

Effect of fungal suspensions in NaDCC disinfectant on the corrosion behaviour AISI 304 stainless steel

Maricica Stoica, Geta Cârâc, Clemansa Tofan,
Oana Emilia Constantin, Georgeta Enache

¹ „Dunarea de Jos” Galați University, 111, Domnească St., 800201, Galați, Romania

Received: 03 October 2009; Accepted: 30 November 2009

Abstract

Fungi are an important group of microorganisms in food microbiology. They are utilized for the industrial fermentation of beer and wine, production of enzymes, antibiotics, vitamins, and organic acids. These processes going on in bioreactors and fermentors which are the heart of systems used in biotechnologies. They are usually made from 316L stainless steel, but some of them are made from less expensive type, as 304 stainless steel (or 304L). For majority of processes, the reactors are operated in batch mode, under sterile or monoseptic conditions. Cleaning and sterilizing are one of the most important activities carried out in the fermentation process. Industrial bioreactors should be sterilized before inoculation because the contamination is a common cause of process failure. Stainless steels will suffer on aside the pitting and crevice corrosion when was exposed to disinfectants and on the other hand, a fungal attack by survival fungi after disinfection procedures of reactors. The effect of fungal suspensions in a disinfectant solution containing 0.25% NaDCC on the corrosion behaviour of type AISI 304 stainless steel was evaluated by linear polarization. The measurements at room temperature were made in a conventional three-electrode chemical cell, using a platinum (CE) electrode, calomel (RE) and an electrode of AISI 304 stainless steel as working electrode (WE). The corrosion process was examined by linear polarization using a Bio-Logic SP-150 potentiostat. The results shown that a synergistic effect between the (sodium dichloroisocyanurate) active substance from the disinfectant solution and fungal suspensions it is an important effect to take in account by the hygienists from food industry.

Keywords: disinfectant; fungi; corrosion; stainless steel

1. Introduction

It is Biotechnology which can simply be defined as the application of living microorganisms and their components to industrial products and processes is not an industry in itself, but an important technology that will have a large impact on many different industrial sectors in the future. Some of the most important microorganisms used in biotechnology are fungi. The fungi consist of moulds and yeast [1]. They are an important group of microorganisms in food microbiology [2] and in food technology, because of their ability to produce toxic metabolites (undesirable effects) or positively modify food characteristics, as well as playing a role in the development of antibiotics

and other beneficial by-products (enzymes, antibiotics, vitamins, and organic acids). These processes going on in bioreactors and fermentors which are the heart of systems used in biotechnologies. They are usually made from 316L stainless steel, but some of them are made from less expensive type, as 304 stainless steel [3]. For majority of processes, the reactors are operated in batch mode, under sterile or monoseptic conditions [4]. Cleaning and sterilizing are one of the most important activities carried out in the fermentation process [5]. In all operations, any solids adhering to the inner surfaces of all vessels must be removed between batches or at routine intervals in continuous operations, not only to facilitate heat transfer, but to remove potential pockets of contamination.

Industrial bioreactors should be sterilized before inoculation, because the contamination with infinitely small quantities of undesirable microorganism is a common cause of process failure [6]. Control of microorganism 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 efficient method to maintain effective sanitation is use of cleaning-in-place (C.I.P.) systems used with closed equipment. Sanitation of equipment between batches with chlorine solutions may be done from a smaller tank.

The aim of this work was to evaluate the effect of fungal suspensions in a disinfectant solution on the corrosion behaviour of type AISI 304 stainless steel by linear polarization.

3. Results and discussion

2.1. Samples characterization and pretreatment procedures.

Tests were performed using stainless steel AISI 304 (SS), coupons were mechanically polished with abrasive paper of increasingly finer grit from between 80 and 1200 μm and chemically cleaned [7]. The coupons were adapted as the working electrode. The exposed area of the working electrode both faces was 12 cm^2 .

2.2. Disinfectant solution

The commercially disinfectant (Actisept namely) with active substance of sodium dichloroisocyanurate, NaDCC (source of chlorine) was studied. This disinfectant are approved by the Romanian National Register of Biocide Products [8] to be used in processing areas. The working concentration of the disinfectant established by the suppliers, was 0.25% sln. with a pH 5.85. The measurements were made using the WTW INOLAB 720 pH-meter.

