Simulation of deposit Listeria monocytogenes Bacteria 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 electrochemical in a 3.5% NaCl solution to generate the pits. The study used optical microscopy to measure the diameter of the pits on the sample surface and found that the average pit diameter varied over time, indicating a progressive pits growth. All samples were incubated for Listeria monocytogenes bacteria for five days to simulate the bacteria deposit in the pit. The SEM images found bacteria inside small clusters and pits of pitting corrosion on the surface of stainless steel, including within the smallest pits with pit mouth diameters of 18 μm. The results found the size of the pit mouth diameter was significant to the amount of bacteria deposited. As the diameter was larger, the amount of bacteria deposited in the pit also increased. These findings suggest that pitting corrosion on stainless steel surfaces can be

a habitat for bacteria. The results of this study have implications 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 of bacteria in 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 bacteria 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, which is the leading cause of foodborne listeriosis in humans, which 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, the infectious dose for humans is thought to contain up to

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

This study aims to simulate pitting corrosion and investigate the effect of bacteria deposits in the pitting as the difference in pit size of the stainless steel grade 304 surface 2B Finish. Pitting corrosion will be induced on the stainless steel samples by electrochemical immersion in a 3.5% NaCl solution. After the corrosion is generated, the samples will be incubated with Listeria monocytogenes (DMST 23136) bacteria, commonly found in food processing environments, and can cause serious health risks if not properly controlled (Yoon *et al.*, 2019). In addition, the morphology of the bacteria deposited in the pits will be examined using scanning electron microscopy (SEM) (Hočevar *et al.*, 2014). The results of this study will purpose of this study is to demonstrate the impact of bacterial deposits within pitting corrosion on stainless steel, with a focus on varying pit sizes.

2. Materials and Methods

The material utilized in this study was austenitic stainless steel grade 304 surface 2B Finish as received condition, which had a thickness of 1.5 mm. The chemical composition of the material was evaluated by optical emission spectrometer BAIRD-

DV6S model P/N 081646 CI-4 and shown in Table 1. The corrosion properties of a material were using the potentiodynamic polarization test technique outlined in the 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 Fig. 1(a). The arrangement of the electrode apparatus was shown and labeled in Fig. 2(b). The reference electrode was an Ag/AgCl reference electrode, and the counter electrode was a platinum wire, it was utilized to carry out the evaluation, using an initial voltage parameter of -0.4 to final 0.4 volts, a scan rate of 0.0005 volts per second, and sensitivity of $1x10^{-3}$ A/V, tested at the room temperature 25°C±2, a masking technique was implemented to focus the test on the as-receive surface 2B finish have an opening area of 28.27 mm^2. The simulated generated pitting corrosion on the stainless steel followed the procedure of Picha Panmongkol & Et al. (Picha *et al.*, 2023). The test solution was 3.5% NaCl. The same equipment, electrodes, and solution were used to determine pitting potentials 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 varied in five sets, ranging from 5, 10, 15, 20, and 30 seconds in a 3.5% NaCl solution. Pit diameter measurement of the average pit mouth diameters of all samples using the OM image top view to measure the %RA was within 95% confidence. The microbiology experiment apparatus is shown in Fig. 2.

After the pitting corrosion on all samples transferred into a polypropylene centrifuge tube with 10 mL of TSB broth, the samples were incubated with Listeria monocytogenes (DMST 23136) in Tryptone Soya Broth (TSB) broth in a centrifuge with a parameter of 150 rpm for five days at 37 ºC in the control temperature room. After incubation, culture

plates were removed from the incubators, and viable colonies were counted and recorded. The amount of colony-forming units is 1.37×10^9 CFU/ml, which was counted on tryptic soy agar from a measure of viable clonogenic cell amounts. After that, the samples with bacteria underwent fixation and dehydration to prepare 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. For the manual counts of the bacteria deposited in the pit hole, 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, were presented in Fig.3

3. Results and Discussion

Simulated generated 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 obtained polarization curves showed that the pitting potentials (Epit) occurred at approximately 0.269 Volts, as shown in Fig. 4. Beyond this point, pitting corrosion occurred as transpassive corrosion. The pitting potential values were then used as a reference to simulate pitting corrosion on the surface of the stainless steel sample by applying a transpassive pitting potential through potentiostat polarization. Finally, the pitting potential value was used to generate pitting corrosion under potentiostat polarization control, with a transpassive pitting potential value voltage of 0.269 Volts as the critical pitting potential with different time periods in five sets, ranging from 5, 10, 15, 20, and 30 seconds.

