Effect of Bacteria Diversity and Succession on Microbiologically Induced Corrosion of Steel in a Freshwater Sediment

Ononiwu Prosper Ikechukwu[1], Tudararo-Aherobo Laurelta E[2].

[1,2]Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Nigeria.

Corresponding Email: prince3iyke75@gmail.com

Abstract— *This study evaluated the influence of freshwater sediment on the corrosion rates of carbon steel and the indigenous bacterial groups and diversity during growth and biofilm formation on carbon steel coupons embedded in freshwater sediment in anaerobic conditions. The diversity evaluation was done using 16S rRNA sequencing method over time and their associated corrosion rate was determined quarterly of inserted coupon using weight loss method. The overall trend of corrosion rates was similar across three quarterly intervals, meanwhile, an initial increase in the first quarter, a sharp decline in the second, and a sharp increase in the third for unsterilized samples was observed while the sterilized samples (Control) showed a slight decrease in corrosion rate. Actinobacteriota and Firmicutes dominated the biofilms 16SrRNA gene sequence at all the experimental quarterly intervals while at all the intervals about 4% was affiliated to the Unknown category of the phylum taxa. Firmicutes were the most diverse and dominant surface-colonizing species detected on 16S rRNA analysis with Clostridium spp having the highest percentage abundance (more than 15%). Proteobacteria, Desulfobacteriota, Acidobacteriota, Bacteroidota, Deferrisomatota, Chloroflexi, Latescibacterota and SAR324_clade were among the phyla of bacterial that increased in abundance, some of which can metabolise Iron (Fe) and sulphur. The results describe differences in microbial diversity and abundance over time, highlighting certain bacterial species that persisted for most of the experiment, through a competitive complex association between bacteria and metal surfaces, which contributed to the development and maintenance of favourable conditions that accelerate corrosion processes. The results show thoughtful changes in bacterial diversity over time in the presence of steel that may relate to their capacity to interact with Fe to cause corrosion. The detection of an Unknown bacterial category at phylum and species level in the biofilm obtained poses a critical problem in the study of microbial induced corrosion (MIC) of metals in any environment owing to their unknown metabolic roles in the corrosion process. Hence, we can not totally relay on culture-dependent methods in the study of MIC in an environment.*

Keywords— *Bacterial diversity: Biocorrosion: Biofilms: Corrosion rate: Environment, weight loss.*

I. INTRODUCTION

Corrosion is both electro-chemical and biogeochemical natural occurring process powered by physical, chemical or biological processes working together. Environmental factors around the metal are the most common causes of physicochemical corrosion. Factors like characteristics of the metal, pH, temperature, salinity, chloride and SO_2 deposition rates, humidity and length of exposure are key triggers of physical and chemical corrosion in metal installations [1,2]. Biocorrosion, also known as microbially influenced corrosion (MIC), is the permanent deterioration of metal by the activities of microorganisms. MIC results from the complex interactions of microbial cells, their cellular metabolites, the metal surface and environmental factors [1]. Different groups of microorganisms often adhere to surfaces by means of biofilms; MIC develops beneath these biofilms via mechanisms such as direct electron transfer, cathodic depolarization, build-up of a concentration gradient or galvanic cell formation [1,3]. Microbial participation has been reported to speed up corrosion rates by up to ten times [4].

The oil and gas and marine installations uses steels for the construction of platforms and transportation infrastructure for the transport of petroleum and water amongst other uses. Most pipelines are constructed with carbon steel. Although highly susceptible to corrosion and attack by microbes, carbon steel is preferred as it yields easily to welding, endures bending well

and is less liable to cracking under stress. Also, it is regarded as durable and of relatively low cost. Mild steel and stainless steel, though not as popular as carbon steel, are also used. Stainless steel is considered expensive and less malleable [1]. Steel corrosion is widely recognized as a prominent concern that results in huge economic deficits in different areas of productive sectors. The metal deterioration generates damage that affects various sectors of industry, causing huge losses to the world economy and numerous problems for countries [5]. Studies establishes a 4% and 20% reduction in national Gross Domestic Product (GDP) in developing and developed countries respectively due to corrosion in industry [6,7,1]. Arena-Ortiz *et al.* [8] fixed the global corrosion cost including management measures at $4 trillion.

Currently, the participation of microorganisms in the corrosion process has been mostly reported in the scientific literature as a process denominated by Microbiologically Influenced Corrosion (MIC) [5]. Although the functional features of different groups of the MIC community are generally studied, little is known about the succession of colonizing species over time, nor the complex relationships between these microbes during the formation of biofilm involved in the corrosion process. Recent studies have shown that during in situ incubations of mild steel, iron oxidizing bacteria (IOB) contribute to the initial colonization, taking an earliest role in biofilm formation and are followed by a change in diversity of the surface microbial population, together with

the further development of SRB populations. However, IOB on steel surfaces could serve as substrates for further colonization by diverse microbial populations, including anaerobes such as sulfate-reducing bacteria, which results in accelerated MIC population [5,9,10,11]. SRB and iron bacteria often co-exist on buried metal infrastructure and tend to exert a more aggressive corrosive effect as a team than as individual colonisers of the metal surface [12].

Bacteria can generate a condition that accelerates corrosion through alteration of pH and redox potential, corrosive metabolites secretion, direct or indirect enzymatic reduction or oxidation of corrosion products or cathodic depolarization through H_2 metabolism [13]. It is reported that corrosion by microorganisms can accelerate the process rates through surface colonization and subsequent biofilm establishment which provides several advantages to these organisms and favours critical ecological functions in the changing environment [10]. Corrosion of carbon steel was reported to be about 6 times greater when SRB are involved with the corrosion pit depth 7.7 times deeper than without SRB [14].

Among two microbial corrosion conditions are recognized, aerobic and anaerobic processes, anaerobic corrosion has been given more attention frequently linked to the activities of sulphate-reducing bacteria (SRB) and thiosulfate reducing bacteria (TRB) [15]. Aerobic corrosion is characterized by an electrochemical process in which a transfer of electrons occurs by metal oxidation process at the anode with concurrent reduction at the cathode, usually oxygen. These electroactive bacteria are able to accept/give electrons from/to an electrode, an insoluble substrate of particles, macroscopic conductive surfaces, electrodes, and are As a result, it is capable of generating a small amount of electricity [5, 15]. The mechanism employed by bacteria present in electroactive biofilms for capture and utilization of energy from environmental sources involves electron flows from negative to more positive potentials. These biofilms are composed of several families of bacteria and even fungi, yeast and algae [16, 17].

Six classes of bacteria; Sulphate Reducing Bacteria (SRB), Acid Producing Bacteria (APB), Sulphate Oxidizing Bacteria (SOB), Iron Reducing Bacteria (IRB), Iron Oxidizing Bacteria (IOB), Manganese Oxidizing Bacteria were targeted and recorded based on literature sources as the main microbial groups involved in MIC [18,19,20,21,22,23,24,25]. Khouzani et al. [26] discourse that SRB are the main causes in severe biodeterioration and rupture of buried pipelines. SRB are anaerobic but can exist underneath deposits of soil, sediment or rust on the pipeline or even beneath an already formed biofilm where oxygen is present, thereby creating the oxygen-deficient microenvironment they require [14].

On freshwater sediment, the most diverse and significant microorganisms are the bacteria and are usually known as early colonizers on metal surfaces. In this study, indigenous bacterial groups and diversity during growth and biofilm formation on carbon steel coupons embedded in freshwater sediment in anaerobic conditions was evaluated. The diversity evaluation was done by 16S rRNA sequencing over time and their associated corrosion rate was also determined.

