



Biocorrosion of Mild Steel in Culture of Aerobic Bacteria

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ABSTRACT

The biocorrosion of mild steel in a culture of aerobic bacteria (Pseudomonas sp.) has been investigated under laboratory conditions. Freshwater samples were collected and subjected to physicochemical and bacteriological analysis in which Pseudomonas sp were cultured using the serial dilution method and used for the study. Selected physicochemical and bacteriological properties including temperature. pH. total dissolved solids (TDS). dissolved oxygen (DO), and total bacterial count (TBC) were monitored throughout the period of the experiment, which lasted for a total of 5760 hours. The corrosion rate of mild steel, determined using the conventional weight-loss method, showed that the average corrosion rate of mild steel in the culture of Pseudomonas sp. was approximately 73% higher than that without inoculum. This result establishes that microbial activities undoubtedly promote corrosion in an environment where they are predominant.

KEYWORDS: Characterization; Microbial Corrosion; Corrosion Rate; *Pseudomonas sp.*

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1. INTRODUCTION

Biocorrosion of freshwater systems can be described as a condition in which metal surfaces are colonized by microorganisms that adhere to their surfaces as they contact the said metal. Generally, biocorrosion or microbiologically influenced corrosion or microbial corrosion is the term used to address the phenomenon in which corrosion is initiated or accelerated by the activities of microorganisms (Amadi, 2015; Amadi *et al.*, 2010; Fernance *et al.*, 2007; Jeon *et al.*, 2001). It is also a kind of electrochemical corrosion in which certain microorganisms either initiate or enhance corrosion (Reza, 2004).

Biocorrosion is the initiation or enhancement of corrosion caused by a living system (Royer & Unz, 2005).

Corrosion of because the occurs physicochemical interaction of metals with their environment. It is also an electrochemical reaction between a material, usually metal and its environment that results in the deterioration of the metal and its property. The extent to which a corrosion process will proceed is determined by a number of factors, which could be biotic (living) or abiotic (non-living) (Videla and Herrera, 2005). Yuan et al. (2008) in their investigation of the presence and absence of marine aerobic Pseudomonas bacterium using electrochemical studies showed that 304 SS underwent different corrosion attacks by Pseudomonas bacteria. They further opined that the presence of Pseudomonas biofilm on the metal coupons gave rise to an increase in the micro-pitting and corrosion rate.

Biocorrosion occurs in various industrial environments. Microorganisms are abundant in soil and water, and they influence the characteristics of metal structures (Puyate *et al.*, 2009, Jeon *et al.*, 2001). In the Niger Delta, Nigeria, the problem of biocorrosion cannot be over-emphasized. Biocorrosion is associated with the numerous rupture and failures of pipelines used to transport petroleum and its products, even though some of these failures are attributed only to corrosion (Odokuma & Ugboma, 2012).

In freshwater environments, there exist numerous microorganisms such as sulphatereducing bacteria, aerobic bacteria, and Sulphur bacteria among others (Pitonzo *et al.*, 2004; Lee & Newman, 2003). The activities of Journal of Newviews in Engineering and Technology (JNET) Volume 5, Issue 1, April 2023



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microorganisms in the corrosion process can cause numerous effects. These effects result from interactions between the environment surrounding the metal surface and the microorganisms, which undoubtedly cause damage to the metal (Amadi *et al.*, 2010).

The microorganisms in freshwater environments utilize the available nutrient sources to generate extracellular polymeric substances. These substances form layers on the metal surfaces thereby inhibiting or accelerating corrosion (Aruliah *et al.*, 2011). Further studies by Aruliah *et al.* (2011) on the influence of aerobic bacteria, *Bacillus megaterium* and *Pseudomonas sp.*, on 304 SS showed that both bacteria caused the metal to undergo severe pitting attack.

Smith and Hashemi (2006) in their study revealed that mild steel is used for the construction of some equipment in the manufacturing sector because of its numerous advantages including strength, ductility, and weldability. However, they added that mild steel is susceptible to corrosion attacks, which informed the decision to use mild steel in this study.

The failure of most pipelines and other engineering structures in freshwater environments has always been attributed to abiotic (non-living) factors with less emphasis on the role played by biotic (living) factors in corrosion processes. It is also noted that most studies on biocorrosion have been centered more on the soil environment with less attention on the activities of aerobic bacteria (Rim-Rukeh, 2007). The need to fill this knowledge gap in existing literature prompted the present study.

