IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 19, Issue 11 Ser. 1 (November 2025), PP 39-51
www.iosrjournals.org

The Influence of Bacteria Diversity on Microbially Induced Corrosion of Carbon Steel in an Estuarine Sediment

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Abstract
The study investigated Microbiologically Influenced Corrosion (MIC) of carbon steel exposed to Marine/Estuarine Sediment (MWSS) in Delta State, employing a combination of physicochemical analysis, corrosion rate assessments, and 16S rRNA gene sequencing (metagenomics). This research established a robust framework linking specific microbial communities to material degradation. Physicochemical characterization of MWSS revealed high salinity and sulphate concentrations conducive to sulphide-related corrosion. The corrosion testing demonstrated that biofilms significantly accelerated material loss, with fluctuating corrosion rates observed over three quarterly intervals, attributed to biofilm maturation and metabolic shifts. The corrosion rates of metal coupons immersed in MWSS after the first and third quarterly analyses, 2.89 mpy and 1.53 mpy respectively, were observed while maintaining a consistent corrosion risk due to the stable dominance of Sulfate Reducing Bacteria (SRB). Scanning Electron Microscopy (SEM) identified pitting corrosion as the dominant failure mode in biologically active sample, while elemental analysis (SEM-EDS) confirmed MIC through the detection of biogenic elements such as sulphur, phosphorus, and manganese in corrosion deposits, indicating active microbial metabolism. Metagenomic analysis revealed a diverse microbial community within the biofilms, predominantly comprising the phylum Desulfobacterota (up to 24% relative abundance), associated with sulphide production, followed by Proteobacteria (about 16% abundance) and Firmicutes (about 14% abundance), validating the predominance of iron-reducer and acid-producer in the environment. Importantly, a significant proportion of sequences were classified as "Unknown," suggesting the involvement of novel and uncharacterized strains in the corrosion process. This highlights a gap in research regarding these microbial agents. In conclusion, the study elucidates the complex interactions between microbial diversity and corrosion in estuarine environments, advocating for further investigation of the unidentified "Unknown" strains. Such efforts may enhance our understanding of MIC dynamics, informing better materials protection strategies in marine and estuarine applications.

Keywords*: Biocorrosion, Biofilms, corrosion rates, marine environment, weight loss, metagenomics*

Date of Submission: 10-11-2025 Date of Acceptance: 21-11-2025

I. Introduction

Corrosion as a natural process, converts a refined metal to its oxide or hydroxide or another compound which is a more stable form or can be generally defined as the gradual destruction of materials by microorganisms or by chemical and electrochemical reactions with their environment. Different types of corrosion were reported such as galvanic [1], pitting and uniform [2], erosion [3], lamellar [4], crevice and microbial corrosions. Corrosion poses a significant global industrial risk, with the cost of inspection, correction, and prevention estimated to reach $2.5 trillion annually [5]. As the planet's most pervasive life forms, microorganisms are ubiquitously distributed in nature and interact with virtually all human activities. They are critical agents in biogeochemical cycles and fundamental to ecosystem health. In materials science, the role of microbes is critical, particularly given their multifaceted and complex influence on both corrosion and corrosion inhibition [6,7].

Microbiologically influenced corrosion (MIC), also known as microbial corrosion, biocorrosion, or biological corrosion, describes the deterioration of materials resulting from the direct or indirect corrosive actions of microbes (including bacteria, archaea, and fungi) and their metabolic byproducts [8,9]. Full understanding of the diversity of microbes colonizing metal surfaces and the heterogeneity of biofilms has persisted as one of the

main challenges in the study of cause-and-effect relationships underlying microbial induced corrosion [10]. Thus, this molecular technology denaturant gradient gel electrophoresis (DGGE), DNA sequencing, has led to insights into the understanding of bacterial communities and heterogeneity of biofilms colonizing metal surfaces in the various oilfield ecological environments.

Microorganisms are capable of either accelerating or mitigating the rate of material degradation [11]. The environments where MIC occurs are diverse. In soil environments, underground pipelines face multiple corrosive factors, with microorganisms participating in many of these processes [12]. In marine environments, microbes such as bacteria, fungi, and archaea are known to promote corrosion. The production of organic acids by some of these organisms during growth can intensify corrosion on materials like carbon steel and Al/Mg alloys [13].

Carbon steel, a widely used material in oil and gas pipelines, is vulnerable to both abiotic and biological corrosion [14]. The breakdown of submerged oilfield facilities in waters, sediments, creeks and soils because of corrosion are unavoidable issue for owners and asset managers worldwide, as they shorten the life span of these facilities. Spontaneous bursting of submerged facilities and the results of striking damages has been revealed. These facilities are submerged, and this makes their inspection and maintenance hard in a particular site, besides field limitations and environmental alterations [15]. Within pipelines including mechanical clamped points, valves and other couplings/joints on the surface of the pipelines (the uncoated sites of the buried pipelines) and on metal surfaces submerged in the oilfield environments, microbially induced corrosion takes place when complex microbial consortia interact with metallic surfaces. This occurs through the establishment of multispecies biofilms in which different microorganisms influence the corrosion through a cooperative global metabolism [16].

Biofilms are generated by the growth of surface-associated microbial consortia and production of extracellular polymeric substances (EPS) by these microorganisms. It is estimated that almost all species of microorganisms on earth live, at least for a period in their life cycles, in such communities [17]. This phenomenon often leads to irreversible attachment of cells to the surface, which comprises inorganic precipitates derived from the bulk aqueous phase and corrosion products of the metal substratum. Microbial investigations on MIC, especially on metal, have been focused on laboratory experiments with single-species biofilms, even though the synergistic effect of multispecies biofilms is already known. Also, there is an increase in contradictory reports on both accelerating and inhibiting actions on the corrosion process of the same functional group of microorganisms, such as sulfate reducers [18,19], iron reducers [20] and methanogens [21].