II. MATERIALS AND METHODS

A. Study Area Description and Sample Collection

The Freshwater sediment was collected from a point in the Okpare-Olomu River (05º29'32"N, 05⁰54'10"E), situated within the Olomu clan of Ughelli South Local Government Area, Delta State, Nigeria. The freshwater is a vital natural water resource. Flowing through multiple communities, including Otu-Jeremi and Okwagbe, it finally joins the Forcados River. The sediments samples were collected using grabbers into sterile polyethylene bag. The physiochemical characteristics of the sample were determined according to Ryan [27].

B. Experimental Design

The experimental set-up and controls were prepared in duplicates. To simulate the microbial colonization process of a metallic pile structure surface, three prepared and pre-weighed Carbon steel coupons were immersed into 1kg Freshwater sediment sample (FWSS) collected. 1kg of freshwater sediment was sterilized and silver nitrate was added to inhibit microbial growth. A known volume of the sterile water sample from the freshwater environment (500ml) was introduced into the sterile sediment samples and labelled, FWSSC. Metal coupons, three (3) each were inserted into the control, FWSSC.

The whole experimental setup was tightened with lids and kept at room temperature at static incubation for a research period of three (3) quarters in the year. At end of each quarter, a coupon was brought out, scraped for biofilm recovery and further analyses, cleaned and final weights taken. One coupon is removed at 3 months interval of exposure for a period of 12months.

C. Metal Coupons preparation

Carbon steel coupons used in this study were purchased and had dimensions of 12.5cm × 1.4cm × 0.1cm. The metal coupons were prepared according to Wu et al. [28]. Each metal coupon was washed with a brush in distilled water, degreased with ethanol, and dried with acetone. All the carbon steel coupons were kept in a desiccator before the measurement. The coupons were sterilized in dry form rapped with aluminium foil paper. The initial weight and final weight were taken before use and after use respectively. The coupons were also labelled, and initial weights were noted. The exposed surface area of each coupon is 16.95 cm^2.

D. Corrosion Rate by Weight Loss Method

Corrosion rates of the biofilms formed at varying periods in the sample were determined by weight loss method. Each labelled coupon was immersed in triplicates at once in the samples labelled in the experimental set-up for the period of the research at static incubation as one coupon per time was removed at 3month interval of exposure for further analyses. The sample labelled FWSSC was sterilized (without bacterial growth) sample from the same location and serve as Control for the test samples. FWSSC in Figure 1 records the corrosion rates of metal coupons without bacterial presence, thus showing the roles of the physical and chemical features of the environment in the corrosion activities on the metal coupons. While the unsterilized environmental sample; FWSS records the

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corrosion rates of metal coupons with the bacterial biofilm formation on the surface of the metal coupons. The coupons were scraped and cleaned using alcohol and acetone. They were dried in airtight desiccators. Final weights were taken, and weight loss was determined by subtracting initial weights from the final weights after corrosion. Corrosion rate (CR) was measured by assuming uniform corrosion over the entire surface of the specimens. Corrosion rate determination in a specific environment is of fundamental importance in corrosion engineering [29]. The corrosion rate in millimetre per year (mm/y) was determined from the weight loss method, using this formula below [29].

$$CR = \frac{W}{D \times A \times t} \times K$$

Where, W = weight loss in grams, K = Constant (8.76×10^4), D = Metal density in (g/cm^3), A = Surface area (cm^2), t = Time (hrs)

All experiments were carried out in triplicates and the standard deviation was calculated using the software Microsoft Excel 2010.

E. Biofilm Preparation and Bacteria DNA Extraction

This experiment was conducted to examine the temporal succession and diversity of bacterial species during the initiation of corrosion on steel coupons. The microbial diversity at 1st, 2nd and 3rd quarters was studied. In each quarter, one coupon of the setup was removed, and the biofilm aggregation was removed by scraping and re-suspended in 3 mL of phosphate-buffered saline (PBS) in a sterile tube. These samples were sent to Inqaba Biotec West Africa Ltd (Ibadan, Nigeria) for DNA extraction and sequencing. Genomic DNA was extracted from the samples received using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Catalogue No. D4300).

F. PCR Of 16S rRNA Gene Amplification, Library Construction and Sequencing

Using a universal primer pair 27F and 1492R, which target the V1–V9 region of the bacterial 16S rRNA gene, genomic DNA samples were PCR amplified. The resulting amplicons were barcoded using Pacbio M13 barcodes in preparation for multiplexing using a few PCR cycles. The generated barcoded amplicons were quantified and combined in equal amounts, and an AMPure PB bead-based purification procedure was carried out. The combined amplicons were used to make the PacBio SMRTbell library in accordance with the manufacturing procedure. The reports contain the summarized metagenomic analysis of full length 16s gene amplicons. Samples were sequenced on the Sequel IIe system by PacBio (www.pacb.com). Raw sub-reads were processed through the SMRTlink (v11.0) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were then processed through vsearch (https://github.com/torognes/vsearch) and taxonomic information was determined based on QIMME2.

III. RESULTS

A. Physicochemical Characteristics of the sample

The physical and chemical characteristics of the freshwater sediment sample (FWSS) collected from Okpare-Olomu river is presented in Table I.

TABLE I: The Physicochemical and Heavy Metal features of sediment sample

Parameters	Okpare-Olomu River (FWSS)
pH	6.00±0.01
Temperature (℃)	28.30±1.10
E. Conductivity (µs/cm)	120.00±4.00
Redox Potential (mV)	42.34±2.12
CO_3 (mg/kg)	0.24±0.02
Chloride as Salinity (mg/kg)	55.83±1.04
Nitrate (mg/kg)	0.73±0.06
Nitrite (mg/kg)	<0.01
Phosphate (mg/kg)	0.97±0.11
Moisture Content (%)	15.61±0.04
Total Nitrogen (mg/l)	0.05±0.01
THC (mg/kg)	2510±9.83
TPH (mg/kg)	2112±12.00
TOC (mg/kg)	0.84±0.02
Lead, Pb (mg/kg)	11.20±0.96
Zinc, Zn (mg/kg)	84.10±1.40
Iron, Fe+ (mg/kg)	5660±15.00
Nickel, Ni (mg/kg)	10.02±1.01
Chromium (mg/kg)	8.90±0.50
Sodium, Na (mg/kg)	8.90±1.20
Silver, Ag (mg/kg)	<0.001
Cadmium (mg/kg)	0.23±0.03
Mercury, Hg (mg/kg)	<0.001
Arsenic, As (mg/kg)	<0.001
Magnesium, Mg (mg/kg)	18.30±1.15
Manganese, Mn (mg/kg)	0.03±0.01
Copper, Cu (mg/kg)	21.20±0.60
Cobalt (mg/kg)	<0.001

B. Corrosion Rates

Corrosion potentials of the presence of bacterial biofilms on the metal coupons inserted in the FWSS and FWSSC samples, are presented in Figure 1. In the first quarter of the metal coupon insertion, for the unsterilized samples, FWSS had the highest corrosion rate. The overall trend of corrosion rates was similar across three quarterly intervals, an initial increase in the first quarter (3.33mpy), a sharp decline in the second (2.16mpy), and a further increase (1.59mpy) in the third for unsterilized samples. Sterilized samples showed a slight decrease in corrosion rate. This suggests that both physicochemical properties and bacterial biofilms contribute to corrosion, with the latter potentially accelerating it or changing its dynamics over time.

