and migration of the cytoplasmic content, followed by an electrolytic contamination of the *Neo* biocide. At the same time, the potential lead to the occurrence of some oxidations and reductions, followed by a potential E_{corr} shiftting in negative range with an amplitude of about 180 mV *i.e.* from +390 mV (SCE) at +213 mV (SCE). During the exposure the corrosion potential fluctuated suggesting alternative passivation and activation of the surface and conducting its spontaneous reversible oxidations. The shift of

Ecorr suggests that the *Saccharomyces cerevisiae* suspension added in *Neo* biocide is a depolarization agent for *SS* surface.

The addition of *Saccharomyces cerevisiae* fungal suspension is characterized by an increase of current density with 90 µA/cm², *i.e.* from 24 µA/cm² to 114 µA/cm². During the experiment, the current density showed a value reduction after 6 minutes. This suggests of initiation of partial surface passivation process with insignificantly the amplitude of about 1 µA/cm² at exposure end (Table 4). In electrochemical system *Neo-Saccharomyces cerevisiae*, the polarization is confirmed by the shift in the cathodic (β_c) and anodic (β_a) Tafel slopes (Stoica et al., 2010b). The cathodic (β_c) slope from 182 mV/dec. to 247 mV/dec. and the anodic (β_a) slope from 405 mV/dec. to 449 mV/dec., for the first 3 minutes from exposure moves (Table 4). The shift of the Tafel slopes observed throughout the experiment reveals that the *Saccharomyces cerevisiae* fungal suspension induces a corrosion mechanism on metallic surfaces (Stoica et al., 2010b) and suggests that *Neo-Saccharomyces cerevisiae* system controls the cathodic and anodic reactions, being predominantly anodic.

There is a shift of the potential *Ecorr*, the increase of *jcorr* and the decrease of polarization rezistance, during the experiment (Fig. 6 and Table 4). The results indicate a synergistic effect between H_2O_2 *Saccharomyces cerevisiae* cells and apllied potential. The predominant anodic mechanism observed in this case, is in good agreement with experimental results previously reported for the systems: H_2O_2 – *Aspergillus niger* – electric potential and H_2O_2 – *Candida mycoderma* – applied potential.

5.2.3 Conductivity variations of solutions

Conductivity is simply the ability of a liquid to conduct electricity, which is related directly to the concentration of ions in the liquid. Figure 7 gives the variation of solutions conductivity of *Neo* compared with *Neo* with fungal suspensions tested.
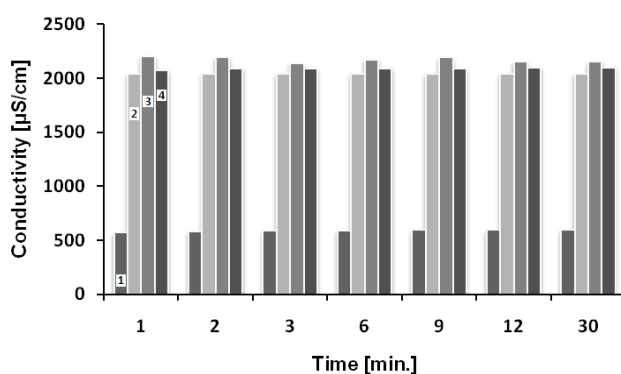

Fig. 7. Conductivity variation in *Neo* (series 1) and *Neo* with different fungal suspension: *Aspergillus niger* (series 2), *Candida mycoderma* (series 3) and *Saccharomyces cerevisiae* (series 4).

The presence of the fungal suspensions can be easily observed in the *Neo* biocide (Fig. 7, series 2-4) with a strong effect on the conductivity values in comparison with those observed for *Neo* without fungal cells (Fig. 7, series 1). This fact could suggest that in *Neo* with fungal cells an electrolytic contamination is produced, which can be explained through synergistic action between H_2O_2 and the applied potential inside the fungal cells, although the ions efflux remains difficult to elucidate (Sukhorukov et al., 2007). As a result the mixtures

consisting of *Neo* biocide with all fungal suspensions are very conductive systems and they can accelerate the corrosion of AISI 304 stainless steel immersed in them.

