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Original Article

Simulation of Listeria monocytogenes bacterial deposits in pitting corrosion on stainless steel grade 304♣

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Abstract

This study investigated and simulated the pitting corrosion on stainless steel grade 304 with a thickness of 1.5 mm and a 2B finish, using 3.5% NaCl solution electrochemically to generate the pits. The diameters of the pits on the sample surface were measured by optical microscopy and the average pit diameter varied over time as pit growth progressed. All the samples were incubated for five days to allow Listeria monocytogenes bacterial deposits in the pits. SEM images showed bacteria in small clusters in pits formed by pitting corrosion on the stainless steel surface, also within the smallest pits with pit mouth diameters of 18 µm. The diameter of the pit mouth was significantly positively correlated with the number of bacteria deposited. These findings suggest that pitting corrosion on stainless steel surfaces can create a habitat for bacteria. This has implications particularly for the food industry, where stainless steel is commonly used in equipment and facilities for food processing and storage. Effective control measures are necessary to prevent the colonization by bacteria of pitting corrosion on stainless steel surfaces.

Keywords: bacteria, corrosion, pitting corrosion, simulation, stainless steel

1. Introduction

Stainless steel is widely used in food and dairy industries due to its excellent mechanical properties, corrosion resistance, and durability. However, pitting corrosion can occur on the surface of stainless steel when exposed to a chloride-containing environment, leading to localized corrosion and failure of the material (Wei *et al.*, 2018). Pitting corrosion refers to localized corrosion that occurs in the form of small holes or pits on the metal surface, which can lead to structural damage and affect the material's performance. In the food industry, if stainless steel equipment develops pitting, it can result in bacterial residues being deposited within the pit holes. Listeria monocytogenes is a type of bacteria found in certain types of food, particularly unpasteurized dairy products. Listeria can also be found in contaminated soil and water, which can lead to the contamination of foods during production and processing. Consuming food contaminated with Listeria can cause a serious infection called listeriosis, and foodborne listeriosis is the dominant form of listeriosis in humans. It can be particularly dangerous for pregnant women, newborns, the elderly, and individuals with weakened immune systems (Jamshidi *et al.*, 2019). Based on retrospective analysis of foodborne listeriosis attributed to pasteurized milk,

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the infectious dose for humans is thought to be approximately 10^9 CFU/ml, enabling a foodborne listeriosis outbreak (FAO/WHO, 2004). Bacteria can become deposited within the pits on a stainless steel surface, forming a biofilm and potentially causing contamination. This problem is particularly significant in industries where cleanliness and hygiene are critical, such as food and pharmaceutical industries. Furthermore, bacterial deposits in pits can accelerate corrosion and cause microbiologically influenced corrosion (MIC) (Li *et al.*, 2019). The presence of bacteria affects also the pH, and the increased rate of weight loss by corrosion damages the material (Hasim *et al.*, 2020).

This study aimed to simulate pitting corrosion and investigate the effects on bacterial deposits in the pitting by pit size, on stainless steel grade 304 surface with 2B finish. Pitting corrosion was induced on the stainless steel samples by electrochemical means in a 3.5% NaCl solution. After corrosion was generated, the samples were incubated with Listeria monocytogenes (DMST 23136) bacteria. They are commonly found in food processing environments, and can cause serious health risks if not properly controlled (Yoon *et al.*, 2019). In addition, morphology of the bacterial deposits in the pits was examined using scanning electron microscopy (SEM) (Hočevar *et al.*, 2014). The results of this study demonstrate the impact on bacterial deposits from pitting corrosion on stainless steel, with a focus on pit size effects.