Routinely, the evolution of biofilms in pipelines is explored by plate counting of significant microbial groups; however, several studies, based on culture and culture- independent strategies, have explored the microbial diversity in biofilms growing within pipelines and metal surfaces from oil facilities which have been associated with biocorrosion damages [22]. Single-species biofilms in laboratory experiments have been used in the microbial investigations on MIC, especially on metal, even though the synergistic effect of multispecies biofilms is already in study. Also, the conventional cultural method of MIC has limited the species of study to only those that are able to grow on synthetic growth media, while also limiting the identification of other important organisms that are unculturable whose role in the corrosion process has been neglected. These indicates that there is an urgent need to focus on the identification of bacteria in biofilms attached on submerged metals using molecular techniques (metagenomic analyses) which is gaining attention for assessing the microbial diversity and heterogeneity in the environments, and it can help explain the biocorrosion process, which is common in the oilfield locations.

In this study, the influence of bacteria diversity on microbially induced corrosion of carbon steel in estuarine sediment 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

Study area description and sample collection

The Estuarine sediment sample was collected from a point near the Benin River Valve Station (BRVS) along the Benin River (05º45'31"N, 05º4'21"E) in the western Niger Delta River region of Nigeria and labelled MWSS (Marine Water Sediment Sample). The sediment sample was collected using grabbers into sterile polyethylene bag. The physiochemical characteristics of the sample were determined according to HACH [23].

Experimental Set-up

The experimental set-up was 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 estuarine sediment sample (MWSS) collected.

The experimental setup was air-tightened with lids and kept at room temperature at static incubation for a research period of three (3) quarters in the year. After 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.

Carbon Steel 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.* [24]. The 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 aluminum foil paper. The initial weight and final weight were taken before use and after use respectively. The coupons were also labeled, and initial weights were recorded. The exposed surface area of each coupon is 16.95 cm^2.

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 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. The corrosion rate (millimetre per year (mm/y)) was determined from the weight loss method, using this equation below [25].

$$CR = W/(D \times A \times t) \times K$$

Where, W = weight loss in grams, K = Constant (8.76 x 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 error of mean was calculated using the software Microsoft Excel 2010.

Scanning electron microscopy- Energy Dispersive X-ray Spectroscopy (SEM-EDS) analyses

After each experimental interval, one of the inserted metal coupons were retrieved and examined with scanning electron microscopy to determine the pitting corrosion and elemental compositions of corrosion deposits on the metal coupons [26].

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).

PCR of 16S rRNA gene amplification, library construction and sequencing

Genomic DNA samples were PCR amplified using a universal primer pair 27F and 1492R - targeting the V1 -V9 region of the bacterial 16S rRNA gene. Resulting amplicons were barcoded with Pacbio M13 barcodes for multiplexing through limited cycle PCR. The resulting barcoded amplicons were quantified and pooled equimolar and AMPure PB bead-based purification step was performed. The PacBio SMRTbell library was prepared from the pooled amplicons following manufacture protocol. 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

Physicochemical Characteristics of the sample

The physical and chemical characteristics of the estuarine sediment sample (MWSS) collected from Benin River is presented in Table 1.

Table 1: The physicochemical and Heavy Metal features of sediment sample

Parameter	(MWSS)
pH	5.80±0.45
Temperature (°C)	27.90±0.32
E. Conductivity (µs/cm)	1320±12.00
Redox Potential (mV)	32.99±7.67
CO_3 (mg/kg)	0.11±0.01
Chloride as Salinity (mg/kg)	829.50±1.90
Nitrate (mg/kg)	0.92±0.04
Nitrite (mg/kg)	<0.01
Phosphate (mg/kg)	0.91±0.05
Moisture Content (%)	18.80±0.22
Total Nitrogen (mg/l)	0.07±0.01
THC (mg/kg)	1480±3.10
TPH (mg/kg)	1395±3.00
TOC (mg/kg)	0.97±0.02
Lead, Pb (mg/kg)	13.46±0.88
Zinc, Zn (mg/kg)	64.20±1.10
Iron, Fe+ (mg/kg)	6880±10.00
Nickel, Ni (mg/kg)	7.20±0.70
Chromium (mg/kg)	11.46±1.02
Sodium, Na (mg/kg)	76.46±1.33
Silver, Ag (mg/kg)	<0.001
Cadmium (mg/kg)	0.18±0.01
Mercury, Hg (mg/kg)	<0.001
Arsenic, As (mg/kg)	<0.001
Magnesium, Mg (mg/kg)	22.40±1.00
Manganese, Mn (mg/kg)	0.02±0.01
Copper, Cu (mg/kg)	28.70±1.05
Cobalt (mg/kg)	<0.001

Corrosion rates

Corrosion rates of the metal coupons inserted in the MWSS after the bacterial biofilms and the corrosion products have been scraped, are presented in Figure 1. The first quarter of the metal coupon insertion showed the highest corrosion rate. The corrosion rates across three quarterly intervals, an initial increase after the first quarter (2.89 mpy), a sharp decline after the second (1.78 mpy), and a further decrease (1.53 mpy) after the third interval. This suggests that both physicochemical properties and bacterial biofilms contribute to corrosion, with the latter potentially accelerating it or changing its dynamics over time.