After the simulation of the 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 pit diameter of the actual pit's mouth on the sample surface. The average diameter of the pit was determined by calculating the percentage of relative accuracy. Based on the calculation results, all specimens had a relative accuracy value of more than 90%. The results indicated that the methods used in this study were accurate. The average pit mouth diameters for the samples with generated pits duration times of 5, 10, 15, 20, and 30 seconds, pit mouth diameters were 30, 44, 57, 64, and 68 μm, respectively. The average diameter of pits was found to have growth evolution. It did not have the same size over a longer duration but changed with time during pit growth, as shown in Fig. 5. It found that the pit growth on the stainless steel is continuous for a longer duration under a 3.5% sodium chloride solution. The results indicate that the pit mouth diameter varied with the duration time of pit growth formation, and this relationship had a significant R-square value of 0.9923. This is due to sodium chloride being a corrosive agent that can accelerate the rate of pitting corrosion on stainless steel. The presence of chloride ions in the solution can cause the breakdown of the passive layer on the stainless steel, leaving it susceptible to attack by the corrosive agents. Once the 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 underwent growth propagation, and new pitting corrosion was initiated while existing pitting corrosion propagated with more duration time. This result is consistent with previous reports on the corrosion pit growth on austenitic stainless steels in sodium chloride solution (González *et al.*, 2012).

Table 2 analyzes ANOVA, a single factor of average pit mouth diameters with generated pits duration times. The result showed that the generated pit's duration times interaction with the average pit mouth diameters with at the level of confidence 95% P-

Value of factors was 0.000 (<0.005). Therefore, the explained variability of the generated pit's duration times affected the significance of the average pit mouth diameters. The corrosion morphologies of the samples were studied using OM Fig. 6, which presented the pit mouth diameter of samples the pit on the surface samples.

Bacteria Deposit in Pitting Corrosion: The corrosion morphologies of the samples were studied using SEM, as shown in Fig. 7 and 8, representing the Listeria monocytogenes bacteria formation at 37°C after five days. The SEM image morphologies of stainless steel surfaces after incubation in the Listeria monocytogenes show that the bacteria are distributed on the stainless steel surface, as shown in Fig. 7. (The arrows indicate the bacteria on the stainless steel surface and deposit in the pits). Bacteria were found to be small gram-positive rods (0.5–4 μm in diameter and 0.5–2 μm in length) (Jamshidi *et al.*, 2019). This result is consistent with other report study 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 confirmed that the stainless steel material had small holes or pits on the metal surface in the food industry at the product contact. Bacteria can also thrive in the nutrient-rich environment created by the corrosion process, 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 surface of the metal, which can act as a protective layer against external threats, such as disinfectants, and provide a habitat for bacteria to grow and multiply. This mass of bacteria then becomes large enough to entrap organic and inorganic debris and other

microorganisms, leading to the formation of a microbial biofilm. These biofilms may be a few micrometers or several millimeters thick in food processing environments. These biofilms can also play a role in the further corrosion of the metal, as they can produce acidic metabolites that contribute to the corrosion process (Hasim et al., 2020; & Yoon et al., 2019).

The SEM images in Fig. 8 (The arrows indicate the bacteria deposit in the pits) revealed the presence of small clusters of Listeria monocytogenes bacteria inside the smallest pits size, with an average pit mouth diameter of 18 µm (Fig. 8(A)), the middle pits size average pit mouth diameter of 44 µm (Fig. 8(B)) as well as inside the biggest pits size, with an average pit mouth diameter of 68 µm (Fig. 8(C)). These findings showed that bacteria were formed in pitting holes of different sizes, particularly concerning the trapping of bacteria within pitting corrosion. Therefore, the experiment presented the effect of bacteria able to form deposit in the pit holes under the pitting corrosion on stainless steel. The foodborne pathogen Listeria monocytogenes is a concern in food safety because of its ability to form biofilm and persist in the food industry (Colagiorgi et al., 2017). Therefore, the authors want to present the impact of bacteria able to develop deposit in the pit holes, which is the leading cause of foodborne listeriosis in humans.