2.3. Fungi strains

The behaviour of three microorganisms were tested in this work: a) *Aspergillus niger* ATCC 16404 provided by the "Ion Cantacuzino" Institute (Bucharest-Romania), with the spore concentration of 1.41×10^6 spores/mL; b) *Candida* isolated from spoiled wine, of suspension containing 1.5×10^6 cells/mL, c) *Saccharomyces cerevisiae* (Pakmaya, Rompak), the number of

cells in the suspensions of 2.4×10^6 cells/mL. The cell concentrations was measured using a Thoma cytometer.

2.3. Experimental conditions

The electrochemical investigations were performed in a glass electrochemical-cell (Metrohm, Switzerland) with three electrodes: Pt (CE), a saturated calomel electrode (SCE) and AISI 304 stainless steel as the working electrode (WE), at room temperature. The entire three-electrode assembly was placed in a Faraday cage to limit 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.

3. Results and Discussion

The SS coupons prepared as WE were carefully cleaned and rinsed with bidistilled water before their immersion in the electrochemical cell (50 mL capacity). In the electrochemical cell were putted a disinfectant solution (0.25%) and the electrodes were fixed. After that a fresh fungal suspension presented above were putted in electrochemical cell. The measurements were made using LP technique. For polarization measurements the potential range was between -1 mV to $+1$ mV vs./SCE (RE). The immersion time before each measurement was 60s to access an equilibrium open potential. Linear Polarization (LP) investigations were performed using slow scan rate of 20 mVs^{-1} [9], to evaluate the behaviour of stainless steel coupons in disinfectant solution, with an injection in the solution of the fungal suspensions (10% vol.). The point of intersection of anodic and cathodic reactions establishes the open circuit corrosion potential (E_{corr}) of the system and indicates the magnitude of corrosion current (I_{corr}).

From LP curves, it is possible to determine corrosion potential (E_{corr}) and parameters, such as corrosion current (I_{corr}) and the polarisation resistance (R_p). These parameters could be useful referring of the surface resistance of the environment. Results are shown in the Figures 1-4 and electrochemical parameters are summarized in the Tables 1-4. The Fig. 1 shown shifted of the potential E_{corr} in more negative range and decreasing in time at the contamination with *Aspergillus niger*, but I_{corr} increasing significantly at the begging and it reduced in time because of the active action of the disinfectant up 12 min.

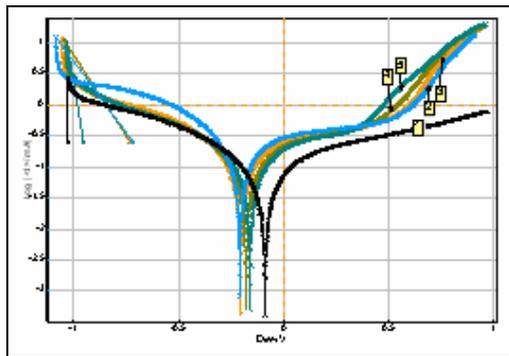


Figure 1. Linear polarization curves at the disinfectant action in different contact time from the artificial contamination with *Aspergillus niger* suspension (10% vol): 1) without fungal suspension; 2) 3 min.; 3) 6 min.; 4) 9 min.; 5) 12 min.

Table 1. Tafel fit. parameters at the disinfectant action with *Aspergillus niger* suspension (10% vol.)

Solution	Time (min.)	AISI 304 Stainless Steel		
		E_{corr} (mV)	I_{corr} ($\mu\text{A}/\text{cm}^2$)	V_{corr} (mmpy)
Disinfectant	-	-92.35	39.80	0.035
Disinfectant + <i>Aspergillus niger</i>	3	-	100.82	0.090
	6	207.64	-	74.25
	9	203.81	65.88	0.059
	12	181.98	64.08	0.057
		166.38		

Figure 2 shown the effect of the disinfectant with *Candida* from wine suspension and could be observe a little shifted in positive range for E_{corr} but I_{corr} remained almost constant. This observation indicates that the disinfectant limited the corrosion effect of *Candida*, thus confirming of corrosion rate values.