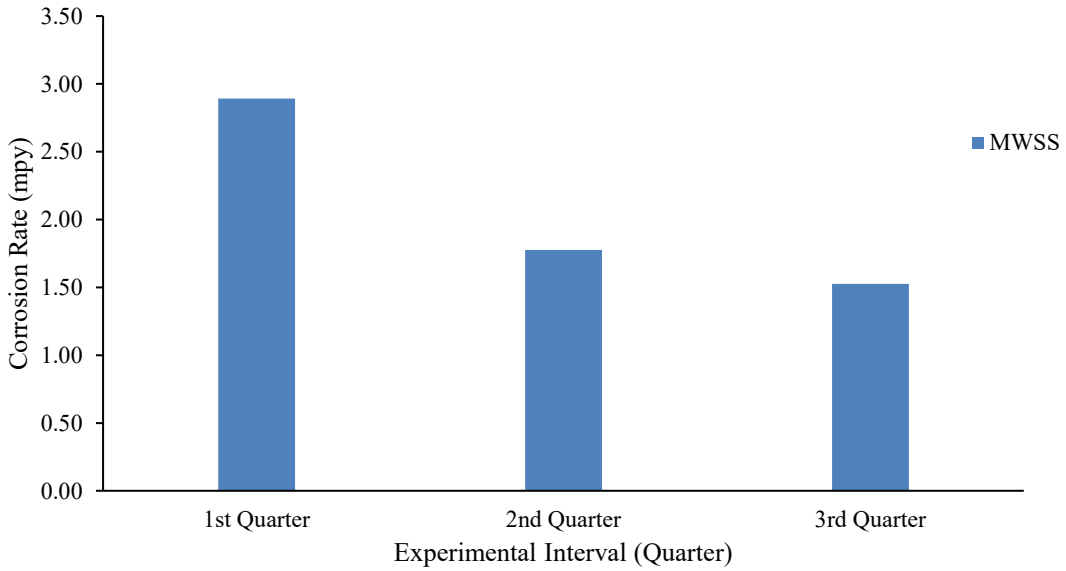

Figure 1. Corrosion rates of metal coupons submerged in freshwater sediment over 3 quarters of the year. SEM-EDX showing the elemental composition of the corrosion deposits/products

Scan Electron Microscopy (SEM-EDX) analysis of corrosion deposits on metal coupons provided insights into the elemental changes over time and the role of microbial activity. The first quarter and third quarter results of the elemental compositions of corrosion deposits on metal coupons immersed in MWSS was presented

in Table 2. In the first quarter, MWSS exhibited significantly lower iron concentrations at 50.82% and low carbon content (0.40%). Phosphorus, Sulphur, and Manganese were present in varying amounts in the MWSS. The lower concentrations of iron and carbon in the MWSS was attributed to microbial activities, suggesting deterioration of the metal and incorporation of carbon into microbial structures or byproducts. The presence of elements like sulphur (1.55 %), manganese (0.20%) and phosphorus (1.16%) in MWSS indicates their involvement in microbial metabolic processes and the formation of corrosion products, reinforcing the microbial influence on corrosion product composition. By the third quarter, the MWSS continued to exhibit lower iron concentrations (45,00%) and carbon content (0.91%). The presence of elements like sulphur, phosphorus and Manganese in MWSS, remained consistent, indicating their persistent involvement in microbially mediated corrosion processes. This suggests that microorganisms are actively metabolizing and transforming these elements, leading to their accumulation and incorporation into the corrosion products or biofilms.

Table 2: Elemental Composition of corrosion deposits on metal coupons for MWSS after the 1st and 3rd Quarter of experimental set-up.

Element	1st Quarter		3rd Quarter	
	MWSS		MWSS	
	A Conc. (%)	W. Conc. (%)	A Conc. (%)	W. Conc. (%)
Iron	24.08	50.82	22.49	45.00
Carbon	0.88	0.40	3.79	0.91
Oxygen	69.48	41.97	60.77	46.60
Silicon	1.13	1.24	5.80	3.23
Zinc	0.00	0.00	0.00	0.00
Sodium	0.48	0.42	1.84	0.86
Chromium	0.30	0.27	0.00	0.00
Aluminium	0.49	0.54	0.48	0.22
Titanium	0.00	0.00	0.00	0.00
Manganese	0.10	0.20	0.30	0.34
Sulfur	1.50	1.55	1.89	1.20
Magnesium	0.42	0.39	1.62	0.50
Phosphorus	0.14	0.16	0.40	0.27
Silver	0.00	0.00	0.00	0.00
Tin	0.00	0.00	0.00	0.00
Copper	0.00	0.00	0.00	0.00
Nickel	0.00	0.00	0.00	0.00
Calcium	0.00	0.00	0.00	0.00
Potassium	0.00	0.00	0.00	0.00
Vanadium	0.00	0.00	0.00	0.00
Chlorine	1.00	2.05	0.62	0.87
Cobalt	0.00	0.00	0.00	0.00

Scan Electron Microscopy Results showing Pitting Corrosion

Scanning Electron Microscopy (SEM) micrographs gives visual evidence of corrosion pitting on the metal coupons. A key observation was that pitting was the dominant feature of corrosion induced by the MWSS, which bore bacterial biofilms. The SEM micrographs, spanning both the first and third quarterly immersion periods, consistently showed both corrosion products and pitting corrosion on the metal coupons (Figure 2). The presence of bacterial biofilms was clearly associated with a higher incidence and severity of pitting directly explains the enhanced corrosion potentials attributed to these biofilms.