C. Bacterial Phyla Relative Abundance

For FWSS, the obtained 16S rRNA gene sequences showed similarity to thirty-six (36) known bacteria species and an Unknown category of species within fourteen (14) known phyla of bacteria: *Actinobacteriota, Acidobacteriota, Firmicutes, Deferrisomatota, Desulfobacterota, Omnitrophota, Proteobacteria, Myxococcota, Bacteroidota, SAR324_clade, Campylobacterota, Chloroflexi, Caldisericaeota, Latescibacterota* and an Unknown category (Figure 2). The result showed that about 35% of all the species in the second experimental interval were affiliated to Firmicutes, 14% and 11% to *Actinobacteriota* and *Desulfobacterota* respectively, while at all the intervals about 4% was affiliated to the

Unknown category. *Actinobacteriota* and *Firmicutes* dominated in the biofilms 16SrRNA gene sequence at all the experimental intervals.
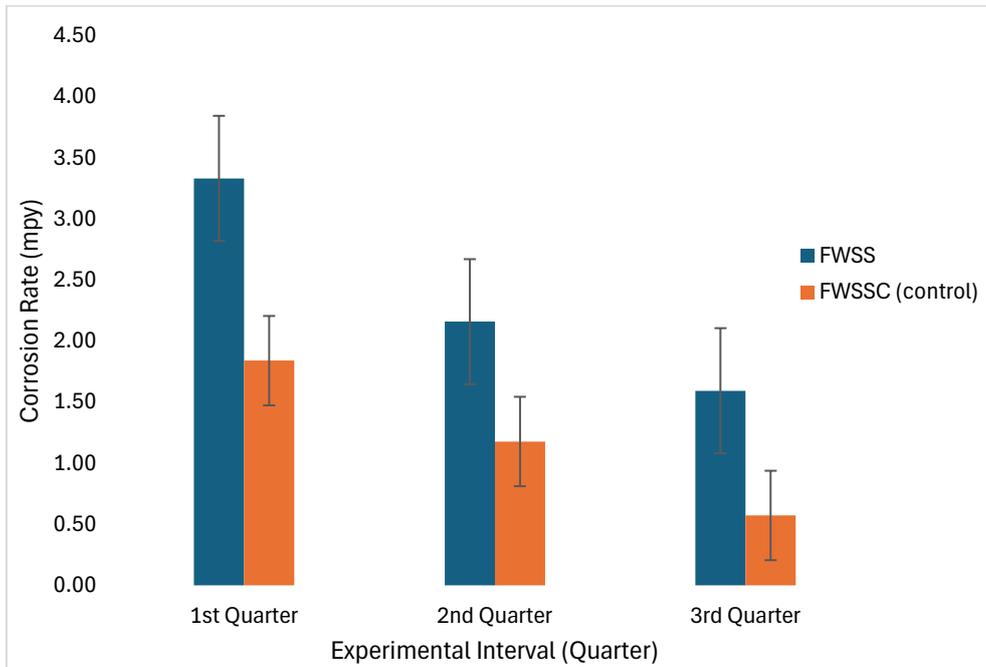

Figure 1. Corrosion rates of metal coupons submerged in freshwater sediment over 3 quarters of the year.
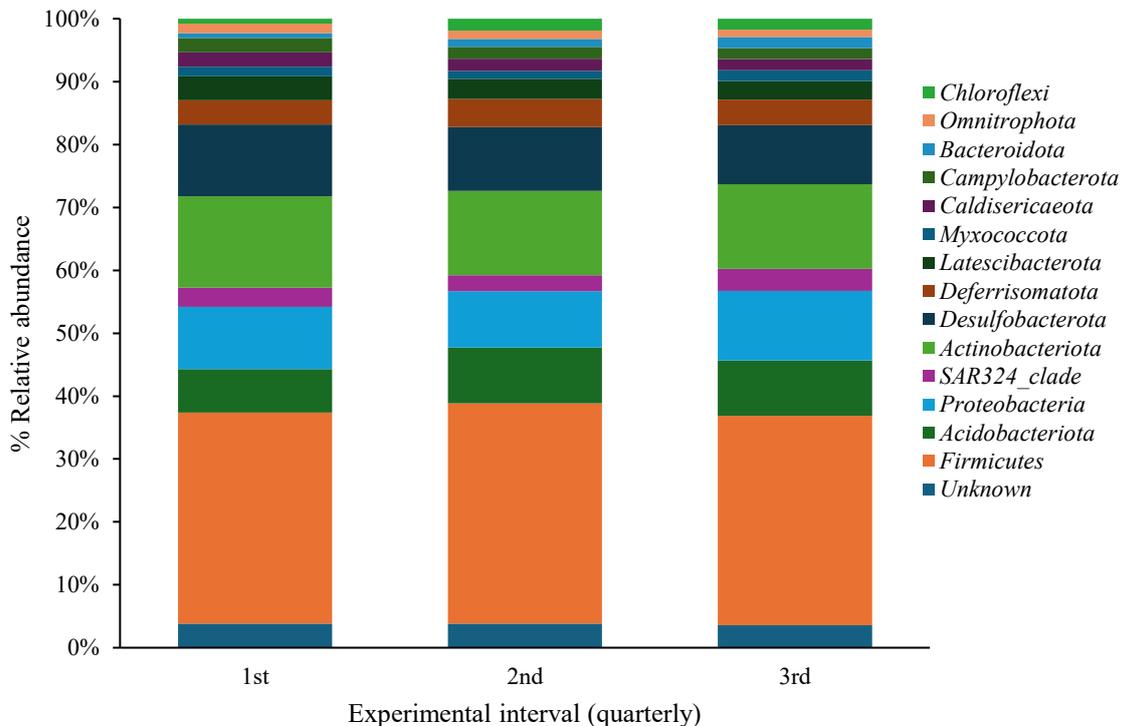

Figure 2. % Relative abundance of 16SrRNA reads matching from bacteria phyla detected on metal coupons throughout analysed periods. The y-axis describes in percentage the relative abundance of 16S genes belonging to different phyla throughout the experiment in FWSS

D. Bacterial Diversity and Succession Analysis

In the analysis of the microbial successions assessment in metal coupon biofilm from FWSS after the first quarter of metal coupon insertion, 131 sequence reads were obtained from which thirty-three (33) known bacteria species and an Unknown category of species were characterized within

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fourteen (14) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, Caldisericaeota, Omnitrophota, Myxococcota, Latescibacterota, SAR324_clade* and *an Unknown category*. The *Firmicutes* were the most dominated phylum in FWSS with more than 33% sequence reads containing eight (8) species distributed in three families, *Bacillaceae Clostridiaceae* and *Alicyclobacillaceae.* Four species from the *Firmicutes* dominated the sequence reads; *Clostridium_sensu_stricto_2_Clostridium_frigidicarnis* with about 7% representativeness, *Bacillus_acidiceler* (about 6%), *Clostridium_sensu_stricto_13_Clostridium_argentinense* (more than 5%) and *Clostridium_sensu_stricto_12_Clostridium_magnum* with more than about 5% sequence reads. *Acidothermus_uncultured_bacterium* from the