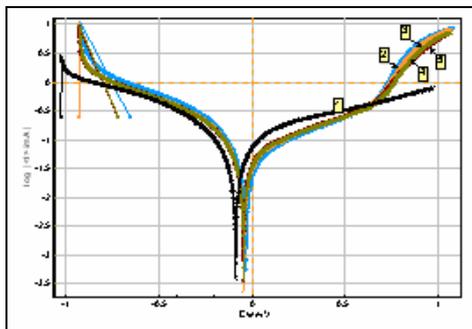


Figure 2. Linear polarization curves at the disinfectant action in different contact time from the artificial contamination with *Candida* (10% vol): 1) without fungal suspension; 2) 3 min.; 3) 6 min.; 4) 9 min.; 5) 12 min.

For *Saccharomyces cerevisiae*. LP curves indicated a increasing of the I_{corr} and corrosion rate also (Fig. 3; Table 3). The cells induce a corrosion effect of the stainless steel and the disinfectant action is limited.

Table 2: Tafel fit. parameters at the disinfectant action with *Candida* from wine suspension (10% vol.)

Solution	Time (min.)	AISI 304 Stainless Steel		
		E_{corr} (mV)	I_{corr} ($\mu\text{A}/\text{cm}^2$)	V_{corr} (mmpy)
Disinfectant	-	-92.35	39.80	0.035
Disinfectant + <i>Candida</i>	3	-	33.74	0.030
	6	16.53	37.10	0.033
	9	32.05	39.25	0.035
	12	39.27	40.60	0.036
			41.10	

Table 3: Tafel fit. parameters at the disinfectant action with *Saccharomyces cerevisiae* suspension (10% vol.)

Solution	Time (min.)	AISI 304 Stainless Steel		
		E_{corr} (mV)	I_{corr} ($\mu\text{A}/\text{cm}^2$)	V_{corr} (mmpy)
Disinfectant	-	-92.35	39.80	0.035
Disinfectant + <i>Saccharomyces cerevisiae</i>	3	2.85	54.58	0.049
	6	-	57.35	0.064
	9	15.63	64.54	0.066
	12	26.43	68.56	0.061
			36.65	

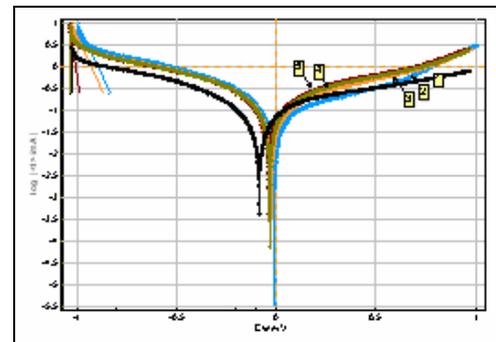


Fig. 3. Linear polarization curves at the disinfectant action in different contact time from the artificial contamination with *Saccharomyces cerevisiae* (10% vol): 1) without fungal suspension; 2) 3 min.; 3) 6 min.; 4) 9 min.; 5) 12 min.

The LP curves for all fungal suspension are presented in Figure 4a for 3 minutes after contamination and Figure 4b after 12 minutes. It could observe that for *Apergillus niger*, the potential E_{corr} is shifted in more negative range compared with *Candida* and *Saccharomyces cerevisiae*. The experimental date are correlating with polarization resistance values (Table 4).

The experimental measured polarization resistance R_p indicates lower resistance of stainless steel at disinfectant action with all fungal suspensions tested. The lower R_p value was obtained at *Apergillus niger* action for first minutes from this fungal contamination, a decreasing with 700 Ω .