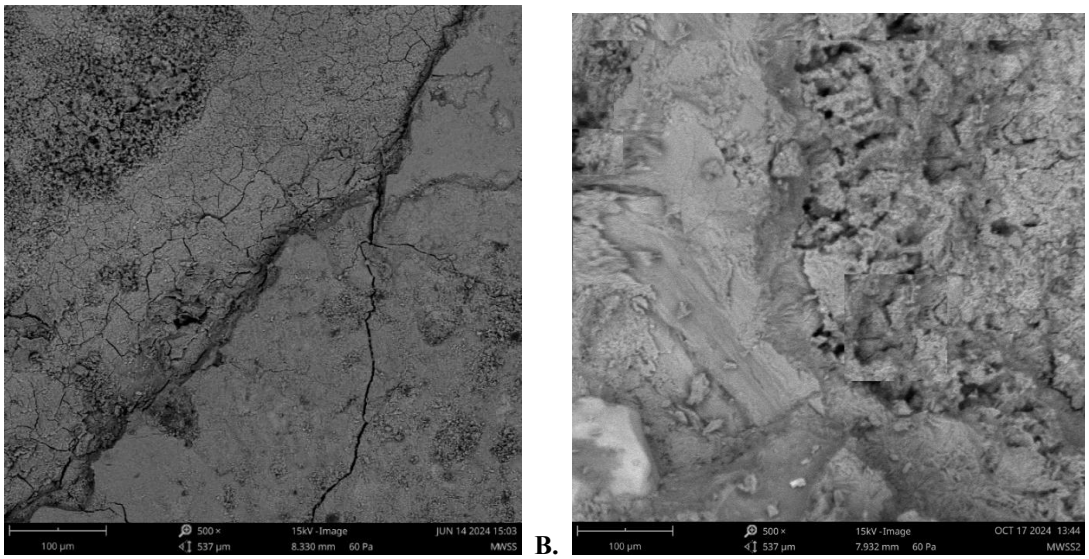

Figure 2: SEM micrograph of biofilm containing microbial cells and corrosion products embedded in extracellular polymeric substance (EPS) on the surface carbon steel coupons exposed to MWSS after. **(A.)** first quarter and **(B.)** third quarter

Relative abundance of 16S rRNA bacterial phyla in the biofilm on the metal coupons

For MWSS, the obtained 16S rRNA gene sequences showed similarity to thirty-nine (39) known bacteria species and an Unknown category of species within thirteen (13) known phyla of bacteria: *Actinobacteriota, Acidobacteriota, MBNT15, Firmicutes, Planctomycetota, Proteobacteria, Latescibacterota, Myxococcota, Spirochaetota, Desulfobacterota, Deferrisomatota, Bacteroidota, Chloroflexi* and *an Unknown category* (Figure 3). The result showed that about 24% of all the species in the first experimental interval were affiliated to *Desulfobacterota,* about 16% and 14% to *Proteobacteria* and *Firmicutes* respectively, 11% to *Latescibacterota* while at all the intervals about 6% was affiliated to the Unknown category. At all the experimental interval, the *Desulfobacterota* dominated in the biofilms 16S rRNA gene sequence.

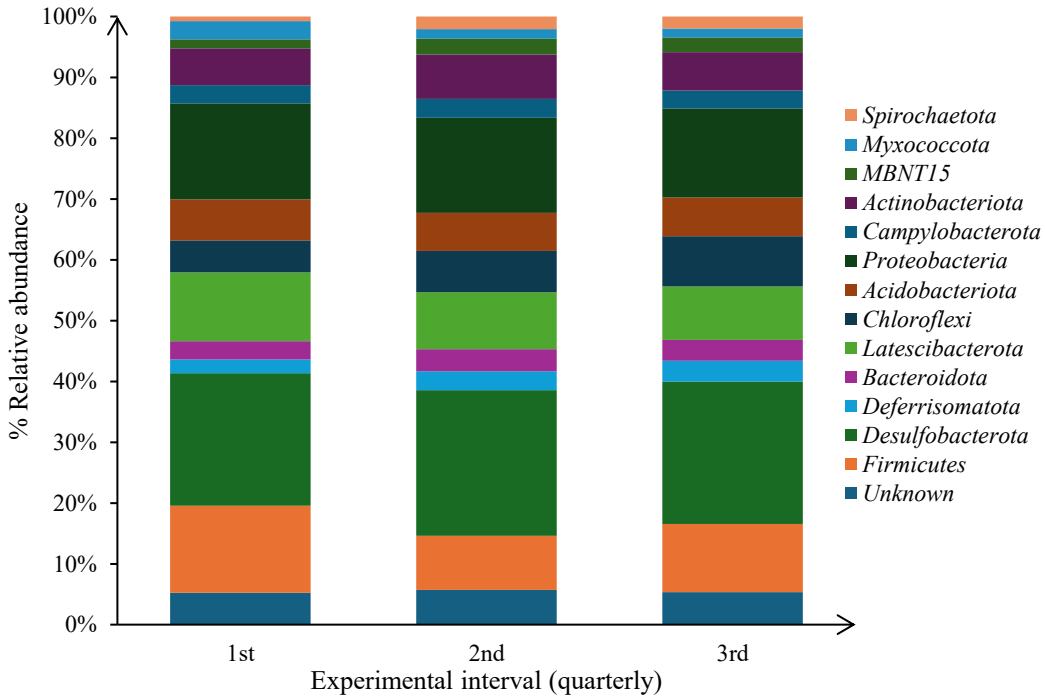

Figure 3: %Relative abundance of 16SrRNA reads matching from bacteria phyla detected on metal coupons throughout analysed periods. The y-axis describes in percentage the relative abundance of 16S genes belonging to different phyla throughout the experiment in MWSS.

