

## MICROBIOLOGICALLY INFLUENCED CORROSION OF MILD STEEL IN CRUDE OIL ENVIRONMENT

Agarry, S. E., Salam\*, K. K., Arinkoola, A. O. & Soremekun, I. O.

Biochemical and Petroleum Engineering Laboratories, Department of Chemical Engineering, Ladoko Akintola University of Technology, P. M. B. 4000, Ogbomoso, NIGERIA  
Corresponding author: kaykaysalam@yahoo.co.uk

### ABSTRACT

In this study, the roles of microorganisms on the corrosion of mild steel in various simulated crude oil environments have been investigated experimentally under three (3) operating conditions namely: pH, salinity and nitrate. Since the presence of water supports microbial life, physicochemical properties and Total Microbial Count (TMC)) of the formation water was determined before adding it to crude oil. Corrosion analyses were performed by weight loss technique, microstructure examination and Fourier Transform Infrared Spectroscopy (FTIR). Microbiological analyses by isolation and identification using appearance factors were conducted on the biofilms formed. The result of physicochemical and biological characterization show that the levels of measured parameters favour the promotion of microbiologically influenced corrosion (MIC). The corrosion rates showed that high acidity ( $\text{pH} < 6$ ) and high alkalinity ( $\text{pH} > 8$ ) favours the growth and activities of microorganisms while increase in salinity and nitrate concentration of crude oil media hinders the growth and activities of microorganisms in the corrosion of mild steel. Microstructure examination depicted more severe pitting corrosion of mild steel in crude oil environment dominant in acidity than salinity and nitrate concentration. FTIR mainly revealed absorption band of  $-\text{OH}$ ,  $\text{COOH}$  and  $\text{NH}_2$  indicating the presence of extracellular polymeric substances (EPS). Seven isolates of bacteria, predominantly negative gram strain (Gram-negative), were observed. In all, this study provides valuable insight into the MIC of mild steel by bacteria in crude oil environments.

**Keywords:** Microbiologically Influenced Corrosion, Mild Steel, Corrosion rate, Crude Oil, Biofilm, Bacteria, FTIR.

### INTRODUCTION

Throughout the world, pipelines play a highly crucial role in the transportation of petroleum (crude oil) and its allied products at all stages of production ranging from downhole to surface and processing facilities (Abd El-Lateef *et al.*, 2012). In the oil industry, production, processing and transportation of crude oil has been estimated to consume about 8% of metals found in the world with the same industry characterized by high rate of corrosion (Anshul and Siddharth, 2012). Most of the pipelines in the world are constructed using mild steel, as it allows the pipes to not only be easily welded in place, but also permits bending and avoids cracking and breaking under pressure. It is the least expensive and most common steel used, very hard, and although easily corrosive, it is also very durable (Prakash *et al.*, 2006). It is estimated that 40% of all internal pipeline corrosion in the oil industry can be attributed to microbial corrosion (Rajasekar *et al.*, 2010). Corrosion related problems worldwide have been estimated to cost as much as 1.8 trillion US dollars with microbial influenced corrosion contributing to approximately 50% of this total (Lin and Ballim, 2012).

The influence of microorganisms in corrosion of metals in numerous systems such as reservoir, oil pipelines, surface equipment, underground structures, wastewater and other effluent treatment facilities have been well documented (Babu *et al.*, 2006; Abd El-Lateef *et*

*al.*, 2012; Anshul and Siddharth, 2012; Rim-Rukeh, 2012). The process in which microorganisms initiate, enhance, facilitate or aggravate corrosion processes of metals is what is termed microbiologically influenced corrosion (MIC) (Beech *et al.*, 2000; Lin and Ballim, 2012; Rim-Rukeh, 2012). Studies have shown that bacteria are the primary causative agents of MIC. The common types of bacteria involved in corrosion processes are Sulphate-Reducing bacteria (SRB) (*Desulfovibrio spp.*), Nitrate Reducing Bacteria (NRB) (*Pseudomonas spp.*) Iron Oxidizing Bacteria (IOB) (*Gallionella spp.*) and Acid-Producing Bacteria (*Thiobacillus spp.*) (Beech *et al.*, 2000; Al-Tai, 2011; Rim-Rukeh, 2012).