Effect of Size Pit on The Amount of Bacteria Deposit in The Pit: The study presented evidence that the size of the pit mouth diameter affects the amount of bacteria that can survive in the pit, as shown in Fig. 8. Specifically, in Fig. 9, the average amount of bacteria deposit in pits with average pit mouth diameters of 18 µm, 44 µm, and 68 µm was found the average amount of bacteria deposit in pits to be 18, 54, and 109, respectively. In addition, the results of the analysis of variance (ANOVA) showed in Table 3 that the relationship between the average size of the pit mouth diameter and the

amount of bacteria deposited in the pit was statistically significant, with a p-value of less than 0.005. The result found that as the pit mouth's diameter was larger, the amount of bacteria deposited in the pit also increased. This means that the size of the pit mouth diameter explained significant variability in the amount of bacteria deposited in the pit. Overall, the study suggests that the size of the pit mouth diameter is an important factor affecting bacteria survival in pits.

4. Conclusions

From the results of this study, the study simulated pitting corrosion on stainless steel grade 304 with a thickness of 1.5 mm and a 2B finish, using electrochemical immersion in a 3.5% NaCl solution. The study identified pitting corrosion on stainless steel samples and employed optical microscopy to measure the diameter of the pits on the sample surface. The analysis revealed that the average pit diameter varied over time, indicating a progressive growth of the pits rather than a consistent size throughout the duration of the experiment. After five days of Listeria monocytogenes bacteria incubation, the samples were detected inside small clusters in pits of pitting corrosion and on the surface of stainless steel. The bacteria were also found to be deposited within the smallest pits on the surface, and the size of the pit mouth diameter is an important factor that affects the survival of the bacteria deposit. As the results of the pit mouth's diameter are larger, the amount of bacteria deposit in the pit also increases. The results of this study have practical applications in the dairy industry, where pitting corrosion on stainless steel surfaces can lead to bacterial colonization and food contamination.

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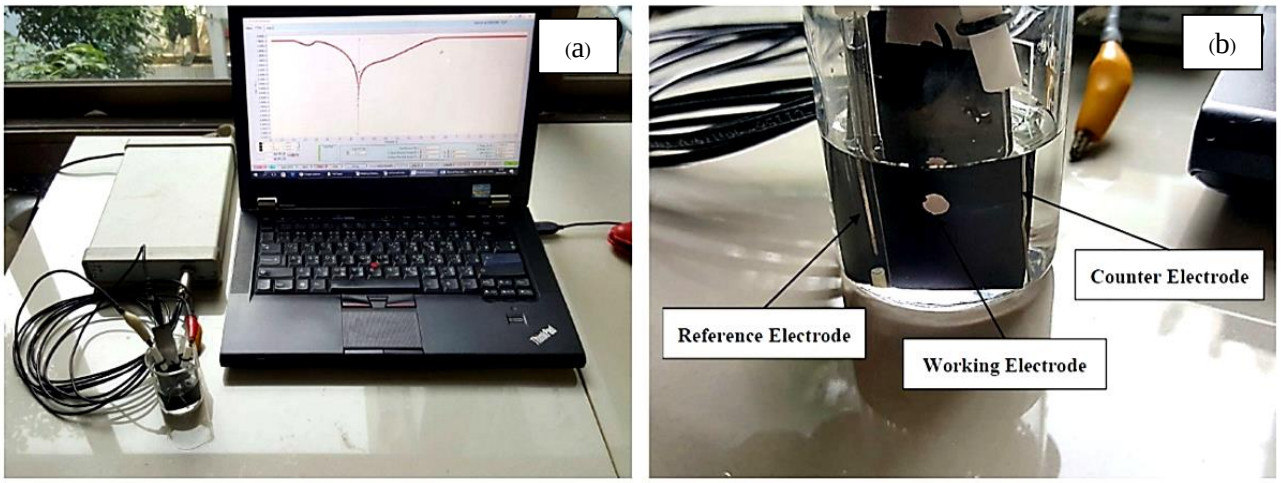


Figure 1 Potentiodynamic Polarization Test; (a) DY2300 Potentiostat program DY2322Potentiostat, (b) Experimental electrode apparatus parts.