2. Materials and Methods

The material utilized in this study was austenitic stainless steel grade 304 with 2B surface finish in as received condition, with a thickness of 1.5 mm. The chemical composition of the material was evaluated using optical emission spectrometer BAIRD-DV6S model P/N 081646 CI-4 and is shown in Table 1. The corrosion properties of the material were determined using the potentiodynamic polarization test outlined in ASTM G5 standard. The pitting potential (Epit) was determined by analyzing the polarization curve obtained from the test. The DY2300 potentiostat, with DY2322 software, is shown in Figure 1(a). The arrangement of the electrode apparatus is shown and labeled in Figure 2(b). There was an Ag/AgCl reference electrode, and the counter electrode was a platinum wire, and the evaluation used an initial voltage of -0.4 V, scanning to the final 0.4 V at a scan rate of 0.0005 volts per second. The sensitivity was $1x10^{-3}$ A/V, and testing was done at 25±2°C room temperature. Masking was implemented to focus the test on the as-received surface with 2B finish over an open area of 28.27 mm^2. Generating pitting corrosion on the stainless steel followed the procedure of Picha Panmongkol (Picha *et al.*, 2023). The test solution was 3.5% NaCl. The same equipment, electrodes, and solution used to determine pitting potential were also used to generate pits. The electrochemical generation of pits was performed using the amperometric i-t mode. Pits were generated under potentiostat polarization control applied as transpassive. The duration of exposure to the pit generation process was varied among 5, 10, 15, 20, and 30 seconds in a 3.5% NaCl solution. Pit diameter measurement and determining the average pit mouth diameters for all samples used the optical microscopy (OM) image in top view to determine the relative accuracy (%RA) with 95% confidence. The microbiology experiment apparatus is shown in Figure 2.

Table 1. Chemical composition of base metal (wt.%)

Material	C	Mn	Si	Cr	Ni	Mo	P
SUS304	0.09	1.04	0.44	17.75	7.69	0.13	0.009

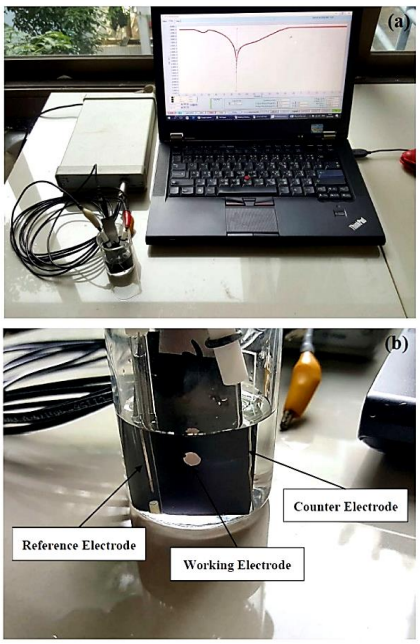

Figure 1. Potentiodynamic polarization test equipment: (a) DY2300 potentiostat program and DY2322 potentiostat, and (b) experimental electrode apparatus parts

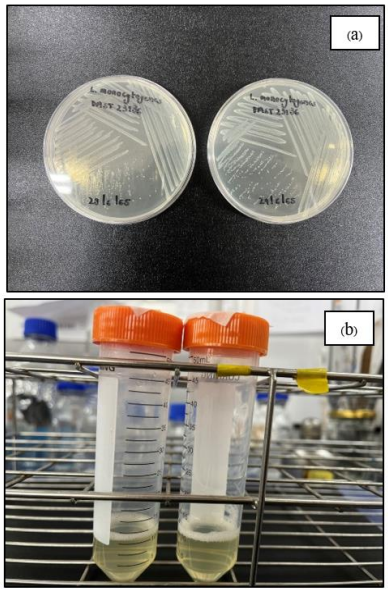

Figure 2. (a) Listeria monocytogenes on blood agar culture plates, and (b) stainless steel 1.5 mm samples incubated with Listeria monocytogenes bacteria

After pitting corrosion all the samples were transferred into polypropylene centrifuge tubes with 10 mL of

TSB broth, and the samples were incubated with Listeria monocytogenes (DMST 23136) in Tryptone Soya Broth (TSB) in a centrifuge run at 150 rpm for five days at 37 ºC in a temperature-controlled room. After incubation, culture plates were removed from the tubes, and viable colonies were counted and recorded. The count of colony-forming units was 1.37×10^9 CFU/ml, which was counted on tryptic soy agar from a measure of viable clonogenic cell colonies. After that, the samples with bacteria underwent fixation and dehydration to prepare them for surface analysis. The stainless steel samples were coated with gold and observed under SEM (Thermo Fisher Scientific, Prisma E SEM) to examine the surface morphology and corrosion features, including pitting corrosion and bacteria. In manual counts of the bacteria deposited in the pit holes, more than three pit holes were counted per condition, and the %RA was within 95% confidence, using Image J software for the counts of bacteria presented in Figure 3.