Microorganisms, through their adhesion and growth on metal surfaces, influence the kinetics of the corrosion processes of the metals through the formation of corrosion cells. They seek irregularities on metal surfaces where they can attach themselves and secrete corrosive by-products such as hydrogen sulphide, sticky polymers, enzymatic products and other metabolites which can deteriorate metals (Sreekumari *et al.*, 2005; Puyate and Rim-Rukeh, 2008; Lin and Ballim, 2012). However, Lewandowski and Beyenal (2008) reported that microorganisms have two major roles in MIC: its acceleration or inhibition. The major factors involved in the enhancement and inhibition of corrosion (formation of biofilms), are the properties of the metal in question and the environment in which it is exposed to (Puyate and Rim-Rukeh, 2008).

Microorganisms perform corrosive actions in the form of biofilms which are a complex microbial community formed from colonization of different species. These biofilms which have many stages of formation such as microbes transportation to the metal surface, formation of films from absorption of organic molecules, attachment of the microbes to themselves with the aid of exopolymers and subsequently expansion, have been observed to contain over 90% water, extracellular polymeric substance (EPS) and inorganic matter (Augustinovic *et al.*, 2012). MIC has been described as an interfacial process in that biofilms alter the properties such as pH, salinity, oxygen levels and nutrients at the interface between the metal and the environment (Rim-Rukeh and Ierhievwie, 2012).

Rajasekar *et al.* (2004) worked on bacterial degradation of naphtha and its influence on corrosion in a storage tank. Corrosion studies were carried out by gravimetric and weight loss methods which uniform corrosion and higher corrosion rate of coupons observed in naphtha with water than in naphtha only. Naphtha degradation by microbes was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) which revealed the formation of primary alcohol during degradation process with the microbes degrading  $(-\text{CH}_2-\text{CH}_2)_n$  to  $\text{R}-\text{CH}_3$ . Iron bacteria, manganese oxidizing bacteria, acid producers, and heterotrophic bacteria were enumerated and identified in the pipeline while SRB could not be noticed.

Rajasekar *et al.*, (2010) conducted a research on microbial communities in petroleum pipeline and their relationship with biocorrosion. Isolation and enumeration of the bacteria consortia was performed on diesel and naphtha pipelines by culture-dependent techniques and 16S rRNA gene sequencing (bacterial DNA finger printing). The samples obtained from the diesel and naphtha transporting pipelines showed the presence of ten and five bacterial genera respectively.

Rajasekar and Ting (2010) conducted a study on microbial corrosion of aluminium 2024 aeronautical alloy exposed to a simulated aviation fuel storage tank environment by Hydrocarbon-degrading bacteria *Bacillus cereus* ACE4 (Gram-positive bacteria) and *Serratia*

*marcescens* (Gram-negative bacteria) ACE2. Electrochemical impedance spectroscopy and metallographic analysis showed that both bacteria caused pitting corrosion while Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDAX), Atomic Force Microscopy (AFM) and Fourier Transform Infrared spectroscopy (FTIR) of the bacterial biofilm ascertained the presence of EPS and the formation of biofilms as microcolonies. The study thus provided valuable contribution to the problem associated with fuel/water mixtures.

In the present study, an attempt has been made to investigate the effects of microbes on the corrosion of mild steel in simulated crude oil environments (crude oil and formation water mixtures) with different operating conditions (pH, salinity and nitrate). The physicochemical and biological characteristics of the formation water were analysed and corrosion rates were determined using weight loss technique; microstructure analysis was applied to identify the presence of pitting corrosion on the metals; the bacterial species were identified up to gram staining level and characterized using FTIR.

## MATERIALS AND METHOD

### Sample Collection and Coupon preparation

The crude oil and formation water samples used for this study were obtained from Nigerian Petroleum Development Company (NPDC) located at 62/64, Sapele Road, Benin City, Edo State, Nigeria.

The elemental composition of mild steel sheet used for this study was tabulated in Table 1. mild steel was cut into coupon ( $4\text{ cm} \times 2\text{ cm} \times 0.1\text{ cm}$ ) and machine to remove any superficial rust at the Mechanical Engineering Workshop of Ladoke Akintola University of Technology, Ogbomosho, Nigeria, Thereafter, the coupons were sterilized by immersing them in pure ethanol, cleaning them with cotton wool and drying them using a desiccator. The exposed surface area of each coupon is  $17.2\text{ cm}^2$  and is calculated as:

$$A = 2(LB + LH + BH) \quad (1)$$

where

$L = 4\text{ cm}$  (length of each mild steel coupon)

$B = 2\text{ cm}$  (width of each mild steel coupon)

$H = 0.1\text{ cm}$  (thickness of each mild steel coupon).