The objective of this study was to investigate the influence of aerobic bacteria (*Pseudomonas species*) on metal corrosion under laboratory conditions. This study also considered variations in the physicochemical and bacteriological characteristics of the freshwater samples at regular intervals to determine the extent to which

the freshwater is susceptible to promoting biocorrosion.

2. MATERIALS AND METHODS

2.1 Materials

The following materials were used during this investigation. They include mild steel coupons, thread, emery paper, ethanol, acetone, oven, desiccator, freshwater sample, plastic container, hydrochloric acid, McCartney glass bottle, water quality monitor, saline, test tubes, glass spreader, nutrient agar, petri dishes, plates, nutrient broth, bowls, polypropylene rope, and weighing balance.

2.2 Methods

The methods adopted in this study are consistent with those of the American Public Health Association (APHA, 2000). The selected physicochemical and bacteriological properties of the freshwater determined during this investigation were temperature, pH, total dissolved solids (TDS), dissolved oxygen (DO), and total bacterial count (TBC). These properties are good environmental impact indicators for biocorrosion assessment (Rim-Rukeh, 2007; Amadi *et al.*, 2010; Odokuma & Ugboma, 2012).

2.2.1 Preparation of Corrosion Coupons

The mild steel used in this study was obtained from a corrosion monitoring and control company in Port Harcourt, Nigeria. From our previous study (Amadi et al., 2010), the elemental composition of the mild steel is given as C = 0.14%; Si = 0.18%; Mn = 0.44%; S = 0.4%; Pt = 0.7\%; Sn = 0.05\%; Cr = 0.01\%; Cu = 0.05%, and Fe = 98.03%. The 1-mm thick mild steel sheet was cold-cut into smaller sheets of dimension 50 mm x 25 mm (length x width). The cold-cut technique was used to maintain the integrity of the steel and to avoid the probable effect of heat on the coupons. An eyelet for the polypropylene rope was created at the top of each coupon midway along the width. The coupons were thoroughly polished with emery paper, sterilized by dipping in absolute ethanol and





degreased by washing in acetone. The coupons were then dried in an oven at a temperature of 80°C for 20 minutes and allowed to cool in a desiccator. The average mass of the prepared corrosion coupons ranged from 13.27-13.82g. A total of sixteen corrosion coupons were prepared and used for the study. The method used in preparing the corrosion coupons is consistent with protocols in the literature (Amadi, 2015; Avwiri & Tay, 1999).

2.2.2 Water Sample Collection

The freshwater sample was collected from a stream in Port Harcourt, Nigeria. The freshwater sample was collected in a glass vial, pre-treated with 0.1M dilute hydrochloric acid and air-dried. The freshwater sample was collected at a depth of about 1.0 m. The choice of 1.0 m was based on the fact that the variability of the physicochemical and bacteriological properties of the freshwater at such depths is negligible (Puyate & Rim-Rukeh, 2007; Amadi, 2015).

The sample for microbial analysis was collected in a sterilized McCartney glass bottle and stored in a cooler containing ice packs while other properties including temperature, pH, TDS, and DO were determined in situ with a multiparameter water quality monitor. The freshwater sample was then transported immediately to the laboratory for analysis.

2.2.3 Isolation and Identification of *Pseudomonas sp.* from Freshwater Sample

The bacteriological analysis of the freshwater sample involved enumeration and isolation of aerobic bacteria in the freshwater. The serial dilution method was used for the enumeration and isolation of *Pseudomonas sp.* in freshwater. In this method, 1 ml of freshwater was transferred to 9 ml of sterile normal saline as a diluent in a test tube. Then 1 ml of the mixture was transferred into another 9 ml diluent and mixed properly. This tenfold serial dilution continued until the required dilution was obtained. Aliquots of 0.1 ml of the appropriate dilution of water samples were spread on a plate, using a sterile glass spreader over the surface of sterile dried nutrient agar in petri dishes. The nutrient agar is composed of 10.0 g peptone, 10.0 g meat extract, 10.0 g sodium chloride, 15.0 g agar, and 1 L of distilled water with a pH of 7.0 (APIRP, 1975). The inoculated plates were incubated at 37°C for 24 hours. The bacterial colonies that developed were counted and expressed as colony-forming units per ml (CFU/ml). Suspected colonies were subcultured onto fresh sterile nutrient agar and used for the characterization identification and of Pseudomonas sp. Standard characterization tests including gram staining, motility, methyl-red, Vogues Proskaver, indole, citrate utilization, and sugar fermentation were adopted. The Pure culture was identified based on its cultural, morphological, and physiological characteristics recommended in several pieces of literature.