Actinobacteriota phylum, *Desulfomonile_uncultured_bacterium* and *Desulfovibrio_uncultured_bacterium* from the *Desulfobacteriota* phylum, and *Sulfuriferula_uncultured_bacterium* from the *Proteobacteria* phylum read more than 4% each rendering them the most abundant in their respective phylum population. The *Unknown category*, recorded about 4% reads, *Candidatus_Solibacter_uncultured_bacterium* from the *Acidobacteriota* phylum, *OPB41_uncultured_Coriobacteriaceae* from the *Actinobacteriota* phylum, *Deferrisoma_uncultured_delta* from the *Deferrisomatota* phylum, *Latescibacterota_uncultured_bacterium* from the *Latescibacterota* phylum also recorded about 4% representativeness each in the sequence reads (Figure 3).
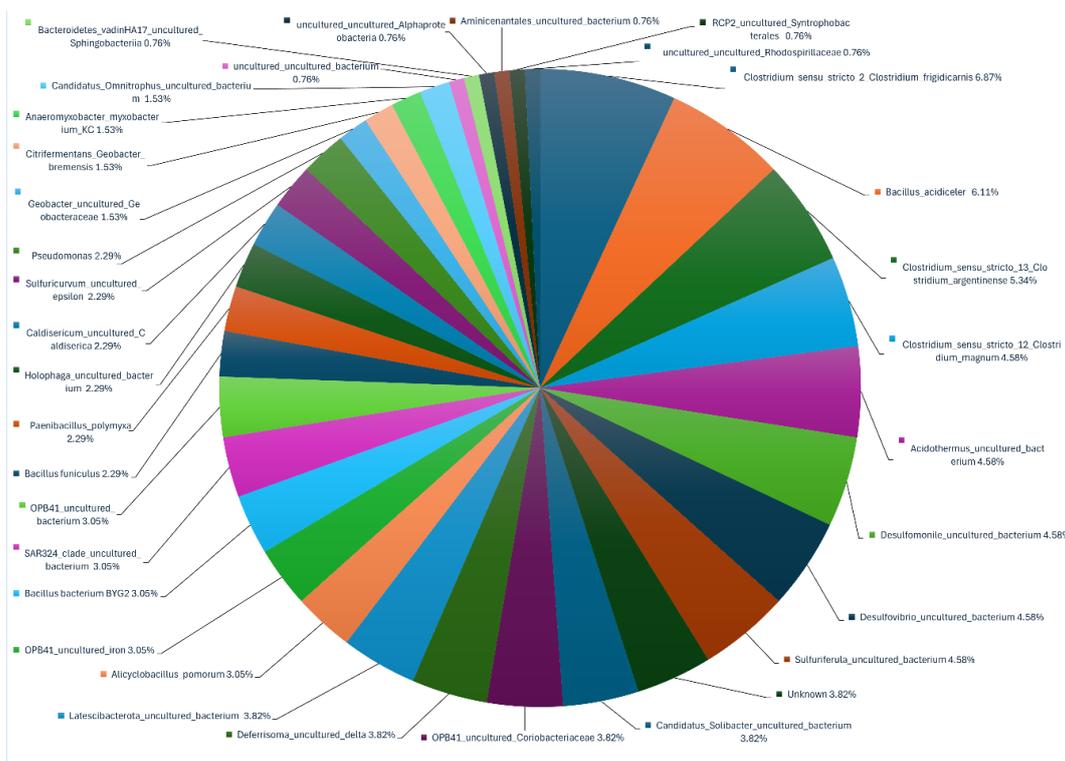

Figure 3. Overview of abundance of 16S rRNA bacterial species after first quarter from metal coupon biofilms in FWSS

In the analysis of the microbial successions assessment in metal coupon biofilm from FWSS after the second quarter of metal coupon insertion, 157 sequence reads were obtained from which thirty-six (36) known bacteria species and an Unknown category of species were characterized within fourteen (14) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, Caldisericaeota, Omnitrophota, Myxococcota, Latescibacterota, SAR324_clade* and *an Unknown category*. The *Firmicutes* still were the most dominated phylum in FWSS with about 35% sequence reads containing eight (8) species distributed in three families, *Bacillaceae Clostridiaceae* and *Alicyclobacillaceae.* Four species from the *Firmicutes*

dominated the sequence reads; *Clostridium_sensu_stricto_2_Clostridium_frigidicarnis* with more than 6% sequence reads, *Bacillus_acidiceler* and *Clostridium_sensu_stricto_13_Clostridium_argentinense* with about 6%, also *Clostridium_sensu_stricto_12_Clostridium_magnum* and *Candidatus_Solibacter_uncultured_bacterium* from the *Acidobacteriota* phylum with more than about 5% sequence reads each. *Deferrisoma_uncultured_delta* recorded more than 4% reads, *Acidothermus_uncultured_bacterium* and *OPB41_uncultured_Coriobacteriaceae* from the *Actinobacteriota* phylum, *Desulfomonile_uncultured_bacterium* and *Desulfovibrio_uncultured_bacterium* from the

Desulfobacteriota phylum, and *Sulfuriferula_uncultured_bacterium* from the *Proteobacteria* phylum read about 4% each rendering them the most abundant in their respective phylum population. The *Unknown category,* recorded about 4% reads. *Latescibacterota_uncultured_bacterium* from the *Latescibacterota* phylum, *Bacillus funiculus* and *Pseudomonas spp* also recorded more than 3% representativeness each in the sequence reads. *Sphingobacterium_griseoflavum* from the *Bacteroidota* phylum, *Conexibacter_woesei* from the *Actinobacteriota* phylum, *Leptolinea_uncultured_Chloroflexi*

from the *Chloroflexi* phylum were detected for the first time with 0.64% representativeness each in the FWSS metal coupon biofilm sequence read (Figure 4). This category classified as "Unknown" both at the phylum and species level, signifies a substantial portion of the microbial community whose specific generic identity could not be determined. This is a critical finding in the metal corrosion biofilm metagenomics, as it means that some of the microbial community cannot be assigned to any known or even named uncultured genus. It strongly suggests that the environment harbors a significant proportion of truly novel bacteria.
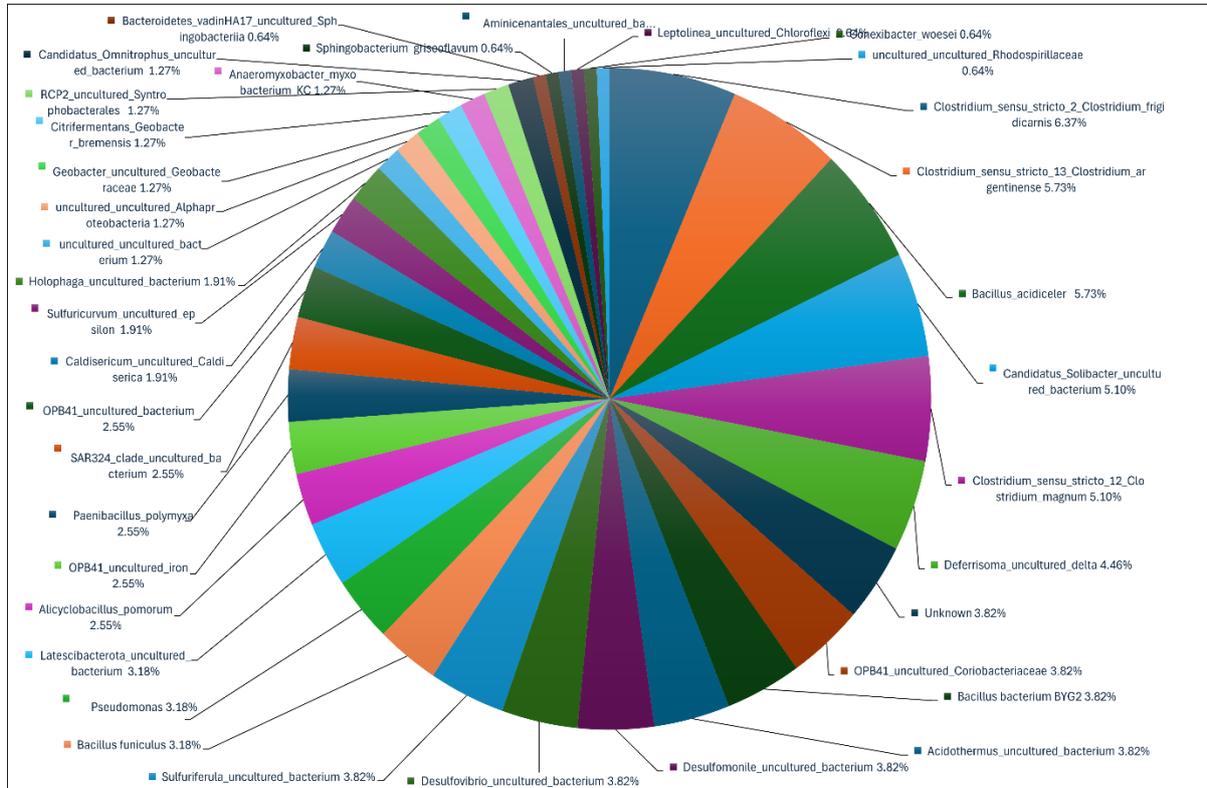