The prepared coupons were weighed before each test using a weighing balance (Setra BL-410S) with the mass of each coupon determined to the nearest 0.001 g.

**Table 1:** Elemental Composition of Mild Steel Used

C (%)	Mn (%)	Si (%)	P (%)	S (%)	Cr (%)	Ni (%)	Mo (%)	V (%)	Ti (%)	Al (%)	Fe (%)	Cu (%)
0.06	1.05	0.27	0.006	0.002	0.02	0.02	0.008	0.05	0.02	0.05	9.424	0.02

### Analysis of Formation water

The formation water was characterized for the following physicochemical parameters: pH, redox potential, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), nitrate ions ( $\text{NO}_3^-$ ) and sulphate ions ( $\text{SO}_4^{2-}$ ); and for the biological parameter - Total Microbial Count (TMC). These parameters are good environmental impact indicators for microbiologically influenced corrosion problems (Puyate and Rim-Rukeh, 2008; Rim-Rukeh and Ierhievwie, 2012). pH was determined with Sension MM-150 multimeter; redox potential with Perk America 300; BOD, COD and DO were determined by

titration; nitrate and sulphate with UV/VIS spectrometer (Jenway-6405) and TMC with rapid agar dipstick method. The rapid agar dipstick method was used based on its simplicity and reliability. Physicochemical characterization of the formation water obtained from a functioning reservoir was done in order to find out the root cause of corrosion (Martinez *et al.*, 2012).

### **pH, Salinity and Nitrate Variation Analysis with Immersion Time**

Four different pH values were analysed for corrosion in this study: 4, 6, 8 and 10. Equal quantities of crude oil media were measured into five (5) disposable containers for each of the pH values under study. The initial pH values of the samples were measured with a Senson MM-150 multimeter. The target pH was obtained for each analysis by varying the addition of 2M H<sub>2</sub>SO<sub>4</sub> and 1M NaOH into the media. The salt used for this test was NaCl. 1 g of the salt was also added into equal quantities of crude oil media in each of five (5) disposable containers. The procedure was repeated with 2, 3 and 4 g of the salt. The weights of the salt added to each sample were determined with the aid of the weighing balance (Setra BL-410S). Calcium trioxonitrate (V) tetrahydrate (Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O) was the nitrate used and the same procedure as the salinity analysis was followed.

It is important to note that crude oil media (10 ml crude oil + 5 ml formation water) was maintained throughout the experiment. The analyses for the different operating parameters were conducted for immersion periods of 7, 14, 21 and 28 days after which the coupons were removed, washed in distilled water, cleaned with ethanol and then dried with the aid of a desiccator.

### **Corrosion Studies**

The final weights of the mild steel coupons in the desiccator were determined and their corrosion rates were calculated in mils per year (a mil is a thousandth of an inch) as specified by Bradford (1993) in Equation 2.

$$C = \frac{k\Delta M}{A\rho t} \quad (2)$$

Where

$C$  = corrosion rate of rectangular coupon (mils/yr.)

$k$  = corrosion rate constant ( $3.45 \times 10^6$  mils/yr.)

$\Delta M$  = weight loss of coupon (g)

$A$  = total exposed surface area of coupon (cm<sup>2</sup>)

$\rho$  = density of coupon (g/cm<sup>3</sup>)

$t$  = time of immersion (h)

It is important to note that the corrosion rates were calculated assuming uniform corrosion over the entire surface area of the mild steel coupons (Akpabio *et al.*, 2011). The corrosion studies were carried out considering the variation of different parameters viz: pH, salinity, nitrate concentration.

### **Microstructure Examination**

After the expiration of the last corrosion period (28 days), the corroded mild steel coupons used for this analysis were selected based on their relatively high corrosion rates compared with other samples (as obtained from the corrosion studies). The coupons were withdrawn from their respective crude oil media and the biofilms formed on them were removed with a

surgical knife. The microstructure was examined for both the control and corroded coupons using inverted metallurgical microscope (XJL-17) with fitted digital camera at a magnification of ( $\times 10$ ).