Figure 2 (a) Listeria monocytogenes on blood agar culture plates, and (b) Stainless steel 1.5 mm samples incubation in the Listeria monocytogenes bacteria.

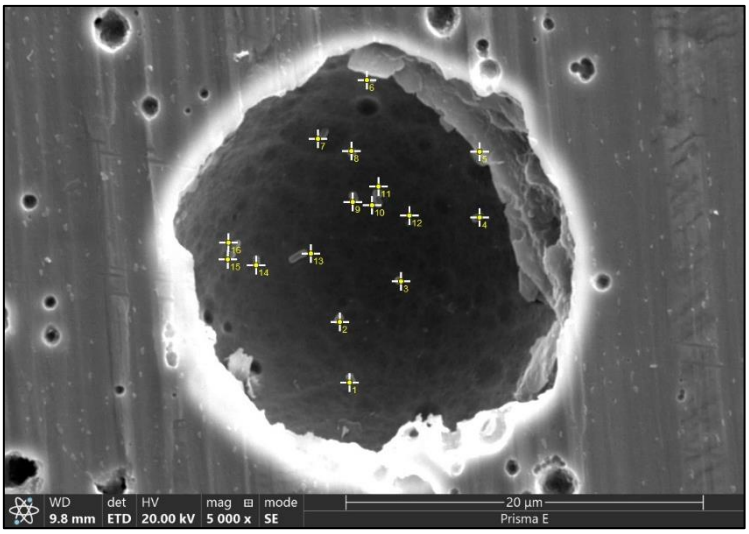


Figure 3 Manually counts the bacteria deposit in the pit using Image J software for the counter.

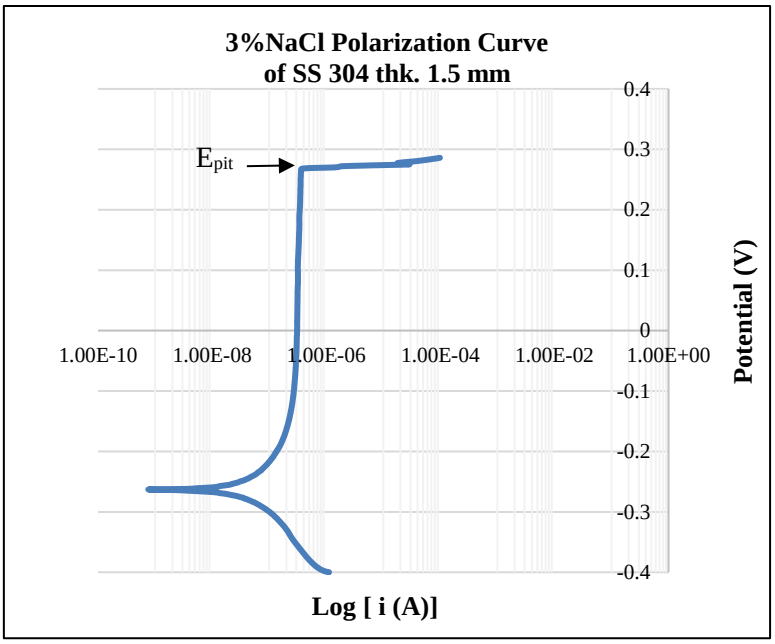


Figure 4 Polarization curve of the stainless steel grade 304 thickness 1.5 mm in 3.5% NaCl solution.