2.2.4 Bacterial Culture and Inoculum Preparations

The bacterial inoculum for the studies was grown in a complex medium of nutrient broth. The bacterial culture was done by inoculating the *Pseudomonas sp.* isolated from the freshwater into 500 ml of nutrient broth medium (APIRP, 1975).

2.2.5 Biocorrosion of Mild Steel in Media

Ten liters of the freshwater sample were divided into two equal parts and put into two bowls, labelled Bowl 1, and Bowl 2. The culture of Pseudomonas sp. in the nutrient broth was added into Bowl 1 and 5 ml of the prepared broth culture medium was added into Bowl 1 on a weekly basis throughout the period of the experiments. No bacterial culture medium was added to Bowl 2, which was used as the control. Eight prepared coupons were put into Bowls 1 and 2 with the help of polypropylene rope tied at the edge of the perforated coupons. At every 720hour (30 days) interval, a corrosion coupon was retrieved from both Bowl 1 and Bowl 2. The retrieved coupon in each case was carefully washed off with de-mineralized water, cleaned,





dried, and weighed. This process was repeated until all the corrosion coupons in Bowls 1 and 2 were exhausted. The experiments lasted for a total period of 5,760 hours (8 months).

The freshwater samples of Bowls 1 and 2 were regularly tested in situ using a multi-parameter water quality monitor and the readings were noted, especially on each day of retrieving the coupons and the total bacterial count determined for each of the experimental setups. The properties assessed include temperature, pH, TDS, and DO.

2.3 Determination of Corrosion Rate of the Mild Steel

The corrosion rate of mild steel was calculated using Equation 1 (Amadi and Wami, 2009):

Corrotion Rate (MPY) =
$$\frac{\Delta M \times 3.45 \times 10^6}{A \times \rho \times t}$$
 (1)

Where, ΔM = Weight loss (g); 3.45 x 10⁶ is the corrosion rate constant in Mils Per Year (MPY); A = The surface area of the exposed coupon (cm²); ρ = The metal density in g/cm³; and T = Time taken for the loss of the metal (hrs.).

3. RESULTS AND DISCUSSION

3.1 Physicochemical and Bacteriological Characteristics of the freshwater sample

Tables 1 and 2 show the results of the physicochemical and bacteriological properties of the freshwater samples on each day coupons were retrieved from the inoculated and uninoculated media in Bowls 1 and 2, respectively. The temperature in Bowl 1 was in the range of 26.90 to 28.50°C; while in Bowl 2, the temperature was in the range of 26.90 to 28.70°C as shown in Tables 1 and 2, respectively. The temperature values in both reactors are in the range that is suitable for bacterial growth Though, (Amadi, 2015). the optimum temperature for bacterial growth lies between 25 - 30°C (Amadi et al., 2010). The pH in Bowl 1 had values in the range of 5.76 to 6.65; while in

Bowl 2, the value was in the range of 6.65 to 6.87 (see also Tables 1 and 2). Both Bowls' results showed that the pH of the two media was slightly acidic. The acidity was more pronounced in Bowl 1 than in Bowl 2. The TDS values in Bowl 1 were in the range of 50.20 to 170.10 mg/l (Table 1); while in Bowl 2 the values were in the range of 76.30 to 170.10 mg/l (Table 2). The values of DO in Bowl 1 as seen in Table 1 varied between 2.84 to 4.50 mg/l; while in Bowl 2, the values were in the range of 3.90 to 4.50 mg/l as shown in Table 2.

Table 1: Physicochemical and BacteriologicalProperties of Inoculated Medium in Bowl 1.

Time (Hrs.)	Temp (⁰ C)	pН	TDS (mg/l)	DO (mg/l)	TBC (CFU/ml
0.00	26.90	6.65	170.10	4.50	1.2x10 ⁵
720	27.70	6.50	106.50	4.42	1.6×10^{6}
1440	27.80	6.50	97.10	4.10	2.3×10^{5}
2160	28.20	6.30	84.60	3.96	2.6×10^7
2880	28.10	6.25	75.40	3.72	1.4×10^{6}
3600	27.80	6.27	64.95	3.21	2.1×10^{5}
4320	27.00	6.20	62.00	3.03	1.7×10^{4}
5040	27.40	5.94	55.80	2.97	1.6×10^4
5760	28.50	5.76	50.20	2.84	1.5×10^4

CFU, Colony Forming Units; DO, Dissolved Oxygen; TBC, Total Bacterial Count; TDS, Total Dissolved Solid

Table 2: Physicochemical and BacteriologicalProperties of Uninoculated Medium in Bowl2.