## Characterization of Microorganisms

### Isolation of Microorganisms

The biofilms formed on the surfaces of the same mild steel coupons used for the microstructure examination were each separately gently scraped into distilled water in different beakers. The sample was serially diluted (10 fold) using 9 ml of sterile distilled water-blanks and the sample was plated by the pour plate technique. The nutrient agar medium was used to isolate the bacteria. The sample was further serially diluted up to  $10^{-2}$  dilution. 1 ml of the sample was poured into sterile petri-dish. The prepared sterile medium was also poured into the petri-dish. The plate was gently swirled so that the medium might be distributed evenly in the plate. The plate was incubated at room temperature for 24 hours. Morphologically dissimilar colonies were selected randomly from the plate and isolated colonies were purified using an appropriate medium by streaking methods. The isolated pure cultures were maintained in test tubes as slant culture for further analysis. The strains were maintained at  $40^{\circ}\text{C}$  in Laboratory Incubator DNP-9082 to keep the bacterial strain viable.

The isolated bacterial cultures were identified through morphological characterization by gram staining. A loop was used to pick a little of the bacterial isolates and each was smeared on different slides which were dried with heat from a flame. Crystal violet was added to cover the slides for thirty (30) seconds after which it was rinsed with water for five (5) seconds. Addition of iodine followed which was allowed to stand for sixty (60) seconds and rinsed for five (5) seconds as well. The stained slides were decolourized with 95% ethanol for thirty (30) seconds and then counter stained with safranin for sixty (60) seconds. XJL-17 Microscope was used to view the stained slides. Observation of purple/violet colouration indicated the presence of Gram positive (G +ve) bacteria while pink/red colouration indicated the presence of Gram negative (G -ve) bacteria.

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier Transform Infrared Spectroscopy (FTIR) was used for the analysis of the biochemical characteristics of three (3) of the isolated bacterial cultures with the aid of Buck M-530 Infrared Spectrophotometer. The spectrum was taken in the mid IR region of  $400 - 4000 \text{ cm}^{-1}$ . The spectrum was recorded using Attenuated Total Reflectance (ATR) technique. A portion of the sample was directly placed in the zinc selenide crystal and the spectrum was recorded in the transmittance mode.

## RESULTS AND DISCUSSION

### Physicochemical and Biological Characterization of Formation Water

The results of the physicochemical and biological characterization tests of the formation water sample are given in Table 2.

**Table 2:** Results of the Characterization Tests for Formation water (Formation Water)

Parameter	Value
pH	6.8
Redox Potential (mV)	No result
BOD (mg/l)	100

COD (mg/l)	1000
DO (mg/l)	300
NO <sub>3</sub> <sup>-</sup> (ppm)	125
SO <sub>4</sub> <sup>2-</sup> (ppm)	18.18
TMC (CFU/ml)	$2 \times 10^4 - 5 \times 10^5$

The results indicated that the formation water was slightly acidic (with a pH of 6.8), which is consistent with the range (4.5 to 9) specified by Puyate and Rim-Rukeh (2008) for microbial activity to take place. The result also gave the value of BOD and COD of the formation water sample to be 100 mg/l and 1000 mg/l respectively. These values showed that the formation water is highly biodegradable such that the need for external organic carbon source is irrelevant for hydrocarbon-degrading microorganisms (Rim-Rukeh, 2012). The concentration of DO in the formation water obtained was 300 mg/l. Rim-Rukeh (2012) asserted that such a high level of DO is indicative of an environment that promotes the growth of facultative or aerobic microorganisms and that the presence of oxygen in an environment plays a key role in the corrosion process as it aids the sustenance of microbial community because microorganisms utilize it as substrate.

The concentrations of nitrate ions (NO<sub>3</sub><sup>-</sup>) and sulphate ions (SO<sub>4</sub><sup>2-</sup>) in the formation water sample were 125 ppm and 18.18 ppm respectively. It has been established that such adequate supplies of nitrate and sulphate are important for the growth and development of microorganisms because nitrogen and sulphur (which are essential elements for cellular metabolism in microbial life) are obtained from nitrate and sulphate respectively (Puyate and Rim-Rukeh, 2008). In particular, sulphate-reducing bacteria (SRB), which are an assemblage of bacteria that can grow in anaerobic environment, do so by oxidizing organic nutrients, with sulphate being reduced to H<sub>2</sub>S. The Total Microbial Count (TMC) in the formation water sample fell within the range ( $2 \times 10^4 - 5 \times 10^5$ ) CFU/ml which indicated that the formation water contained adequate microbial population for the enhancement of effective microbial activity. The high level of microbial count in the formation water sample was an indication of availability of good food supply by the formation water for the microorganisms. This is validated by the established fact that microbial population between  $10^4$  and  $10^6$  CFU/ml in an environment is a concern of potential corrosion problem (Puyate and Rim-Rukeh, 2008; Rim-Rukeh, 2012).