Bacterial diversity and succession analysis

In the analysis of the microbial successions assessment in metal coupon biofilm from MWSS after the first quarter of metal coupon insertion, 133 sequence reads were obtained from which thirty-four (34) known bacteria species and an Unknown category of species were characterized within thirteen (13) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, MBNT15, Spirochaetota, Myxococcota, Latescibacterota,* and *an Unknown category*. *Latescibacterota_uncultured_bacterium* from the *Latescibacterota* phylum dominated the sequence reads with more than 7% representativeness followed by *Clostridium_sensu_stricto_13_Clostridium_argentinense* and *Clostridium_sensu_stricto_1_uncultured_bacterium* from the *Firmicutes* with about 6% and more than 4% representativeness respectively. Among the *Acidobacteriota* phylum, *Candidatus_Solibacter_uncultured_bacterium* was the most abundant with more than 4% representativeness. The *Desulfobacteriota* were the most dominated and diverse phylum in MWSS with about 22% sequence reads containing nine (9) species among which *Desulfobacca_uncultured_delta* recorded a significant representativeness of about 4%, followed by *Desulfatiglans_uncultured_Desulfobacteraceae* and *Geothermobacter_uncultured_soil* with 3% representativeness. The *Proteobacteria* was the second most abundant phylum in MWSS with more than 15% sequence reads containing seven (7) species, among which *Pseudomonas spp, uncultured_uncultured_Alphaproteobacteria* and *Acidisphaera_uncultured_soil* recorded more than 3% representativeness each. The *Unknown category* of the species made a significant contribution, hence being the third dominated species category with more than 5% representativeness in the MWSS sequence reads after the first quarter (Figure 4).

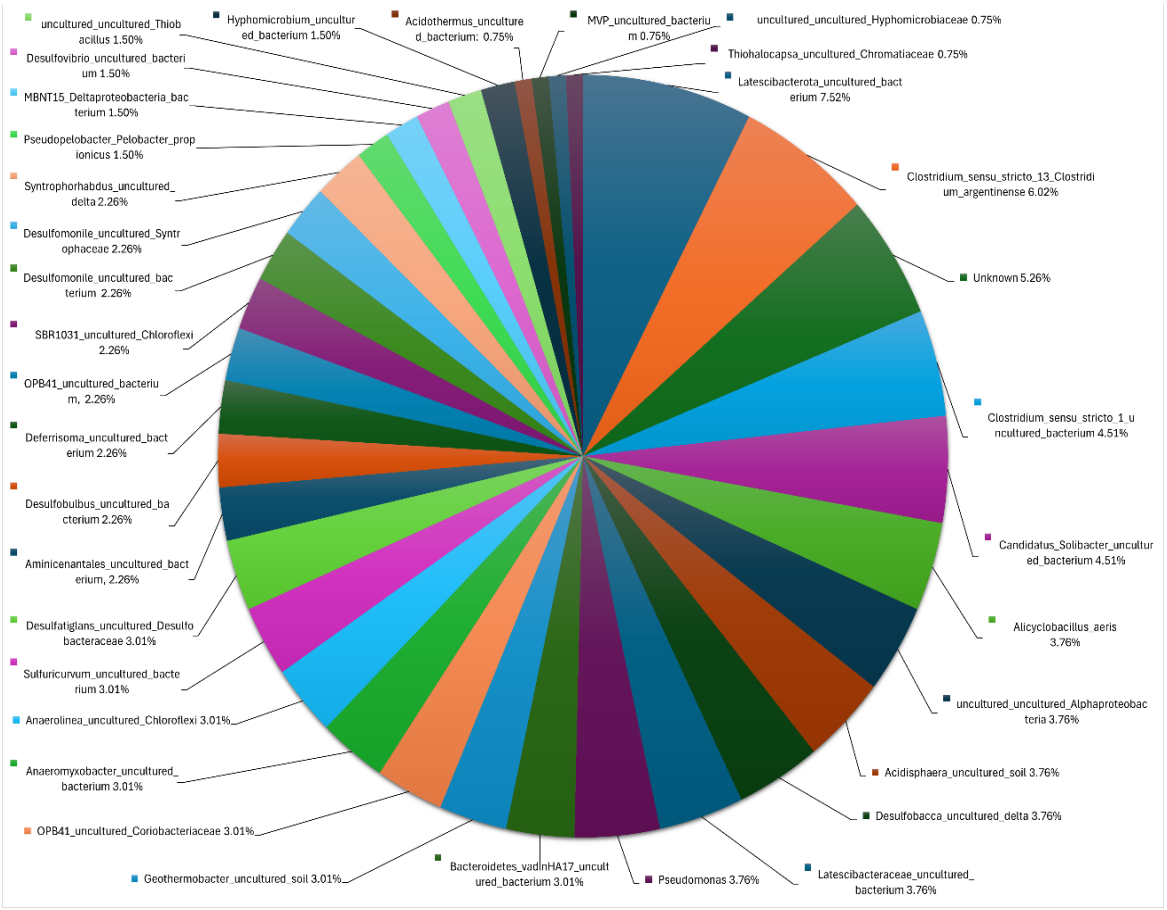

Figure 4: Overview of abundance of 16S rRNA bacterial species after first quarter from metal coupon biofilms in MWSS

In the analysis of the microbial successions assessment in metal coupon biofilm from MWSS after the second quarter of metal coupon insertion, 188 sequence reads were obtained from which thirty-eight (38) known bacteria species and an Unknown category of species were characterized within thirteen (13) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, MBNT15, Spirochaetota, Myxococcota, Latescibacterota,* and *an*

Unknown category. The *Latescibacterota_uncultured_bacterium* from the *Latescibacterota* phylum dominated the sequence reads with almost 6% representativeness followed by the *Unknown category* of the species which topped the second dominated species category with more than 5% representativeness in the MWSS sequence reads.