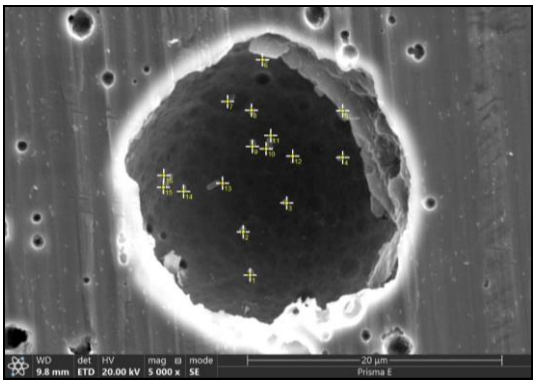

Figure 3. Manual counting of bacteria deposited in a pit, using Image J software

3. Results and Discussion

3.1 Generating pitting corrosion on the stainless steel

The potentiodynamic polarization tests were conducted on a 1.5 mm stainless steel sample in a 3.5% NaCl solution. The polarization curves showed a pitting potential (Epit) of approximately 0.269 Volts, as shown in Figure 4. Beyond this point, pitting corrosion occurred as transpassive corrosion. The pitting potential was used to generate pitting corrosion under potentiostat polarization control, with a transpassive pitting potential of 0.269 Volts as the critical pitting potential, testing the five alternative time periods 5, 10, 15, 20, and 30 seconds.

After the simulation of pitting corrosion, the sample was removed from the electrolyte, washed, rinsed using soap water, and dried with hot air. An optical microscope was used to determine the diameters of the actual pit mouths on the sample surface. The average diameter of pits was determined along with its relative accuracy, which in all cases was better than 90%. The results indicate that the methods used in this study were accurate. The average pit mouth diameters for the samples treated for 5, 10, 15, 20, and 30 seconds were respectively 30, 44, 57, 64, and 68 µm, showing the growth with treatment time, see Figure 5. The pit growth on stainless steel was continuous with duration of treatment in a 3.5% sodium chloride solution, and the coefficient of determination of the model fit was significant at about 0.99. Sodium chloride is a corrosive agent in aqueous solution, and it can accelerate the rate of pitting corrosion on stainless steel. The chloride ions in the solution can cause a breakdown of the passive layer on stainless steel, leaving the material susceptible to attack by corrosive agents. Once a pit has formed, it can continue to grow as long as the corrosive environment is present, leading to progressive damage over time. Therefore, the pit growth propagated, and new pitting corrosion was initiated while existing pitting corrosion propagated with time. This result is consistent with previous reports on corrosion pit growth on austenitic stainless steels in sodium chloride solution (González *et al.*, 2012).

Table 2 summarizes a one-way ANOVA of average pit mouth diameter in relation to treatment time. The results show that the treatment time was statistically significantly associated with the average pit mouth diameter, at P-value 0.000 (<0.005). The corrosion morphologies of the samples were studied using OM imaging (Figure 6).