### Corrosion Studies

The results of the corrosion studies carried out considering the variation of different parameters viz: pH, salinity and nitrate concentration. The graphs of the corrosion rates against immersion time are depicted in Figures 1(a-c).

Figure 1a showed that the more acidic the crude oil environment was, the higher the corrosion rate because at pH of 4, the corrosion rate of the mild steel was higher than that of the mild steel exposed to the crude oil media with pH of 6. The corrosion rates of the coupons experienced at pH of 10 was also greater than those obtained at pH of 8 indicating that the more alkaline the medium, the higher the corrosion rate. This validates the claim by Rim Rukeh (2012) that acidic and alkaline environments with pH < 6 and pH > 8 respectively exhibit more corrosion tendencies than other environments with pH between 6 and 8. It has been established that some metals develop a protective surface of oxide film upon exposure to aqueous solutions. This film is most likely responsible for the corrosion resistance of the mild steel when exposed to the environments with pH 6 and 8 (Al Zubaidy *et al.*, 2011).

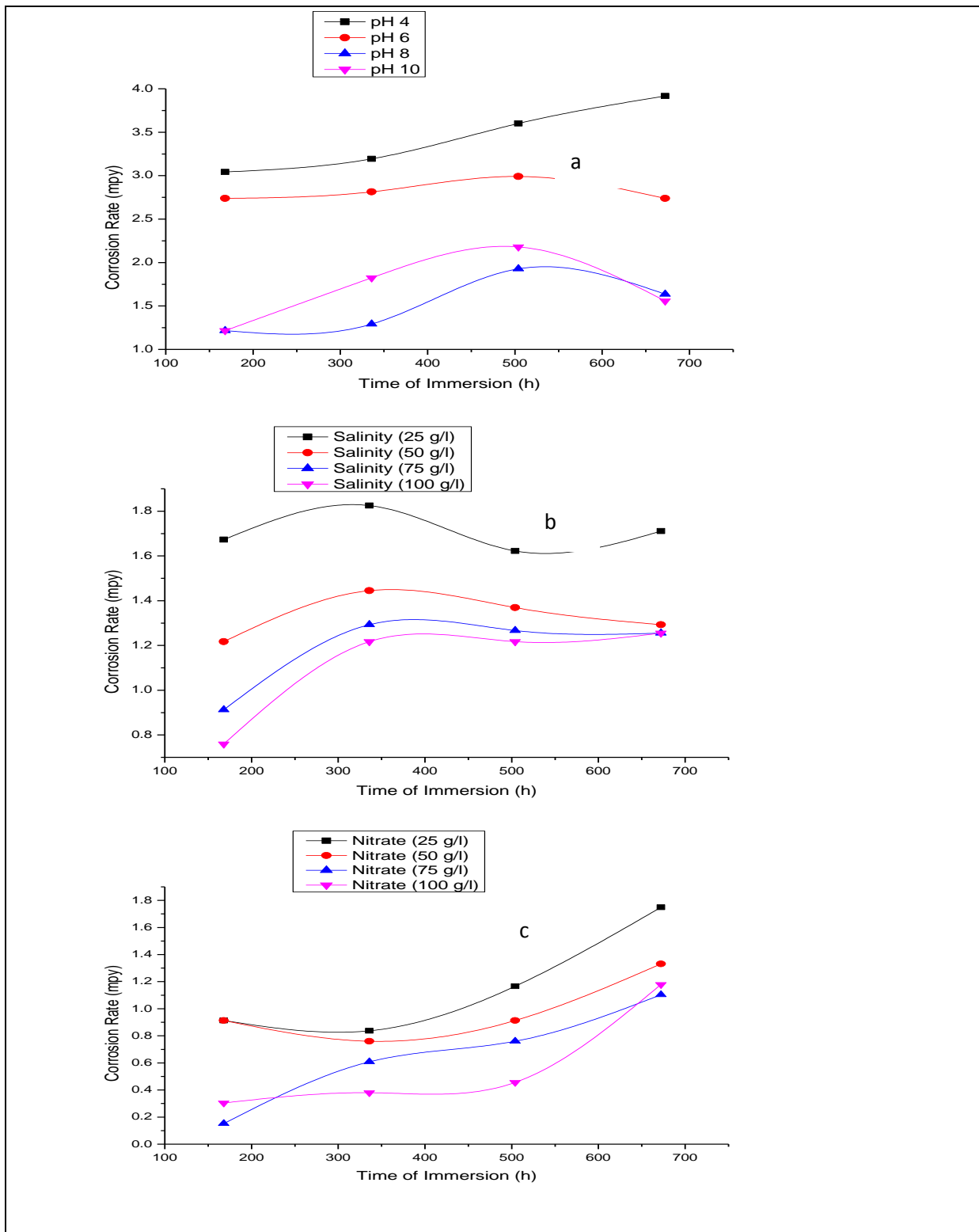


Figure1 Variation of Corrosion Rate with Time of Immersion for Different pH Values, salinity and nitrate concentration