The *Candidatus_Solibacter_uncultured_bacterium* specie was third with more than 4% representativeness. The *Desulfobacteriota* the most dominated and diverse phylum in MWSS recorded more than 24% sequence reads containing ten (10) species, with *Desulfobacca_uncultured_delta* and *Desulfovibrio_uncultured_bacterium* recording more than 3.5% read each. *Bacteroidetes_vadinHA17_uncultured_bacterium* from the *Bacteroidota* phylum persisted with more than 3.5% representativeness together with *Pseudomonas spp* and *Latescibacteraceae_uncultured_bacterium* which recorded the same sequence reads each. The *Firmicutes, Alicyclobacillus_aeris* and *Clostridium_sensu_stricto_13_Clostridium_argentinense* recorded about 3% sequence reads each. These and other species recorded with their sequence reads are shown in Figure 5. The *Proteobacteria* remains the second most abundant phylum in MWSS with about 16% sequence reads containing eight (8) species. The observed abundance of the *Unknown* species category signifies that the bacteria communities that are yet to be assign any metabolic role whose specific generic identity could not be determined could be playing significant roles in the corrosion process of the metal coupon whether directly or indirectly.

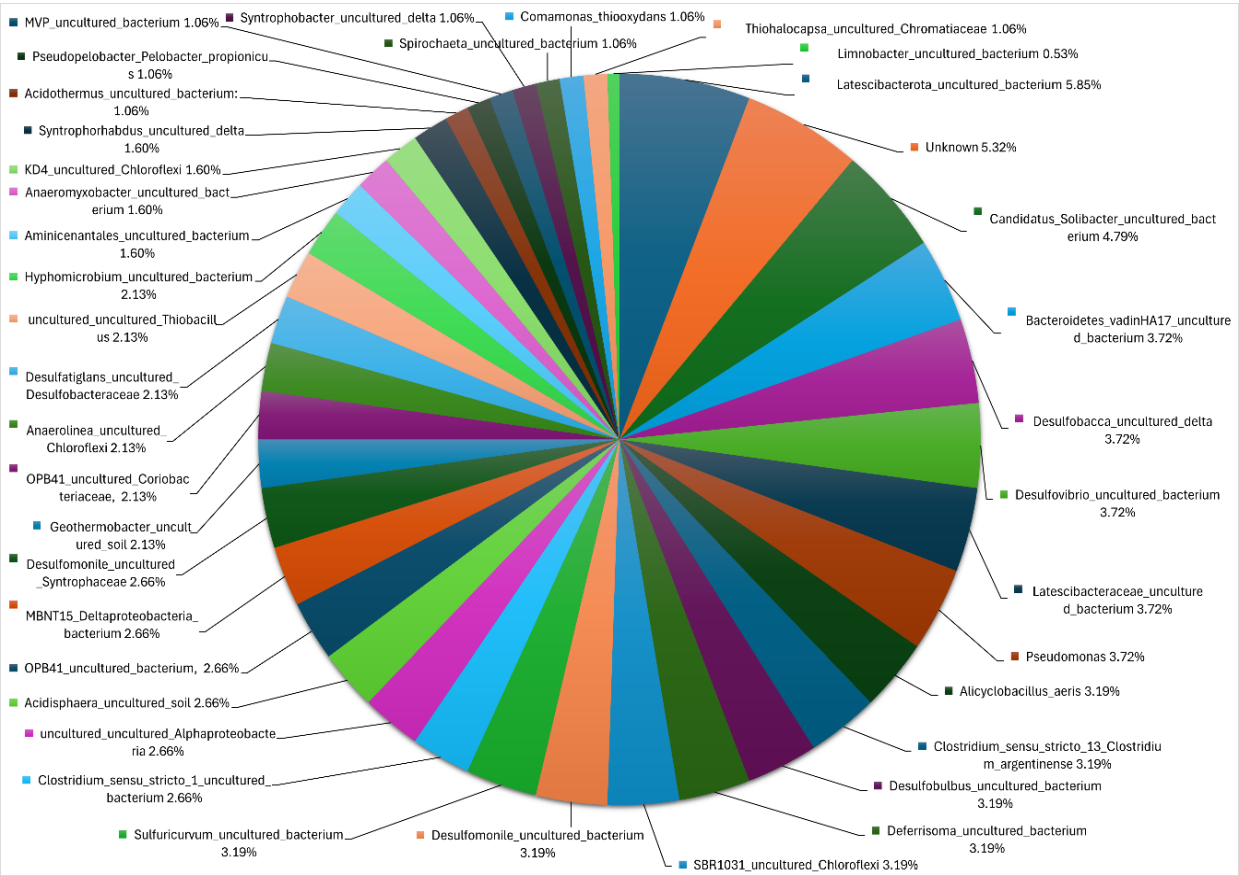

Figure 5: Overview of abundance of 16S rRNA bacterial species after second quarter from metal coupon biofilms in MWSS

The biofilm on the metal coupon from MWSS after the third quarter of metal coupon insertion, 201 sequence reads were obtained from which thirty-nine (39) known bacteria species and an Unknown category of species were characterized within thirteen (13) known phyla of bacteria: *Firmicutes, Proteobacteria, Desulfobacteriota, Acidobacteriota, Actinobacteriota, Campylobacterota, Bacteroidota, Deferrisomatota, Chloroflexi, MBNT15, Spirochaetota, Myxococcota, Latescibacterota,* and *an Unknown category*. The *Latescibacterota_uncultured_bacterium* and the *Unknown* species *category* dominated the sequence reads with more than 5% representativeness followed by the *SBR1031_uncultured_Chloroflexi* from the *Chloroflexi* phylum with about 5% representativeness in the MWSS sequence reads, then *Candidatus_Solibacter_uncultured_bacterium* specie with more than 4% representativeness. The *Firmicutes, Clostridium_sensu_stricto_1_uncultured_bacterium, Alicyclobacillus_aeris* and

Clostridium_sensu_stricto_13_Clostridium_argentinense recorded about 4% sequence reads each. The *Desulfobacteriota* remains the most dominated and diverse phylum in MWSS recording about 24% sequence reads containing ten (10) species, with *Desulfobacca_uncultured_delta* and *Desulfovibrio_uncultured_bacterium* recording more than 3% read each. *Bacteroidetes_vadinHA17_uncultured_bacterium* from the *Bacteroidota* phylum, *Deferrisoma_uncultured_bacterium, Latescibacteraceae_uncultured_bacterium* and *Pseudomonas spp* recorded the same sequence reads of about 3.5% each. These and other species records with their sequence reads are shown in (Figure 6) The *Proteobacteria* remains the second most abundant phylum in MWSS with about 16% sequence reads containing nine (9) species.