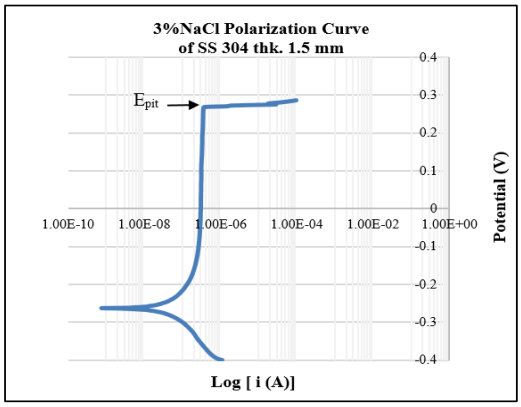

Figure 4. Polarization curve of stainless steel grade 304 (thickness 1.5 mm) in 3.5% NaCl solution

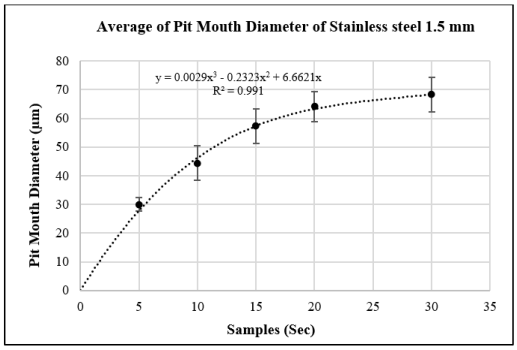

Figure 5. Average pit mouth diameter by treatment time

3.2 Bacteria deposits in pitting corrosion

The corrosion morphologies of the samples were studied using SEM, as shown in Figures 7 and 8, along with the Listeria monocytogenes bacterial formations from exposure at 37°C for five days. The SEM image of stainless steel surfaces after incubation with Listeria monocytogenes

Table 2. One-way ANOVA of average pit mouth diameter by duration of electrochemical corrosion

Summary				
Duration	Count	Sum	Average	Variance
5 Sec	11	330	30	5
10 Sec	11	488	44	37
15 Sec	11	629	57	37
20 Sec	11	705	64	27
30 Sec	11	751	68	36

ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Duration	10748	4	2687	95	0.000	3
Error	1417	50	28			
Total	12165	54				

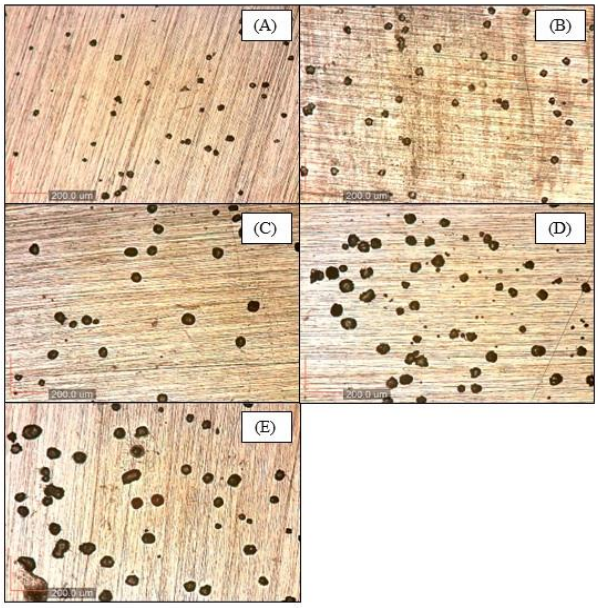

Figure 6. Pits on the sample surfaces subjected to electrochemical corrosion for (A) 5 sec, (B) 10 sec, (C) 15 sec, (D) 20 sec, and (E) 30 sec

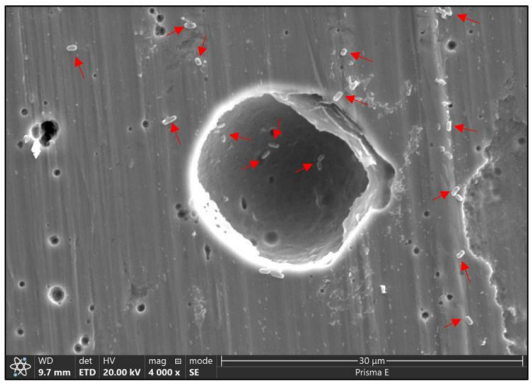

Figure 7. SEM image showing Listeria monocytogenes bacteria on the planar stainless steel surface and as deposits in the pits