The graph also showed that corrosion rate increased with increase in immersion time for the crude oil media with pH of 4 from 3.042 mpy to 3.916 mpy when the immersion period increased from 168 h to 672 h. Also at pH of 6 – 10, there was an increase in corrosion rate with increase in immersion time from 168 h to 504 h. However, after immersion time of 504 h, there was a decline in corrosion rate until the final immersion period (672 h). These

observations showed that corrosion was promoted at low pH and that the less acidic the crude oil environment, the less effective the microbial activities. The decrease in corrosion rate could be attributed to the thickness of biofilm formed on the surface of the metals at high pH (that is 6 - 10) and high immersion time as have been proved that biofilms have the potential of inhibiting corrosion (Lewandowski and Beyenal, 2008; Little *et al.*, 2006; Rim-Rukeh and Ierhievwie, 2012).

Figure 1b showed that the higher the salinity of the environment, the lower the corrosion rate of mild steel exposed to it. This is because the corrosion rate decreased in the order of increasing salt concentration  $25 > 50 > 75 > 100$  g/l. In coherence with the observations of Al Zubaidy *et al.* (2011), corrosion rate increased with increase in salinity and then decreased to a constant value as a result of maximum combination of high conductivity and the oxygen solubility. From the submission of Babu *et al.* (2006), the microbes present consumed organic compounds and degraded the hydrocarbons present in the environment, converting them into organic acids with the creation of anaerobic environment. Thus, corrosion reduced with increase in salinity because the solubility of oxygen decreased. Such reduction in the amount of dissolved oxygen is not suitable for the survival of microorganisms which influence corrosion (Puyate and Rim-Rukeh, 2008). It has also been determined that oxygen depletion in seawater is a corrosion control measure for unprotected metals (Little *et al.*, 2006).

The graph depicted that corrosion rate increased with increase in immersion time of the mild steel coupons from 168 h to 336 h for all the salt concentrations 25 g/l, 50 g/l, 75 g/l and 100 g/l. However, after immersion time of 336 h, there was a decline in corrosion rate until the final immersion period (672 h). The decrease in corrosion rate could be attributed to the thickness of biofilm formed on the surface of the metals as a result of the high immersion period as have been proved that biofilms have the potential of inhibiting corrosion (Lewandowski and Beyenal, 2008; Little *et al.*, 2006; Rim-Rukeh and Ierhievwie, 2012).

Figure 1c showed that the higher the nitrate concentration of the crude oil environment, the lower the corrosion rate of mild steel exposed to it. This is because the corrosion rate decreased in the order of nitrate concentration  $25 > 50 > 75 > 100$  g/l. The results are consistent with the assertion of Little *et al.* (2006), that the addition of extra amount of nutrients may not stimulate microbial growth. The work stated that nutrients containing excess concentration of nitrate can inhibit pitting corrosion as a result of suppression of microbial activities. In addition, it was ascertained that increased addition of nitrate to a medium can influence a shift in its dominant microbial population from sulphate-reducing bacteria (SRB) (which are mostly responsible for MIC) to nitrate-reducing bacteria (NRB), as well as a remarkable 50% reduction in corrosion. Also, laboratory and field experiments have demonstrated that nitrate treatment, which reduces H<sub>2</sub>S production, can be an effective alternative to biocide treatment to reduce the numbers of SRB and their activity (Zakaria *et al.*, 2012).

These observations are however in contrast to the findings made by Muthukumar (2003) that the addition of nitrate can be used to stimulate the degradation of hydrocarbons in crude oil which is a result of microbial activities. In other words, the higher the concentration of nitrate in crude oil environment, the greater the corrosion tendencies it poses as a result of increased microbial population.