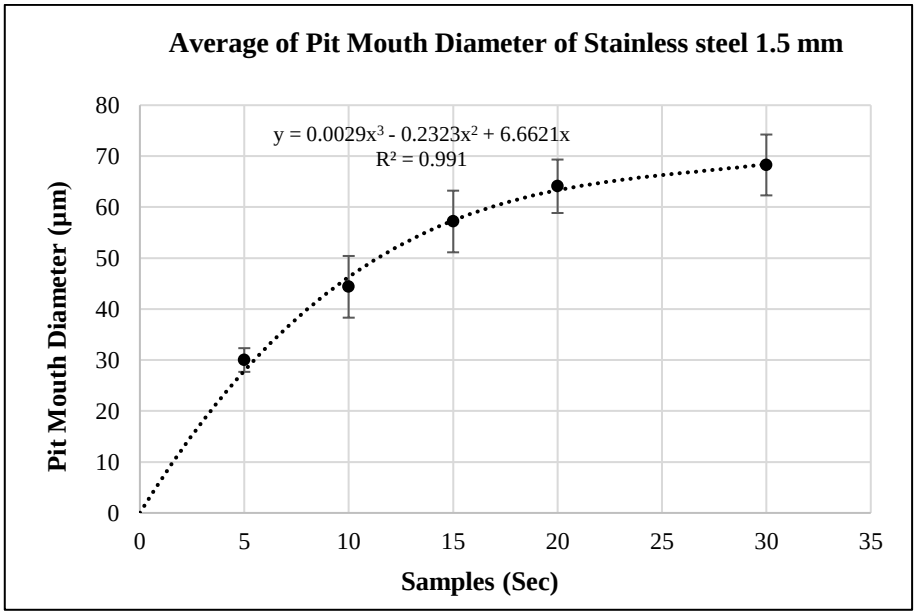


Figure 5 Average of pit mouth diameter of the pit on the surface samples (A) 5 Sec, (B) 10 Sec, (C) 15 Sec, (D) 20 Sec, and (E) 30 Sec.