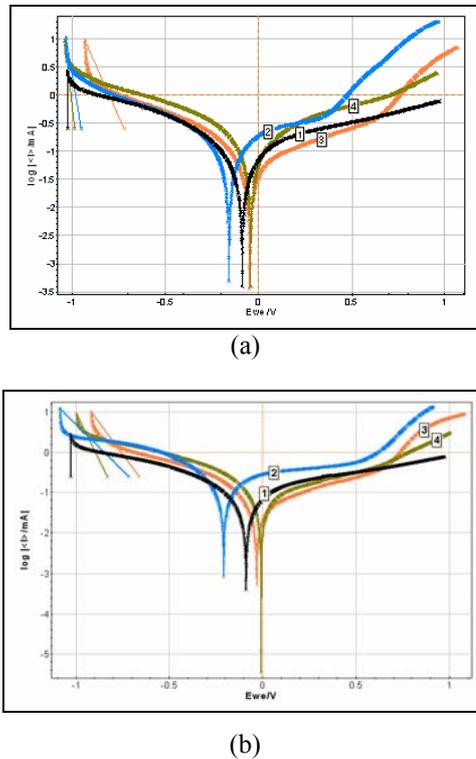


Fig. 4. Linear polarization curves from the artificial contamination: 1) without fungal; 2) with *Aspergillus niger*; 3) with *Candida*; 4) with *Saccharomyces cerevisiae* in different contact time: a) 3 min.; b) 12 min.

Table 4: Polarization Resistance (R_p) at the disinfectant action with fungal suspensions after different contact time

Solution	AISI 304 Stainless Steel	
	R_p (Ω)	
	3 min.	12 min.
Disinfectant	1 221.40	
Disinfectant + <i>Aspergillus niger</i>	482.7	770.5
Disinfectant + <i>Candida</i> from wine	1 359.70	1 202.65
Disinfectant + <i>Saccharomyces cerevisiae</i>	873.46	732.36

The better polarization resistance was obtained at the *Candida* action and not larger variation with the contact time from this contamination was observed. The R_p shown a clear distinction between fungal type, decreasing in the order:

Apergillus niger < *Saccharomyces cerevisiae* < *Candida*.

4. Conclusion

There were evaluated the corrosion resistance of the AISI 304 stainless steel surface at active substance (sodium dichloroisocyanurate) from Actisept disinfectant. The three microorganisms (fungal) suspensions were tested and electrochemical parameters were presented. The E_{corr} and I_{corr} shown a clear distinction between fungal type behaviour at the disinfectant action on stainless steel surface. The R_p decreasing in the order: *Apergillus niger* < *Saccharomyces cerevisiae* < *Candida*.

References

- Maddox, Ian S., *Practical Sanitation in the Food Industry*, 1st ed., Taylor & Francis Ltd, 1994 pp.11.
- Hutkins, R. W., *Microbiology and Technology of Fermented Food*, 1st ed., Ed. Hutkins R.W. Blackwell Publishing, Oxford, 2006, pp.17.
- Tung, H.-L., Chiou, S.-Y., Tu, C.-C., Wu, W.-T., An airlift reactor with double net draft tubes and its application in fermentation, *Bioprocess Engineering* **1997**, 17 (1), 1–5.
- Ratlidge, C., Kristiansen, B., *Basic Biotechnology*, 3rd Edition, Cambridge University Press 2006, pp.183.
- Suryanarayan, S., Current industrial practice in solid state fermentations for secondary metabolite production: the Biocon India experience, *Biochemical Engineering Journal*, **2003**, 13 (2-3), 189-195.
- Junker, B., Lester, M., Leporati, J., Schmitt, J., Kovatch, M., Borysewicz, S., Maciejak, W., Seeley, A., Hesse, M., Connors, N., Brix, T., Creveling, E., Salmon, P., Sustainable Reduction of Bioreactor Contamination in an Industrial Fermentation Pilot Plant, *Journal of Bioscience and Bioengineering*, **2006**, 102 (4), 251-268.
- Compere, C., Bellon-Fontaine, M.N., Bertrand, P., Costa, D., Marcus, P., Poleunis, C., Pradier, C.M., Rondot, B., Walls, M.G., Kinetics of conditioning layer formation on stainless steel immersed in seawater, *Biofouling*, **2001**, 17 (2), 129-145.
- Government Decision no. 545/2008 amending and completing Government Decision no. 956/2005 concerning the placing on the market of biocide products, decision no. 545/2008 Official Gazette of Romania, Part I No. 416/2008.
- Normand, B., Cabrillac, C., Crousier J., Rameau, J.-J., Classical electrochemical characterization methods: description and recommendations for the analysis of metal corrosion, *Revue de Métallurgie*, **2002**, 9, 689 - 700.