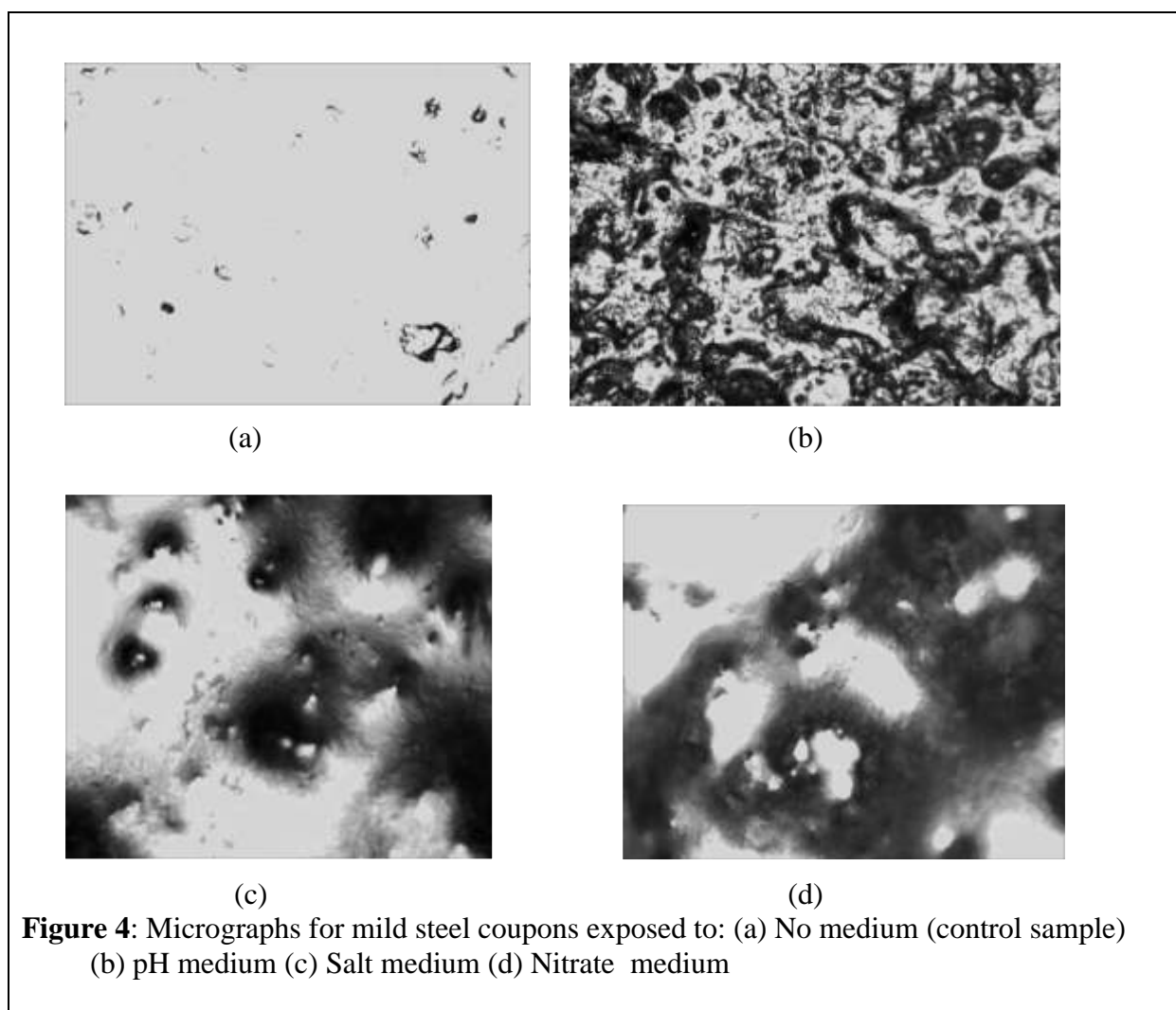
The graph depicted a relative increase in corrosion rate with increase in immersion time of the mild steel coupons from 168 h to 672 h for all the nitrate concentrations 25, 50, 75 and



100 g/l. The increase in corrosion rate could be attributed to the activities of microbial biofilms formed on the surface of the metals as a result of the high immersion period as have been proved that biofilms have the potential of accelerating corrosion (Lewandowski and Beyenal, 2008; Little *et al.*, 2006; Rim-Rukeh and Ierhievwie, 2012).

### Microstructure Examination

The micrographs of the mild steel coupons used in this study before corrosion i.e. (control sample) is shown in Figure 4(a) while those exposed to the selected pH, salinity and nitrate media are shown in Figures 4(b), 4(c) and 4(d). More severe pitting corrosion was observed from the micrograph of the mild steel coupon exposed to the pH medium followed by those exposed to the salinity and nitrate media respectively. These observations corroborate the results of the corrosion studies explained earlier.