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.

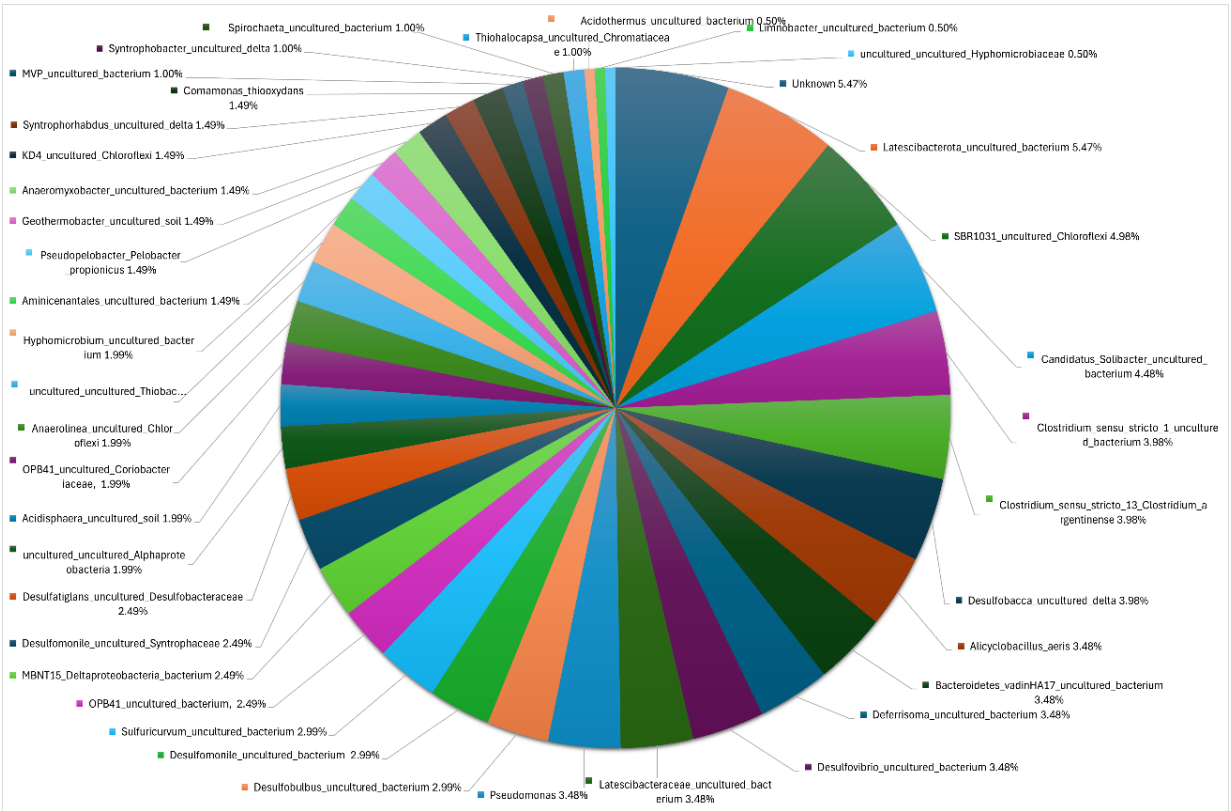

Figure 6: Overview of abundance of 16S rRNA bacterial species after third quarter from metal coupon biofilms in MWSS

IV. Discussions

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 [27]. The high corrosion rates observed in the estuarine 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 [28], 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 [29] Osadebe *et al.,* [28] 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.

Adenosine Triphosphate (ATP) is usually found in all living organisms, which could explain the phosphorus peak relating to cells involved with the corrosion deposit. Phospholipids are found in the membranes of all bacteria and contain a fixed proportion of the biomass as phospholipid as reported by Little *et al*. [30]. Therefore, the presence of phosphorus on MWSS metal coupons was definitive proof of microbial induced corrosion. Also, activities of sulphate reducing bacteria and manganese oxidising bacteria produced surface bound sulphur and manganese respectively. Therefore, the presence of sulphur and manganese peaks was a definitive proof for microbial induced corrosion. The presence of sulphur can create a serious risk to metallic materials. Sulphur aids in pit initiation [30]. The presence of sulphur on the external surface of pipeline is a strong indication of microbiologically influenced corrosion [31]. The sulphur and sulphur compounds in the freshwater can promote proliferation of sulphate reducing or sulphur oxidizing bacteria.

The distinct difference in corrosion morphology, with pitting being prominent in biologically active systems, underscores the localized and aggressive nature of microbiologically influenced corrosion. Bacteria preferentially colonized the grain boundaries on steel, suggesting that intergranular MIC might contribute to the overall corrosion [32]. This observation in Figure 2 was consistent with pit deepening in steel samples exposed to marine biofilms [33,34].

This study described the bacteria population dynamics, present in biofilm build-up, over metal coupons inserted in an estuarine sediment condition. It established the adherence of microorganisms in coupons inserted in estuarine sediment, maybe benefiting from the carbon steel. A distinct succession of 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 [22, 35], with prevalence of *Desulfobacterota* followed by *Proteobacteria* and *Firmicutes* phyla and other significant phyla with a small number of representatives of the Unknown category of bacteria phylum. This observation of the abundance of the Unknown species category, signifies that the bacteria communities that are yet to be assign any metabolic role whose specific generic identity could not be determined could be playing significant roles in the corrosion process of the metal coupon whether directly or indirectly. 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 [36].