show that the bacteria were attached on the stainless steel surface. In Figure 7. the arrows indicate bacteria on the planar stainless steel surface and in the pits. These bacteria are small gram-positive rods, 0.5–2 µm in diameter and 0.5–4 µm in length (Jamshidi *et al.*, 2019). This result is consistent with prior studies on Listeria monocytogenes bacteria on a stainless steel surface (Chavant *et al.*, 2002; & Di Bonaventura *et al.*, 2008). This is important evidence that the bacteria can survive on stainless steel surfaces. The findings confirm that stainless steel material can have small holes or pits on the metal surface, of relevance in the food industry at product contact. Bacteria can also thrive in the nutrient-rich environment created by corrosion, which can provide energy and nutrients for their growth and survival. Additionally, the corrosion process can generate localized changes in pH, which can create more favorable conditions for bacterial growth and lead to microbiologically influenced corrosion (MIC) (Hasim *et al.*, 2020; & Yoon *et al.*, 2019). Moreover, bacteria can form biofilms on the metal surface, which can act as a protective layer against external threats, such as disinfectants, and provide a habitat for bacteria to grow and multiply. As the mass of bacteria becomes large enough to entrap organic and inorganic debris and other microorganisms, this leads to the formation of a microbial biofilm. These biofilms may be from a few micrometers to several millimeters thick in food processing environments. The biofilms can also play a role in the further corrosion of the metal, as they can produce acidic metabolites (Hasim *et al.*, 2020; & Yoon *et al.*, 2019).

In the SEM images in Figure 8, the arrows indicate bacteria deposited in the pits, revealing the presence of small clusters of Listeria monocytogenes bacteria inside the smallest sized pits with average mouth diameter of 18 µm (Figure 8(A)), in the middle sized pits with average mouth diameter of 44 µm (Figure 8(B)), as well as inside the biggest pits with an average mouth diameter of 68 µm (Figure 8(C)). These findings show that bacteria entered in pitting holes of different sizes, provided by pitting corrosion on stainless steel. The foodborne pathogen Listeria monocytogenes is a concern in food safety because of its ability to form biofilms and persist in the food industry (Colagiorgi *et al.*, 2017).

3.3 Effect of pit size on the number of bacteria deposited in the pit

The study provides evidence that the pit mouth diameter affects the amount of bacteria that can survive in the pit, as shown in Figure 8. Specifically, in Figure 9, the