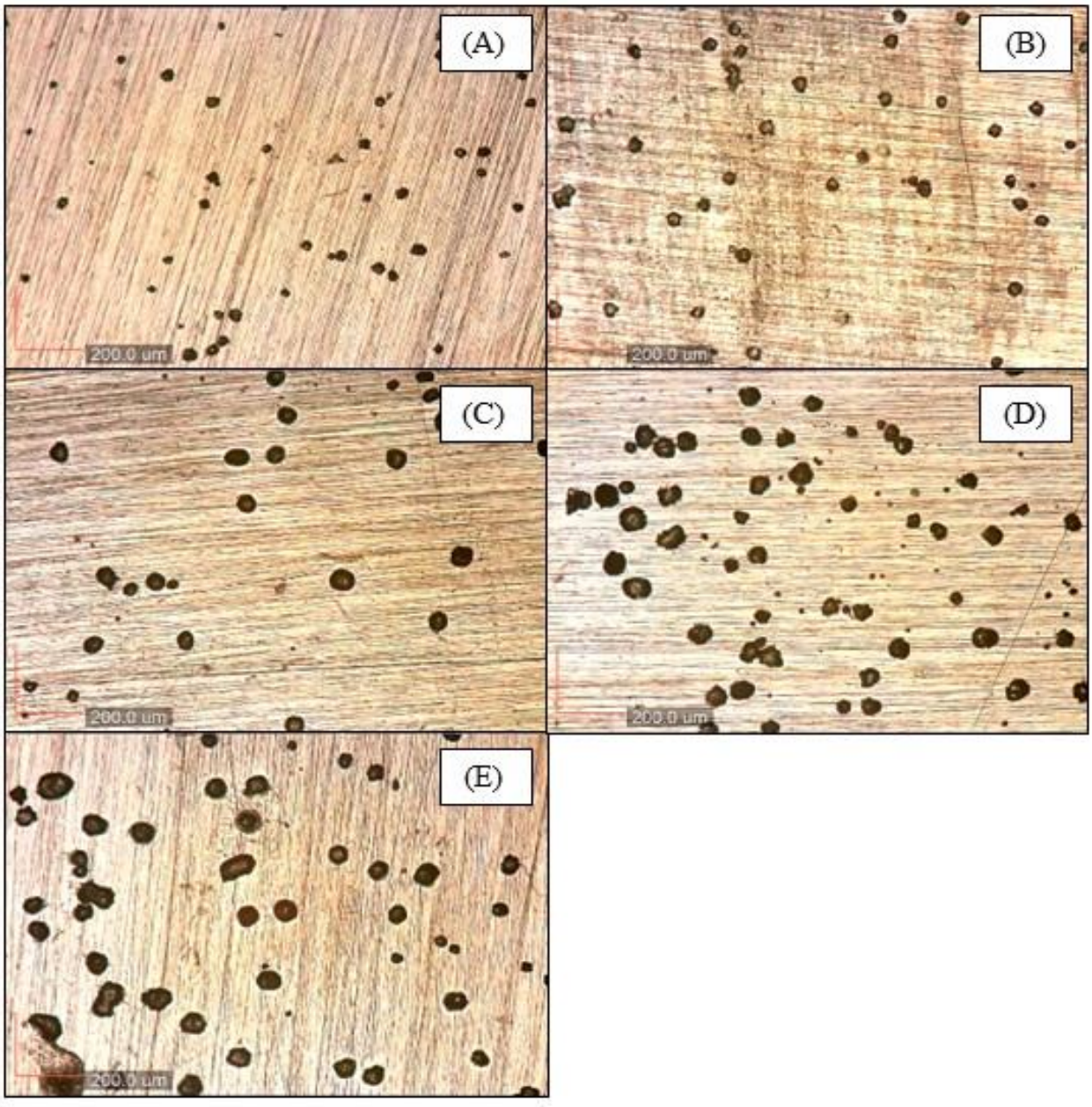


Figure 6 The pit mouth diameter of samples the pit on the surface samples (A) 5 Sec, (B) 10 Sec, (C) 15 Sec, (D) 20 Sec, and (E) 30 Sec.