Time (Hrs.)	Temp (⁰ C)	рН	TDS (mg/l)	DO (mg/l)	TBC (CFU/ml)
0.00	26.90	6.65	170.10	4.50	1.2×10^{5}
720	27.40	6.67	116.70	4.48	4.8×10^4
1440	27.60	6.70	104.60	4.37	4.5×10^{3}
2160	28.00	6.72	95.80	4.30	3.7×10^{3}
2880	27.50	6.78	91.20	4.15	3.6×10^3
3600	28.70	6.80	89.70	4.05	$3.2x10^{3}$
4320	28.40	6.83	85.40	4.00	3.1×10^{3}
5040	28.10	6.85	80.50	3.95	< x30
5760	27.80	6.87	76.30	3.90	< x30

CFU, Colony Forming Units; DO, Dissolved Oxygen; TBC, Total Bacterial Count; TDS, Total Dissolved Solid





3.2 Variation in the Physicochemical and Bacteriological Characteristics of the Freshwater Sample with Time

3.2.1 Variation in Temperature

Figure 1 shows the variation of temperature in Bowls 1 and 2 with time. From Figure 1, it is seen that the effect of temperature on the biocorrosion of mild steel in both experimental setups was the same; hence, the effect of temperature as a contributing factor is neglected.





3.2.2 Variation in pH

Figure 2 shows the variation in pH in Bowls 1 and 2 with time. As stated, the acidity was more pronounced in Bowl 1 than in Bowl 2. The acidity increased more in Bowl 1 than in Bowl 2. The increase in acidity in Bowl 1 could be attributable to the metabolic and microbial activities of Pseudomonas sp. Pseudomonas sp., which was dominant in the medium has the biochemical characteristics of converting glucose and other sugars in the medium to acid (Buchanan & Gibbons, 1994). Bowl 2 acidity was tending towards neutrality because the activities of the micro-organisms in the medium were decreased due to the death of the microorganisms as the nutrients in the Bowl 2 were used up at the beginning of the experiment.



Figure 2: Variation in pH of the Freshwater in Bowls 1 and 2 with Time.

3.2.3 Variation in Total Dissolved Solids (TDS)

Figure 3 shows the variation in total dissolved solids (TDS) in Bowl 1 and Bowl 2. Generally, the TDS decreased at a faster rate within the first 1000 hours and then more slowly afterwards in the two Bowls. The faster decrease in the value of TDS in Bowl 1 is attributable to the depletion of nutrients as the nutrients were used up by the bacteria (*Pseudomonas*) in the medium in their metabolic and microbial activities.



Figure 3: Variation of Total Dissolved Solids with Time

3.2.4 Variation in Dissolved Oxygen (DO)

Figure 4 shows the variation in DO in Bowl 1 and Bowl 2. From Figure 4, it can be observed that the rate of DO consumption in Bowl 1 is higher

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as the experiment progressed. This drastic reduction in dissolved oxygen values in Bowl 1 may be attributable to the presence of *Pseudomonas* organisms, which utilize oxygen as substrate. Similar observations have been made on the rate of oxygen consumption by immobilized nitrate oxidizing bacteria *Nitrobacteria agilis* (Picioreanu *et al.*, 1998) and iron oxidizing bacteria *Leptothrix Discophora* in water environments (Rim-Rukeh, 2007; Puyate *et al.*, 2009).

3.2.5 Variation in Total Bacterial Count (TBC)

Figure 5 illustrates the variation of TBC with time in the two experimental setups. At the beginning of the experiment, the total bacterial counts in both Bowls were the same $(1.2 \times 10^5 \text{ CFU/ml})$. In Bowl 1, there was an observed increase in total bacterial count from 10^5 to 10^7 CFU/ml within 2160 hours of the experiment and which then started reducing from 10^7 after 2160 hours to 10^4 at 5760 hours of the experiment. The observation in Bowl 1 may be attributed to the transformation of initial planktonic cells to sessile cells despite the continuous supply of nutrients (Melchers, 2007). The drastic reduction of total bacterial count in Bowl 2 is due to the depletion of nutrients in the medium.



Figure 4: Variation of Dissolved Oxygen with Time



Figure 5: Variation in Total Bacterial Count (TBC) with Time

3.3 Weight Loss and Corrosion Rate of Mild Steel

Tables 3 and 4 show the results of weight loss and corrosion rate of mild steel immersed in Bowls 1 and 2 in the laboratory experiments, respectively.