5.2.4 Synergic of working parameters

The corrosion behaviour on *SS* was tested in the biocide solution with and without fungal suspensions. The working parameters taken into account in accelerated corrosion tests (LP) were: nature and size of cells, cellular density of suspensions and conductivity of solutions, applied potential and pretreatment of working electrode of *SS*. Eletctrochemical parameters can justify the shift of E_{corr} potential in the anodic direction and increase of i_{corr} for mixtures consisting by *Neo* biocide with fungal suspensions. The corrosion behaviour of *SS* surface is specific to each system and it is made evident through R_p (polarization resistance) and V_{corr} (corrosion rate) variations from Tables 2 - 4.

Figure 8 shows the R_p values of *SS* immersed in *Neo* and *Neo* with fungal suspension at different contact time after immersion.
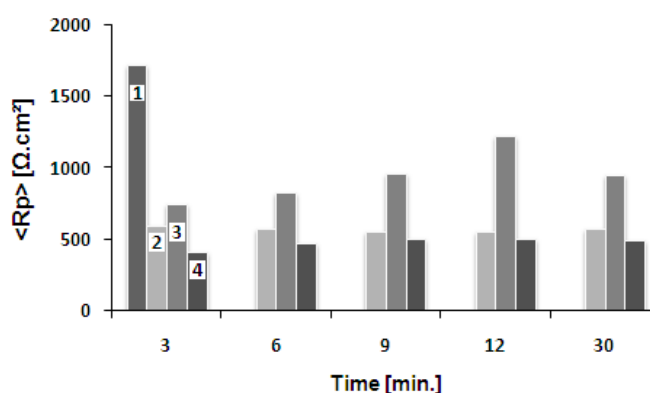

Fig. 8. R_p values of *SS* immersed in *Neo* and *Neo* with fungal suspension: *Aspergillus niger* (series 2), *Candida mycoderma* (series 3) and *Saccharomyces cerevisiae* (series 4).

The R_p values are lower in *Neo* with fungal suspension than in *Neo* only. The order of magnitude of R_p is 10^4 Ω.cm² for *Neo*. In case of *Neo - Aspergillus niger* is observed a greatly decrease of R_p with the increase in time. In *Neo - Candida mycoderma* system the R_p values decrease up to minute 3 and afterwards a significant increase was obtained up to minute 12 followed by a lower decrease at final contact time (Fig. 8, series 3). In *Neo - Saccharomyces cerevisiae* a significant decrease in the R_p values was observed (Fig. 8, series 4). The order of magnitude of R_p is 10^3 Ω.cm² in *Neo* with fungal suspensions.Thus, the *SS* surface is more susceptible in electrochemical system containing H_2O_2 from *Neo* biocide with *Aspergillus niger* suspension, respectively *Saccharomyces cerevisiae* suspension.

Figure 9 presents the V_{corr} values of *SS* immersed in *Neo* and *Neo* with fungal suspension at different contact time after immersion.

In case of *Neo - Aspergillus niger* the V_{corr} values increase more in time up to minutes 6 and afterwards a smaller decrease was obtained up to minutes 30 (Fig. 9, series 2). The V_{corr} values decrease more in *Neo - Candida mycoderma* system up to 12 minutes followed by an increase at final contact time (Fig. 9, series 3). The highest increase of V_{corr} values was obtained when *Saccharomyces cerevisiae* was added in *Neo*, at the beginning of the measurements and then there was a slight decrease up to minute 12 from immersion contact time (Fig. 9, series 4). Thus, the *SS* surface is more corrosive in electrochemical system