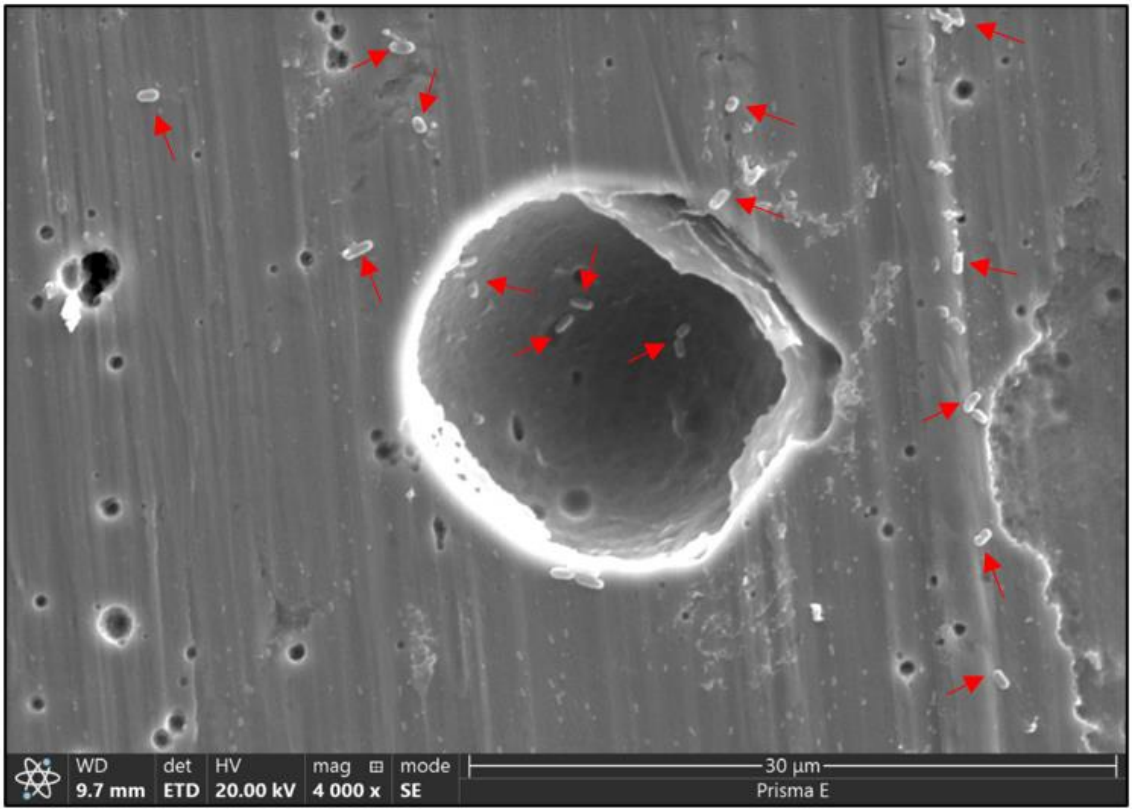
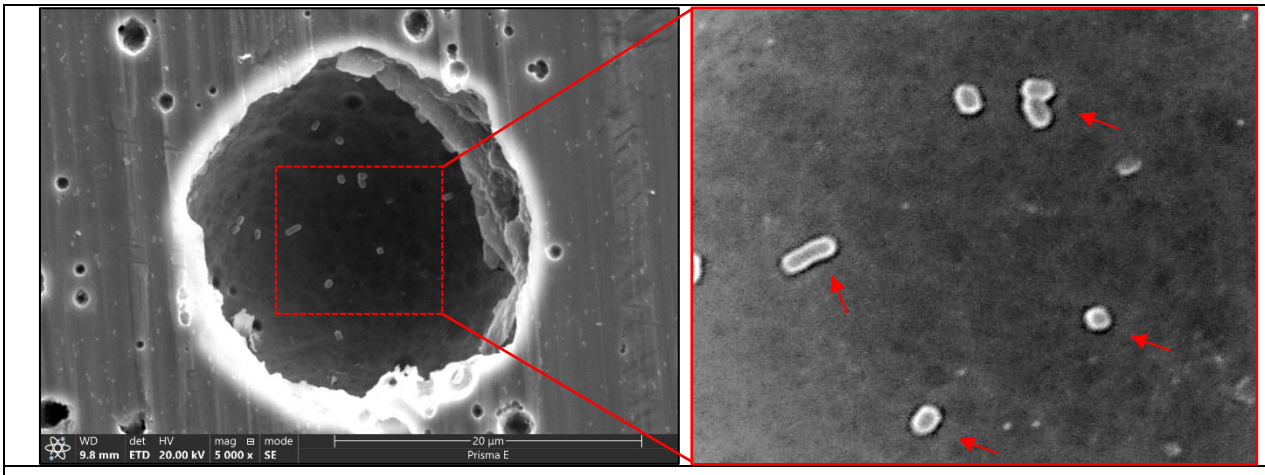
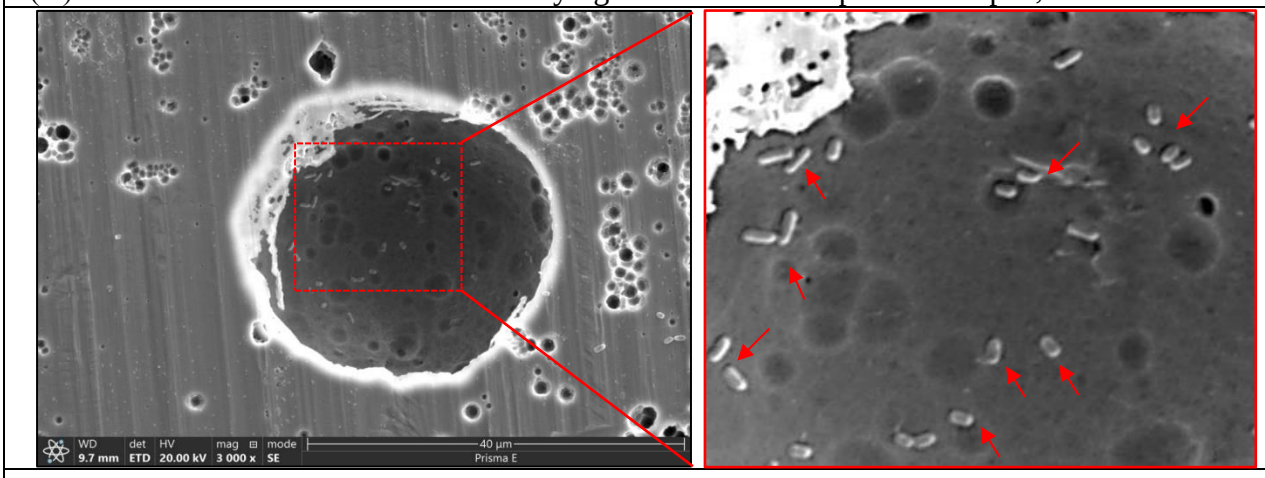