Figure 4. Overview of abundance of 16S rRNA bacterial species after second quarter from metal coupon biofilms in FWSS

In the analysis of the microbial successions assessment in metal coupon biofilm from FWSS after the third quarter of metal coupon insertion, 171 sequence reads were obtained from which thirty-six (36) known bacteria species and an Unknown category of species were characterized within fourteen (14) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, Caldisericaeota, Omnitrophota, Myxococcota, Latescibacterota, SAR324_clade* and *an Unknown category*. The *Firmicutes* still were the most dominated phylum in FWSS with more than 33% sequence reads containing eight (8) species distributed in three families, *Bacillaceae, Clostridiaceae* and *Alicyclobacillaceae.*
Clostridium_sensu_stricto_2_Clostridium_frigidicarnis with about 6% sequence reads, *Bacillus_acidiceler* and *Clostridium_sensu_stricto_13_Clostridium_argentinense, Candidatus_Solibacter_uncultured_bacterium* recorded more

than 5% sequence reads each and *Clostridium_sensu_stricto_12_Clostridium_magnum* with more than about 5%. *Deferrisoma_uncultured_delta* and *Pseudomonas spp* recorded more than 4% reads, *Acidothermus_uncultured_bacterium* and *OPB41_uncultured_Coriobacteriaceae, Desulfomonile_uncultured_bacterium* and *Desulfovibrio_uncultured_bacterium*, *Bacillus bacterium BYG2, Paenibacillus_polymyxa, SAR324_clade_uncultured_bacterium* and *Sulfuriferula_uncultured_bacterium* read about 3.5% each, also, the *Unknown category,* recorded about 3.5% representativeness. *Latescibacterota_uncultured_bacterium*, *Bacillus funiculus* and *Holophaga_uncultured_bacterium* also recorded about 3% each in the sequence reads. *Sphingobacterium_griseoflavum* and *Conexibacter_woesei* recording 1.17% and *Leptolinea_uncultured_Chloroflexi* with 0.58% representativeness were also detected after the third

quarter in the FWSS metal coupon biofilm sequence read (Figure 5).

This category classified as "Unknown" both at the phylum and species level, signifies a substantial portion of the microbial community whose specific generic identity could not be determined. This is a critical finding in the metal corrosion biofilm metagenomics, as it means that most of the microbial community cannot be assigned to any known or even named uncultured genus. It strongly suggests that the biofilm harbors a significant proportion of truly novel bacteria. Also, the dynamics pattern in the bacterial species diversity and population in the metal coupon biofilm suggests that the interaction between the bacterial biofilms with the surrounding environment and the metal coupon is likely characterized by dynamic nutrient cycling, potentially involving significant carbon, iron and Sulphur metabolism which significantly influences the corrosion process, leading to bioavailability of metabolic substrates in anaerobic condition over time, consequently leading to biofilm maturation, metabolic shifts, and accumulation of corrosive byproducts.
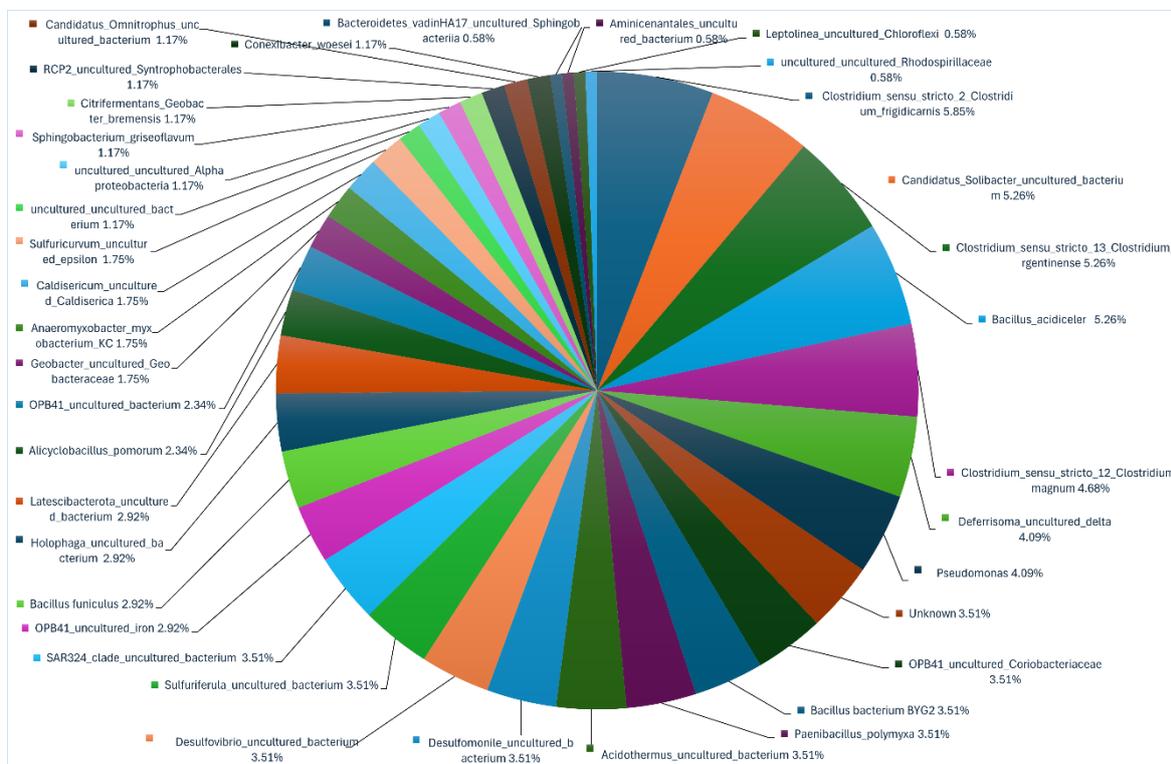