Table	3:	Corrosion	Rate	Results	for
inocula	ted 1	medium (Bov	vl 1)		

Time (Hrs.)	Initial Mass(g)	Final Mass(g)	ΔM (g)	Corrosion Rate (MPY)
0.00	0.00	0.00	0.00	0.00
720	13.62	13.57	0.05	0.83
1440	13.61	13.52	0.09	0.75
2160	13.50	13.30	0.20	1.12
2880	13.56	13.24	0.32	1.33
3600	13.36	12.94	0.42	1.42
4320	13.27	12.74	0.53	1.51
5040	13.29	12.64	0.65	1.58
5760	13.30	12.44	0.86	1.83

MPY, Mils Per Year





Table	4:	Corr	osion	Rate	Results	for
uninoc	ulate	ed med	ium (I	Bowl 2)		
			-		~ .	

Time	Initial	Final	ΔM	Corrosion
(Hrs.)	Mass(g)	Mass(g)	(g)	Rate (MPY)
0.00	0.00	0.00	0.00	0.00
720	13.53	13.51	0.02	0.33
1440	13.82	13.77	0.05	0.41
2160	13.41	13.32	0-09	0.51
2880	13.30	13.17	0.13	0.55
3600	13.55	13.36	0.19	0.63]
4320	13.39	13.15	0.24	0.68
5040	13.71	13.43	0.28	0.66
5760	13.46	13.09	0.37	0.78

MPY, Mils Per Year

3.3.1 Variation in Weight Loss of Mild Steel with Time

Figure 6 illustrates the weight loss of coupons in Bowl 1 and Bowl 2 with time. Figure 6 shows an increase in weight loss of the coupons in both experimental setups with time. Weight loss was more pronounced in the inoculated medium than in the uninoculated medium. The corrosive aggressiveness in Bowl 1 may be due to the noticeable presence of *Pseudomonas* bacteria.



Figure 6: Plot of Weight-loss vs. Time

3.3.2 Variation in Corrosion Rate of Mild Steel with Time

Figure 7 illustrates the variation in corrosion rates of mild steel in Bowl 1 and Bowl 2 with time. Comparing the corrosion rates of coupons in Bowls 1 and 2, it is observed that the corrosion rates of coupons immersed in Bowl 1 were higher than those in Bowl 2. The higher corrosion rate in Bowl 1 was due to the presence of Pseudomonas sp., which enhanced corrosion rates by about 73% when compared with the corrosion rate in Bowl 2. This result is supported by earlier observations by Amadi (2015), Amadi et al. (2010), Rim-Rukeh (2007) that the most direct effect of microbiologically influenced corrosion is the increase in corrosion rate. Costerton (1995) observed that the presence of microorganisms could cause the corrosion rate to increase by about 1000 - 100,000 times or higher than in the absence of microorganisms. After a period of 720 hours of immersion, mosaic deposits of rusty materials (tubercles) were observed on the surfaces of the coupons retrieved from Bowl 1. Physical adsorption of the rusty materials/biofilm (tuberculation) of microbial cells on the surfaces of the coupons after 5760 hours of immersion increased tremendously as seen in Figure 7. On the other hand, the coupons retrieved from Bowl 2 showed no adsorption of rusty materials/biofilms on its surface after 5760 hours as seen in Figure 7. The key feature for the enhancement of corrosion in the present analysis was the alteration of the material surfaces by the presence deposits caused of rusty by Pseudomonas the sp. In presence of Pseudomonas sp., the formation of the insoluble ferric (Fe³⁺) coating on the surface of the metal was prevented, hence, the increase in the corrosion rate of mild steel in Bowl 1 than in Bowl 2. The simultaneous production of Fe^{2+} and S^{2} will undoubtedly add to the corrosiveness of the environment. Sheng et al. (2012) and Jeon et al. (2001) reported on the influence of Fe^{2+} on the corrosion of mild steel. High concentration of Fe^{2+} in cultures of *desulfovibrio sp.* prevented the formation of a protective FeS coating on the coupon thereby increasing the corrosion rate of mild steel. This is the most outstanding way through which Pseudomonas sp. accelerate the corrosion rate of metals (Amadi, 2015).







Figure 7: Plot of Corrosion Rate vs. Time

4.0 CONCLUSION

This study has revealed that the influence of microorganisms is significant in the corrosion process and results in about a 73% increase in the corrosion rate of mild steel. However, it is also shown that a metal immersed in freshwater will always corrode with or without the presence of microorganisms as seen in the case of the control experiment. The findings of the present study have provided data that could be used to mitigate corrosion problems caused by the activities of microorganisms.

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