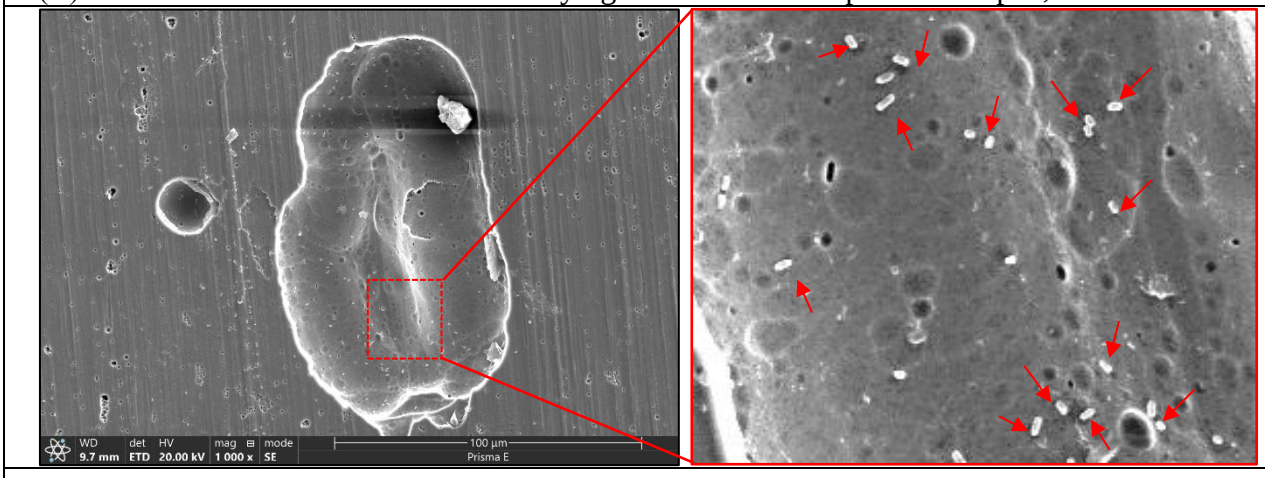
Figure 7 SEM observations of Listeria monocytogenes bacteria formation on the stainless steel surface and deposit in the pits.



(A) SEM observations of Listeria monocytogenes formation deposit in the pits, the smallest size



(B) SEM observations of Listeria monocytogenes formation deposit in the pits, the middle size



(C) SEM observations of Listeria monocytogenes formation deposit in the pits, the biggest size

Figure 8 SEM observations of Listeria monocytogenes formation deposit with different sizes.

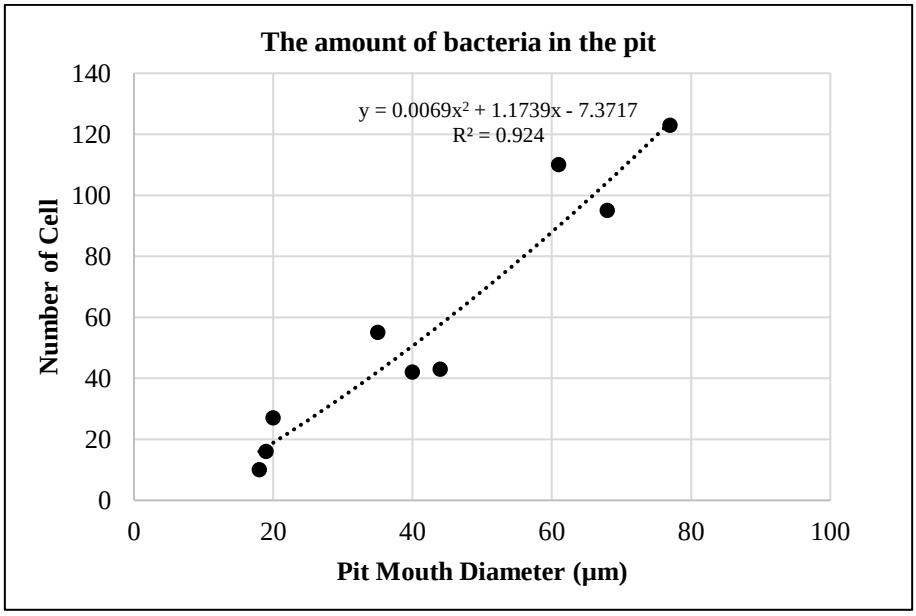


Figure 9 The average amount of bacteria deposited in the pit.

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

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

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				

Table 2 The results of ANOVA; single factor of average pit mouth diameters with generated pits duration times

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				

Table 3 The results of ANOVA; Single factor of average size pit mouth diameter with the amount of bacteria deposited in the pit