Figure 5: Overview of abundance of 16S rRNA bacterial species after third quarter from metal coupon biofilms in FWSS

IV. DISCUSSIONS

In natural freshwater sediment environment, studies on microbial succession on metal coupons might be challenging due to the various factors at work in the experiment, such as the environmental conditions. Recently, the bacteria population growth dynamics under long periods has been evaluated in an aquarium tank-built system for analysis, with a constant flow of seawater [30]. The higher corrosion rates observed in the unsterilized freshwater sediment corresponds with conclusions from a study on the biocorrosion of carbon steel, mild steel and stainless steel across the four environments studied; brackish water sediment, mangrove swamp sediment, lateritic soil, and river water sediment, that microbial involvement tends to be associated with increased corrosion rates [1], while this supports the finding in this study that MIC proceeds at faster rates in anaerobic or low oxygen environments. Several research reported similar microbially induced corrosion rates as recorded in the current study. An evaluation of microbial biofilms on carbon steel found similar corrosion rates of 0.45±0.01-0.12±0.01 mm/year dependent on environmental conditions [31]. Osadebe *et al.,* [1] observed an average percentage weight loss of 0.82% and 0.27% for carbon steel and mild steel respectively during a nine-month study in unsterilized river water sediment environments. Other research, however, recorded corrosion rates much higher than this study. Oparaodu and Okpokwasili, [32] reported a corrosion rate of 3.51 mm/year, 5.58 mm/year, and 0.32 mm/year for Carbon steel, Mild steel and Stainless steel respectively, in water-logged soil and 3.67 mm/year, 3.18 mm/year, and 0.19 mm/year, respectively, in sandy soil.

Our studies described the bacteria population dynamics, present in biofilm build-up, over metal coupons inserted in a freshwater sediment condition. It established the adherence of microorganisms in coupons inserted in freshwater sediment, maybe benefiting from the carbon steel. The dynamic pattern suggests that the presence of bacterial biofilms significantly influences the corrosion process, leading to fluctuating rates over time, likely due to biofilm maturation, metabolic changes, and build-up of corrosive byproducts. A distinct succession of

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bacterial species over time can be shown in phylogenetic study, with species that is abundant during the early colonization and others that persisted during the experiment. The outcomes deduced from this study showed several phyla of bacteria when compared with other similar studies [5,17], with prevalence of *Firmicutes* followed by *Actinobacteriota* and *Desulfobacterota* phyla and other significant phyla with a small number of representatives of the Unknown category of bacteria phylum. This category classified as "Unknown" at the phylum level, signifies a substantial portion of the bacterial community whose specific generic identity could not be determined. This is a critical finding in the metal corrosion biofilm metagenomics, as it means that some of the bacterial population cannot be assigned to any known or even named uncultured genus. It strongly suggests that the environment harbors a significant proportion of truly novel bacteria, whose activities might significantly be contributing to the corrosion process of metals in this environment, yet their mechanism of metabolism has not been proven. The specific conditions and other human activities within the environment of sample collection area can be a determinant of the results. The location is characterised by series of oil and gas activities, thus has some oil company's installations such as pipelines submerged in the water body. It has also been exposed to several environmental concerns due to crude oil bunkering and illegal refining [33]. During refining, residues are disposed off into the water body thereby impacting the quality of the water hence, the residue disseminates down into the water sediments [34]. A study of MIC formed in a flow-through colonization system based in a laboratory described different colonizers for different periods and conditions. For instance, *Deltaproteobacteria* species grow in active flow, while *Gammaproteobacteria* were shown to develop relatively better in stationary conditions [5,30]. Interestingly, when the assessing occurs in in situ conditions, there is a prevalence of bacteria species from the *Zetaproteobacteria* class [10,17].

Firmicutes were the most diverse and dominant surface-colonizing species detected on 16S rRNA analysis. Three species from the *Firmicutes; Clostridium_sensu_stricto_2_Clostridium_frigidicarnis*, *Bacillus_acidiceler*, and *Clostridium_sensu_stricto_13_Clostridium_argentinense* dominated almost throughout the study with a rapid dominance by *Candidatus_Solibacter_uncultured_bacterium species* in the last analysis, after the 3rd quarter of incubation. The significant dynamics in the profile of species present after the 2nd and third quarters, and the corresponding rapid increase in the corrosion rate of the coupons, signifies a clear relationship between the metal corrosion and the variety of persistent and abundant species throughout the incubation period. The presence and prevalence of *Clostridium spp* in this study in an anaerobic condition of corrosion is similar to the finding with Ramos-Monroy *et al.,* [35] and Nolan *et al.* [36], that demonstrated that the bacterial secretion of organic acids contributes to the pitting and general biocorrosion of API XL 52 steel and indicating the existence of reduced zones in the biofilm [37]. *Clostridium spp* are known sporulating Gram-positive bacteria that produces exopolysaccharide and organic acid thus an Acid producing bacteria, also has been identified

as an SRB [38], that are able to produce metal oxides at low pH and thus accelerate the pitting of a steel surface [39]. The prevalence of this species suggests that the ability to form spores is a selective advantage, for example, for survival in high temperature regions or during periods of low nutrient availability. Although low overall cell densities characterized the biofilms, they appear to constitute a heterogeneous environment allowing the establishment of metabolically and physiologically diverse bacterial communities [37]. This has clearly established that APB groups of bacteria are important for the corrosion process especially in an anaerobic condition [35]. Also, *Clostridium spp* has been reported to co-exist with other bacteria groups (SRB, IRB, etc) in biofilm structure, since their secretion of products either provides substrates for other bacteria group or favourable condition for other groups to thrive, hence described as pioneer colonizers during biofilm formation over surfaces in coastal environments [37,39,5]. Biofilm growth is the result of complex processes involving the attachment of microbial cells enabled by the secretion of extracellular polymeric substances (EPS), often referred to as the slime [40]. Resulting from biofilm formation, two types of different behaviour have been observed; microorganisms can act beneficially protecting the materials and thereby, diminishing the corrosion rate [41] or, accelerating the corrosion process with the consequent damage to the material integrity [42,43]. The inert metal wearing is an indirect consequence of the formation of biomass and products, causing a slow biocorrosion in the metal and covering large areas and a considerable amount of metal loss [35].

Other species belonging to several phyla: *Desulfobacterota, Deferrisomatota, Actinobacteriota, Acidobacteriota, Firmicutes, Myxococcota, Planctomycetota, Proteobacteria, Latescibacterota, Spirochaetota, Bacteroidota, Chloroflexi* (*Candidatus_Solibacter_uncultured_bacterium, Deferrisoma_uncultured_delta, Pseudomonas spp, Acidothermus_uncultured_bacterium, Paenibacillus_polymyxa, OPB41_uncultured_Coriobacteriaceae, Desulfomonile_uncultured_bacterium, OPB41_uncultured_iron, Desulfovibrio_uncultured_bacterium, Conexibacter_woesei, SAR324_clade_uncultured_bacterium, Sulfuriferula_uncultured_bacterium, Alicyclobacillus_pomorum, Latescibacterota_uncultured_bacterium, Holophaga_uncultured_bacterium, Sphingobacterium_griseoflavum, Latescibacterota_uncultured_bacterium,* and *Leptolinea_uncultured_Chloroflexi*) were all persisting in the biofilms across the periods of the incubation. The detection of sulphate reducing bacteria (*Desulfomonile_uncultured_bacterium, Desulfovibrio_uncultured_bacterium, Latescibacterota_uncultured_bacterium*), iron reducing bacteria (*Pseudomonas spp, OPB41_uncultured_iron, OPB41_uncultured_Coriobacteriaceae, Deferrisoma_uncultured_delta, OPB41_uncultured_bacterium,*