Despite that *Latescibacterota_uncultured_bacterium* from the *Latescibacterota* phylum dominated the sequence reads with more than 7% representativeness, the Desulfobacteriota were the most dominated and diverse phylum in MWSS with about 24% sequence reads containing ten (10) species among which *Desulfobacca_uncultured_delta* and *Desulfatiglans_uncultured_Desulfobacteraceae* recorded significant representativeness of about 4% each after the third quarter. The Proteobacteria phyla include species; *Pseudomonas spp., uncultured_uncultured_Alphaproteobacteria, Thiohalocapsa_uncultured_Chromatiaceae, Acidisphaera_uncultured_soil, Comamonas_thiooxydans, uncultured_uncultured_Thiobacillus, Hyphomicrobium_uncultured_bacterium, Limnobacter_uncultured_bacterium, uncultured_uncultured_Hyphomicrobiaceae;* that maintained abundance of about 15%. 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 [22, 27]. Interestingly, under in situ conditions of assessment, there is a prevalence of bacteria species from the *Zetaproteobacteria* class [35, 37]. Also, the dynamics pattern in the bacterial species diversity and population mostly for the Proteobacteria and Desulfobacteriota 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, nitrogen, 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.

The outcomes deduced from Figure 3 in this study showed several similar phyla of bacteria when compared with other similar studies [22, 35]. Among the relatively abundant phyla found in the biofilm on the metal coupon, *Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes*, and *Chloroflexi* [20,38,39,40,41,42,43], have been studied and found to be typical corrosive microbial communities [44]. The category classified as "*Unknown*" at the phylum level in the MWSS, 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 environments harbour 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.

Proteobacteria also was one of the most diverse and abundant bacterial phyla studied with respect to carbon steel biocorrosion in seawater, with sequences affiliated mainly to *Rhodobacterales* [37]. In a laboratory system simulating different seawater conditions, such as stagnant and active flux water and mild steel or pyrite as substrate, the presence of P*roteobacteria* representatives was described in all conditions, with a prevalence in conditions where iron material from the bottom of the aquarium was added at the end of the experiment [27]. *Proteobacteria* phylum is among the nine most widely distributed bacterial lineages in marine habitats [45].

Other species belonging to several phyla: *SBR1031_uncultured_Chloroflexi, Desulfobacca_uncultured_delta, Alicyclobacillus_aeris, Bacteroidetes_vadinHA17_uncultured_bacterium, Sulfuricurvum_uncultured_bacterium, MBNT15_Deltaproteobacteria_bacterium, uncultured_uncultured_Alphaproteobacteria, Acidisphaera_uncultured_soil, uncultured_uncultured_Thiobacillus, Hyphomicrobium_uncultured_bacterium, Pseudopelobacter_Pelobacter_propionicus, Anaeromyxobacter_uncultured_bacterium, KD4_uncultured_Chloroflexi, Syntrophorhabdus_uncultured_delta, Comamonas_thiooxydans, MVP_uncultured_bacterium, Syntrophobacter_uncultured_delta, Spirochaeta_uncultured_bacterium, Thiohalocapsa_uncultured_Chromatiaceae, Acidothermus_uncultured_bacterium, Limnobacter_uncultured_bacterium, uncultured_uncultured_Hyphomicrobiaceae, Candidatus_Solibacter_uncultured_bacterium,* were all persisting in the biofilms across the periods of the incubation.

The detection of acid producing bacteria *(Clostridium_sensu_stricto_1_uncultured_bacterium and Clostridium_sensu_stricto_13_Clostridium_argentinense),* sulphate reducing bacteria (*Desulfomonile_uncultured_Syntrophaceae, Desulfatiglans_uncultured_Desulfobacteraceae, Desulfobulbus_uncultured_bacterium, Desulfomonile_uncultured_bacterium, Desulfovibrio_uncultured_bacterium, Latescibacteraceae_uncultured_bacterium),* iron reducing bacteria (*Pseudomonas spp, OPB41_uncultured_bacterium, OPB41_uncultured_Coriobacteriaceae, Deferrisoma_uncultured_bacterium, Geothermobacter_uncultured_soil, Anaerolinea_uncultured_Chloroflexi, Aminicenantales_uncultured_bacterium and Latescibacteraceae_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 [35]. *Bacteroidetes_vadinHA17_uncultured bacterium* is a member of the phylum *Bacteroidota* was 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 [46].

V. Conclusion and Implications

The research established that biocorrosion of metals were significantly enhanced by the selected bacterial groups based on corrosion rate, dynamics in the population of the selected bacterial groups bacteria during biocorrosion, elemental analysis of corrosion deposits and corrosion morphology. All these specified that the bacterial biofilms significantly induced and facilitated corrosion of coupons used in this study. The presence of several bacterial species on the metal coupons proves diverse microbial contributions to corrosion processes. Among other phyla, *Desulfobacterota, Proteobacteria and Firmicutes* dominated MWSS coupons. These bacterial species not only dominate, also they persisted throughout the experimental period, possibly provides favourable conditions and substrates for competitive coexistence in the biofilm structure, emphasizing the complex interplay between microbial communities and corrosion dynamics.

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. Consequently, culture-dependent methods are insufficient for the environmental study of MIC.

Hence, the study elucidates the complex interactions between microbial diversity and corrosion in estuarine environment, advocating for further investigation of the unidentified "Unknown" strains. Such efforts may enhance our understanding of MIC dynamics, informing better materials protection strategies in marine and estuarine applications.

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