containing H_2O_2 from *Neo* biocide with *Aspergillus niger* respectively *Saccharomyces cerevisiae*. This fact suggests that during food line disinfection the corrosion rate of AISI 304 Stainless Steel surfaces exceeds 0.02 mm/y (Fontana, 1987) and reduces the equipments lifetime. It is possible that the killing action showed in these experiments is due to other chemical reactions occurring while the electrical currents pass through the liquid medium. The results obtained could suggest that an electrochemical disinfection is quite attractive as a promising alternative technology. The conductivity and potential sensors constantly monitor the concentration of the biocide solutions with cells contamination are benefic in cleaning the food processing. The *SS* surface behaviour is complex and requires further investigation for its understanding under the action of different biocides and fungal suspensions.
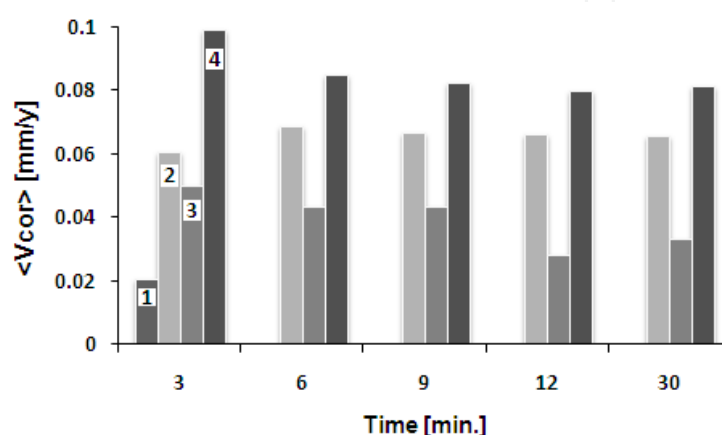

Fig. 9. V_{corr} values of *SS* immersed in *Neo* (1) and trend V_{corr} values of *SS* immersed in *Neo* with fungal suspensions (10% vol.): *Aspergillus niger* (series 2), *Candida mycoderma* (series 3) and *Saccharomyces cerevisiae* (series 4).

5.3 Remarks

The work deals with the corrosion behaviour of AISI 304 Stainless Steel into biocide solution (*Neoseptal* with hydrogen peroxide as active substance) through artificial contamination with three (10% vol.) fungal suspensions as: *Aspergillus niger*, *Candida mycoderma* and *Saccharomyces cerevisiae*. At the applied electrical potential the biocide can work better within the fungal cells, and thus disturbing the present microorganisms homeostasis in several ways such as increasing the environmental conductivity of solutions and the corrosion rate of metallic support. A synergic effect achieved through the mixture of biocide, fungal suspension and applied electric potential, is more destructive than each parameters by its self. The E_{corr} values of AISI 304 Stainless Steel in the mixtures decreased during the contact time after artificial contamination. The fungal suspension has a significant influence on the synergic effect of the AISI 304 Stainless Steel corrosion in the following order: *Saccharomyces cerevisiae*>*Aspergillus niger*>*Candida mycoderma*. A more influence on the synergic effect of the surfaces immersed at the mixture consisting on biocide *Neoseptal* solution with *Saccharomyces cerevisiae* could be explained through the less resistance at the chemical attack from biocide. The synergic effect between the active substance of the disinfectant, fungal suspensions and the applied electric potential should taken into account for the hygienic and safety food bioprocessing industry.

6. Conclusions

Experimental data conclude that the mixtures consisting of biocide with fungal suspensions has an major effect to increase the anode current density, leading to further degradation by corrosion of the AISI 304 Stainless Steel surfaces. The results showed that there is a synergic effect between the active substances from the disinfectant, fungal suspensions and applied electric potential. This chapter could be considered a pioneer research and brinks possible ideas for further researches and raises more issues that need to be explored on the food processing.

7. Acknowledgment

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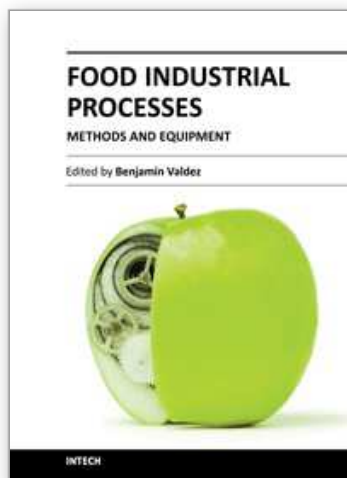
Food Industrial Processes - Methods and Equipment

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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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