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Geobacter_uncultured_Geobacteraceae, Citrifermentans_Geobacter_bremensis, Anaeromyxobacter_myxobacterium_KC, Aminicenantales_uncultured_bacterium and Latescibacterota_uncultured_bacterium) together with iron oxidizing bacteria and sulphur oxidizing bacteria (*Sulfuriferula_uncultured_bacterium* and *SAR324_clade_uncultured_bacterium*), described the roles of the various species in the oxidation and reduction of elemental sulphur and iron, and to use metabolic energy from these reactions hence, the interactions between these groups of bacteria in the corrosion process and reason for the corrosion rate observed on the metal coupons. In a recent study on microbial community succession over mild steel, the dominance of *Proteobacteria* and *Bacteriodetes* was described in different conditions, with the oxidizing iron group *Zetaproteobacteria* within the *Proteobacteria* being the most abundant [17]. On the other hand, another study on microbial corrosion of steel coupons conducted in deep groundwater detected the prevalence of the *Betaproteobacteria* group, mainly *Burkhoderiales* and *Hydrogenophilales* species, which are described as oxidizing iron and hydrogenotrophic, respectively [44].

Sphingobacterium griseoflavum and *Bacteroidetes vadinHA17 uncultured Sphingobacteriia* are members of the phylum *Bacteroidota* also observed to persist on the metal coupon after all period of analysis. There is little reference about the role of *Bacteroidota* involved in corrosion process, but members of this group are efficient surface colonizers and can take some advantage of primary production by chemolithotrophic Fe- and S-oxidizing bacteria during biofilm formation [13].

V. CONCLUSION AND IMPLICATIONS

We used a controlled laboratory setting to investigate the influence of steel on bacterial succession and corrosion in freshwater sediment sample collected from a freshwater environment.

The presence of several bacterial species representing phyla proves diverse microbial contributions to corrosion processes. *Clostridium spp* were more abundant at all the periods (1st, 2nd and 3rd quarter) of analyses, possibly provides favourable conditions and substrates for competitive coexistence in the biofilm structure, highlighting the complex interplay between microbial communities and corrosion dynamics. The results show thoughtful changes in bacterial diversity over time in the presence of steel that may relate to their capacity to interact with Fe to cause corrosion. Detection and identification of the specific bacteria associated with corrosion paves the way for a more targeted approach to reducing corrosion.

The detection of an *Unknown* bacterial category at phylum and species level in the biofilm obtained poses a critical problem in the study of MIC of metals in any environment owing to their unknown metabolic roles in the corrosion process. Hence, we can not totally relay on culture- dependent method in the study of MIC in an environment.

The relationship between the diversity and quantity of sequences present on the metal coupons was observed to be inversely related as not all species were equally competitive with the prevalence of specific bacterial phyla. The observed changes in the % abundance of individual species over time during the experiment despite the level of prevalence of their phylum might be a function of the adaptive ability of these species at a given period of the corrosion process given to the presence of substrate that might be favourable for its growth and metabolism, hence a competitive association. The dynamics pattern in the bacterial species diversity and population in the metal coupon biofilm suggests that the interaction between the bacterial biofilms with the surrounding environment and the metal coupon significantly influences the corrosion process, leading to bioavailability of metabolic substrates in anaerobic condition over time, consequently leading to biofilm maturation, metabolic shifts, and accumulation of corrosive byproducts.

REFERENCES

[1] Osadebe A.U., Olorondu D.C., Okpokwasili G.C. (2021). Environmental and microbial influences on corrosion of selected types of petroleum industry steel. *Environ. Nat. Resour. J.19(4):*310-319.

[2] Yan L, Diao Y, Laang Z, Gao K. (2020). Corrosion rate prediction and influencing factors evaluation of low-alloy steels in marine atmosphere using machine learning approach. *Journal of Science and Technology of Advanced Materials; 21(1):*359-70.

[3] Da Silva PS, de Senna FL, Goncalves MMM, do Lago DCB. (2019). Microbiologically-influenced corrosion of 1020 carbon steel in artificial seawater using garlic oil as natural biocide. *Materials Research;22(4)*:e20180401.

[4] Liu H and Cheng YF. (2018). Microbial corrosion of X52 pipeline steel under soil with varied thicknesses soaked with a simulated soil solution containing sulfate-reducing bacteria and the associated galvanic coupling. *Electrochimica Acta* 2018; 266:312-25.

[5] Moura V., Ribeiro I. Moriggi, P., Capão, A. Salles, C., Bitati, S and Procópio, L. (2018). The influence of surface microbial diversity and succession on microbiologically influenced corrosion of steel in a simulated marine environment. *Archives of Microbiology.* https://doi.org/10.1007/s00203-018-1559-2

[6] Hou B, Li X, Ma X, Du C, Zhang D. (2017). The cost of corrosion in China. *Npj Materials Degradation*; 1:1-10.

[7] Fayomi OSI, Akande IG, Odigie S. (2019). Economic impact of corrosion in oil sectors and prevention: An overview. *Journal of Physics: Conference Series*;1378:022037.

[8] Arena-Ortiz LM, Muhilan M, Reyes-Sosa M, Ortiz-Alcantara J. (2019). Bio-corrosion, sulfate-reducing bacteria in the Yucatan Peninsula. *Journal of Marine Biology and Oceanography*;8(1):1000202.

[9] Bryce C, Blackwell N, Schmidt C, Otte J, Huang Y, Kleindienst S, *et al.* (2018). Microbial anaerobic Fe(II) oxidation-ecology, mechanisms and environmental implications. *Environmental Microbiology*;20(10):3462-83.

[10] Dang H, Lovell CR (2015) Microbial surface colonization and biofilm development in marine environments. *Microbiol Mol Biol Rev 80(1):*91–138

[11] Marty F, Gueuné H, Malard E et al (2014) Identification of key factors in accelerated low water corrosion through experimental simulation of tidal conditions: influence of stimulated indigenous microbiota. *Biofouling 30(3):*281–297

[12] Valencia-Cantero E, Peña-Cabriales JJ. (2014). Effects of iron reducing bacteria on carbon steel corrosion induced by thermophilic sulphate reducing consortia. *Journal of Microbiology and Biotechnology;24(2):*280-6.

[13] Doghri I, Rodrigues S, Bazire A et al (2015) Marine bacteria from the French Atlantic coast displaying high forming-biofilm abilities and different biofilm 3D architectures. *BMC Microbiol 15:*231

[14] Liu H, Meng G, Li W, Gu T, Liu H. (2019). Microbially influenced corrosion of carbon steel beneath a deposit of CO_2-saturated formation water containing *Desulfotomaculum nigrificans*. Frontiers in *Microbiology;10:*1298.

[15] Liengen T, Basseguy R, Feron D et al. (2014) Understanding biocorrosion: fundamentals and applications, 1st edn. Woodhead Publishing, Cambridge

[16] Carvalho ML, Doma J, Sztyler M et al (2014) The study of marine corrosion of copper alloys in chlorinated condenser cooling circuits: the role of microbiological components. *Bioelectrochemistry 97:*2–6

[17] McBeth JM, Emerson D (2016) In situ microbial community succession on mild steel in estuarine and marine environments: exploring the role of iron-oxidizing bacteria. *Front Microbiol 7:*767

[18] Chen, S., Liu, G. and Zhang, D. (2019). Corrosion of Q235 carbon steel in seawater containing *Mariprofundus ferrooxydans* and *Thalassospira* sp. *Frontier Microbiolog* 10: 936-942.