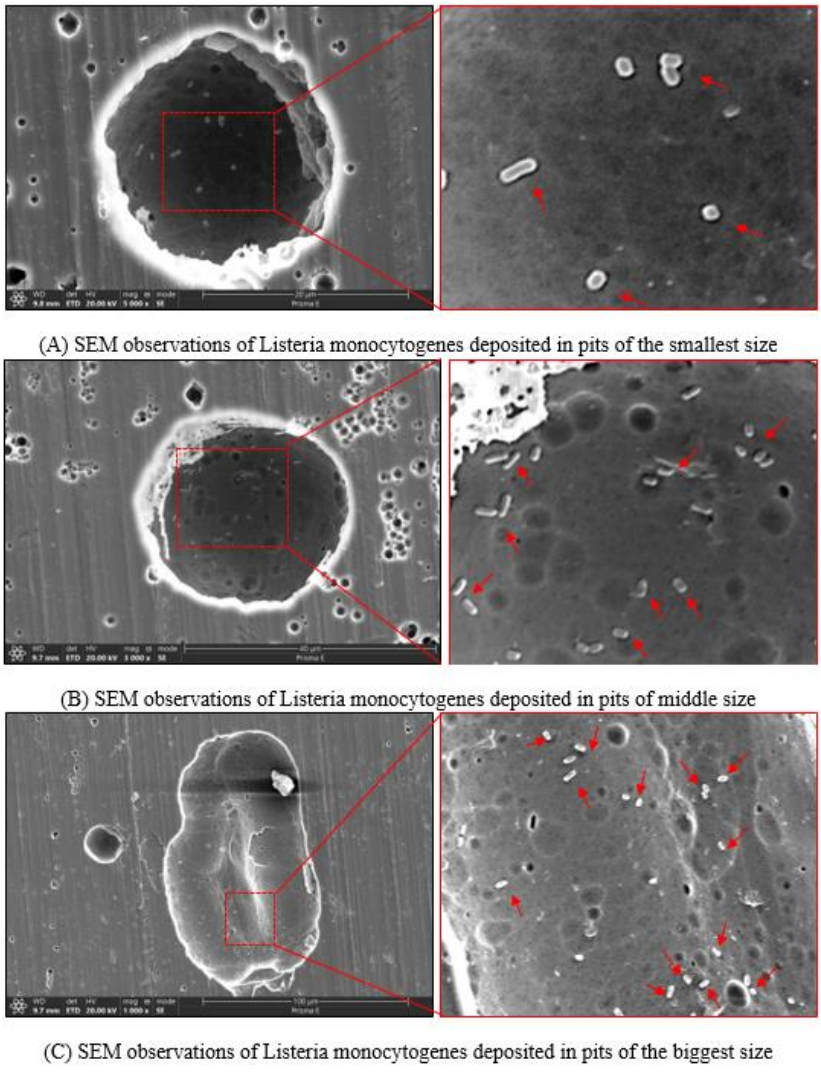

Figure 8. SEM observations of Listeria monocytogenes deposited in pits of different sizes

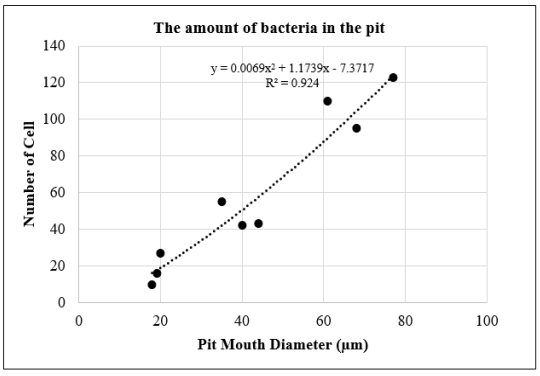

Figure 9. The average number of bacteria deposited in a pit by diameter of pit mouth

average numbers of bacteria deposited in pits with average pit mouth diameters of 18 µm, 44 µm, and 68 µm were respectively found to be 18, 54, and 109. In addition, the analysis of variance (ANOVA) in Table 3 shows that the relationship between the average pit mouth diameter and the count of bacteria deposited in the pit are statistically significantly related, with a p-value of less than 0.005. As the pit mouth was larger, the number of bacteria deposited in the pit also increased. Overall, the study suggests that the pit mouth diameter is an important factor affecting bacterial survival in pits.

4. Conclusions

This study simulated pitting corrosion on stainless steel grade 304 with a thickness of 1.5 mm and a 2B finish, using electrochemical treatment with immersion in a 3.5% NaCl solution. The study identified pitting corrosion on stainless steel samples and employed optical microscopy to measure the diameters of the pits on sample surfaces. The analysis revealed that the average pit diameter had progressive growth with treatment time, rather than a consistent size across the experiments. After five days of incubation with

Table 3. One-way ANOVA of count of bacteria deposited in the pit by average pit mouth diameter

Summary				
Groups	Count	Sum	Average	Variance
18 µm	3	53	18	74
44 µm	3	163	54	121
68 µm	3	328	109	196

ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	12772	2	6386	49	0.000	5
Within Groups	784	6	131			
Total	13556	8				

Listeria monocytogenes bacteria, the samples had small bacterial clusters in pits from pitting corrosion and also on the planar surface of stainless steel. Bacteria were also found to be deposited within the smallest pits on the surface, and the pit mouth diameter is an important factor that affects the survival of the bacteria deposited. As the pit mouth diameter grows larger, the count of bacteria deposited in the pit also increases. The results of this study have practical implications especially for the dairy industry, where pitting corrosion on stainless steel surfaces can lead to bacterial colonization and food contamination.

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