[19] Gu, T., Jia, R., Unsal, T. and Xu, D. (2019). Toward a better understanding of microbiologically influenced corrosion caused by sulfate reducing bacteria. *Journal Material Science and Technology* 35: 631–636.

[20] Dou, W., Jia, R., Jin, P., Liu, J., Chen, S., and Gu, T. (2018). Investigation of the mechanism and characteristics of copper corrosion by sulfate reducing bacteria. *Corrosion Science* 144: 237–248.

[21] Ghiara, G., Spotorno, R., Trasatti, S. P. and Cristiani, P. (2018). Effect of *Pseudomonas fluorescens* on the electrochemical behaviour of a single-phase Cu-Sn modern bronze. *Corrosion Science* 139: 227–234.

[22] Li Y, Xu D, Chen C, Li X, Jia R, Zhang D, Sand W, Wang F, Gu T (2018b) Anaerobic microbiologically influenced corrosion mechanisms interpreted using bioenergetics and bioelectrochemistry: a review. *J Mater Sci Technol 34*:1713–1718

[23] Zhou, E., Li, H., Yang, C., Wang, J., Xu, D., Zhang, D. and Gu, T. (2018). Accelerated corrosion of 2304 duplex stainless steel by marine *Pseudomonas aeruginosa* biofilm. *International Biodeterioration and Biodegradation* 127: 1–9.

[24] Xu D, Xia J, Zhou E et al (2017) Accelerated corrosion of 2205 duplex stainless steel caused by marine aerobic *Pseudomonas aeruginosa* biofilm. *Bioelectrochemistry 113:*1–8

[25] Zhao, Y., Zhou, E., Liu, Y., Liao, S., Li, Z., Xu, D., Zhang, T. and Gu, T. (2017). Comparison of different electrochemical techniques for continuous monitoring of the microbiologically influenced corrosion of 2205 duplex stainless steel by marine *Pseudomonas aeruginosa* biofilm. *Corrosionn Science* 126: 142–151.

[26] Khouzani MK, Bahrami A, Hosseini-Abari A, Khandouzi M, Taheri P. (2019). Microbiologically influenced corrosion of a pipeline in a petrochemical plant. *Metals;9*:459.

[27] Ryan, J., George, E. and Abdul, R. (2001). Soil and plant analysis laboratory manual. 2nd Edition. International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. 230pp.

[28] Wu J, Zhang W, Chai, K. and Yu, A. (2020). Corrosion Behavior of AISI 1045 Steel in Seawater in the Presence of *Flavobacterium* sp. *Frontiers in Microbiology* 11: 303-320.

[29] Bala Manikandan, C., Balamurugan, S., Balamurugan, P., & Beneston, S. L. (2019). Corrosion inhibition of mild steel by using banana peel extract. *International Journal of Innovative Technology and Exploring Engineering, 8(6),* 1372–1375.

[30] Ramírez G.A., Hoffman C.L., Lee M.D. *et al* (2016). Assessing marine microbial induced corrosion at Santa Catalina Island, California. *Front Microbiol 7:*1679

[31] De Melo IR, Filho SLU, Oliveira FJS, de França FP. (2011). Formation of biofilms and biocorrosion on AISI-1020 carbon steel exposed to aqueous systems containing different concentrations of a diesel/biodiesel mixture. *International Journal of Corrosion*; 2011:415920.

[32] Oparaodu, K.O., Okpokwasili, G.C. (2014). Comparison of percentage weight loss and corrosion rate trends in different metal coupons from two soil environments. *International Journal of Environmental Bioremediation and Biodegradation; 2(5):*243-9.

[33] Edjere, O., & Asibor, G. (2020). Evaluation Of Total Petroleum Hydrocarbons (Tphs) And The Effects Of Illegal Bunkering Activities On Okpare River, Delta State, Southern Nigeria. *SAU Science-Tech Journal*, 5(1), 139-151.

[34] Idibie, C. A., & Idibie, O. C. (2021). Physiochemical And Microbiological Analyses Of Crude Oil Impacted Oil Of Okpare-Olomu Community Of The Niger Delta. *IOSR J Environ Sci Toxicol Food Tech, 15(1),* 1-8.

[35] Ramos-Monroy, O. A. & Ordaz, N. R., Hernández-Gayosso M. J., Ramírez, C. J. & Mayer, J. G. (2019). The corrosion process caused by the activity of the anaerobic sporulated bacterium *Clostridium celerecrescens* on API XL 52 steel. *Environmental Science and Pollution Research* https://doi.org/10.1007/s11356-019-06064-3

[36] Nolan, L., Risser, T., Catubig, R., Venugopal, A. Glasson, J., Callahan, D. L., Somers, A., Ackland M. L. and Michalczyk, A. (2025). Bacterial Diversity and Succession in the Presence of Steel and Effects on Corrosion. *Environmental Microbiology Reports pp 1-22*

[37] Kjeldsen K. U., Kjellerup, B. V., Egli, K., Frølund, B., Nielsen, P. H., Ingvorsen, K. (2007). Phylogenetic and functional diversity of bacteria in biofilms from metal surfaces of an alkaline district heating system, *FEMS Microbiology Ecology, 61(2)* 384–397. https://doi.org/10.1111/j.1574-6941.2006.00255.x

[38] Doyle CJ, O'Toole PW and Cotter PD (2018). Genomic Characterization of Sulphite Reducing Bacteria Isolated from the Dairy Production Chain. *Front. Microbiol. 9*:1507. doi: 10.3389/fmicb.2018.01507

[39] Rajasekar, A., Anandkumar, B., Maruthamuthu, S., Ting, Y., and Rahman, P. (2010). Characterization of corrosive bacterial consortia isolated from petroleum-product-transporting pipelines. *Appl. Microbiol. Biotechnol.*, 85(4):1175– 1188.

[40] Torres-Bautista BE, Wikieł AJ, Datsenko I, Vera M, Sand W, Seyeux A, Zanna S, Frateur I, Marcus P (2015). Influence of extracellular polymeric substances (EPS) from *Pseudomonas* NCIMB 2021 on the corrosion behaviour of 70Cu-30Ni alloy in seawater. *J Electroanal Chem 737*:184–197

[41] Jia R, Tan JL, Jin P, Blackwood DJ, Xu D, Gu T (2018). Effects of biogenic H2S on the microbiologically influenced corrosion of C1018 carbon steel by sulfate reducing Desulfovibrio vulgaris biofilm. *Corros Sci 130*:1–11

[42] Feugeas, F., Magnin, J.P., Cornet, A., Rameau, J.J. (1997). Corrosion influence par les microorganisms: influence du biofilm sur la corrosion des aciers, techniques et resultants récents. *J Phys III* 7:631–663

[43] Liu H, Xu D, Dao AQ, Zhang G, Lv Y, Liu H (2015). Study of corrosion behavior and mechanism of carbon steel in the presence of Chlorella vulgaris. *Corros Sci 101*:84–93

[44] Rajala P, Carpén L, Vepsäläinen M *et al* (2015). Microbially induced corrosion of carbon steel in deep groundwater environment. *Front Microbiol 24(6):*647