### Morphological Characterization

A total of seven bacterial isolates were identified in the biofilms scraped from the surfaces of the corroded mild steel coupons. The result of gram staining the ten isolates identified is as shown in Table 3.

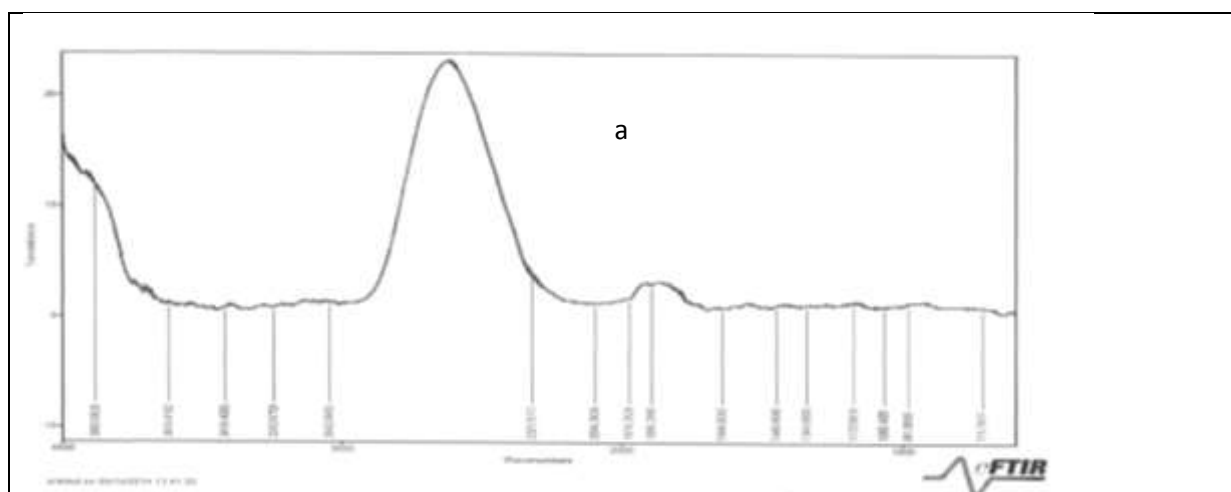
**Table 3:** Morphological Characteristics of the Bacterial Isolates

Parameter	Gram positive (G +ve)	Gram negative (G -ve)
pH	-	3
Salinity	1	1
Nitrate	1	1

According to Akpan and Solomon (2014), Gram positive bacteria possess inherent ability to withstand stressed environment such as oil pipelines due to their thick cell wall which is made up of peptidoglycan. They have up to about 25 sheets of peptidoglycan stacked one upon another in contrast to Gram negative bacteria which have only one sheet. However, Rajasekar *et al.* (2004) concluded that Gram negative bacteria are more active in the degradation of naphtha which is a major hydrocarbon constituent of crude oil. Therefore, from the results obtained, it can be inferred that the dominance of Gram negative bacteria shows that they were more active in the degradation of hydrocarbons in the crude oil thereby enhancing the corrosion process. These characterization tests further validate the claims that bacteria are actually involved in the corrosion processes occurring on the mild steel coupons exposed to crude oil environments.

### Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Figure 6a shows the FTIR spectrum of the biofilm on the mild steel immersed in the pH medium. The IR spectrum analysis showed a peak between 3044 and 3619  $\text{cm}^{-1}$ , which indicates the presence of COOH stretch which is an acid dimer. Figure 6b shows the FTIR spectrum of the biofilm on the mild steel immersed in the salt medium. The spectrum showed a broad peak between 3208 and 3612  $\text{cm}^{-1}$  indicating the presence of OH stretch. Another peak occurs between 2323 and 2358  $\text{cm}^{-1}$ , which indicates the presence of COOH band implying an acid group. C=C cis and vinyl is also noticed at peak 1647  $\text{cm}^{-1}$ . Figure 6c shows the FTIR spectrum of the biofilm on the mild steel immersed in the nitrate medium. The spectrum showed a broad peak between 3039 and 3624  $\text{cm}^{-1}$ , which indicates the presence of OH stretch. It also depicts a peak between 1627 and 1682  $\text{cm}^{-1}$  indicating the presence of a  $\text{NH}_2$  bend.



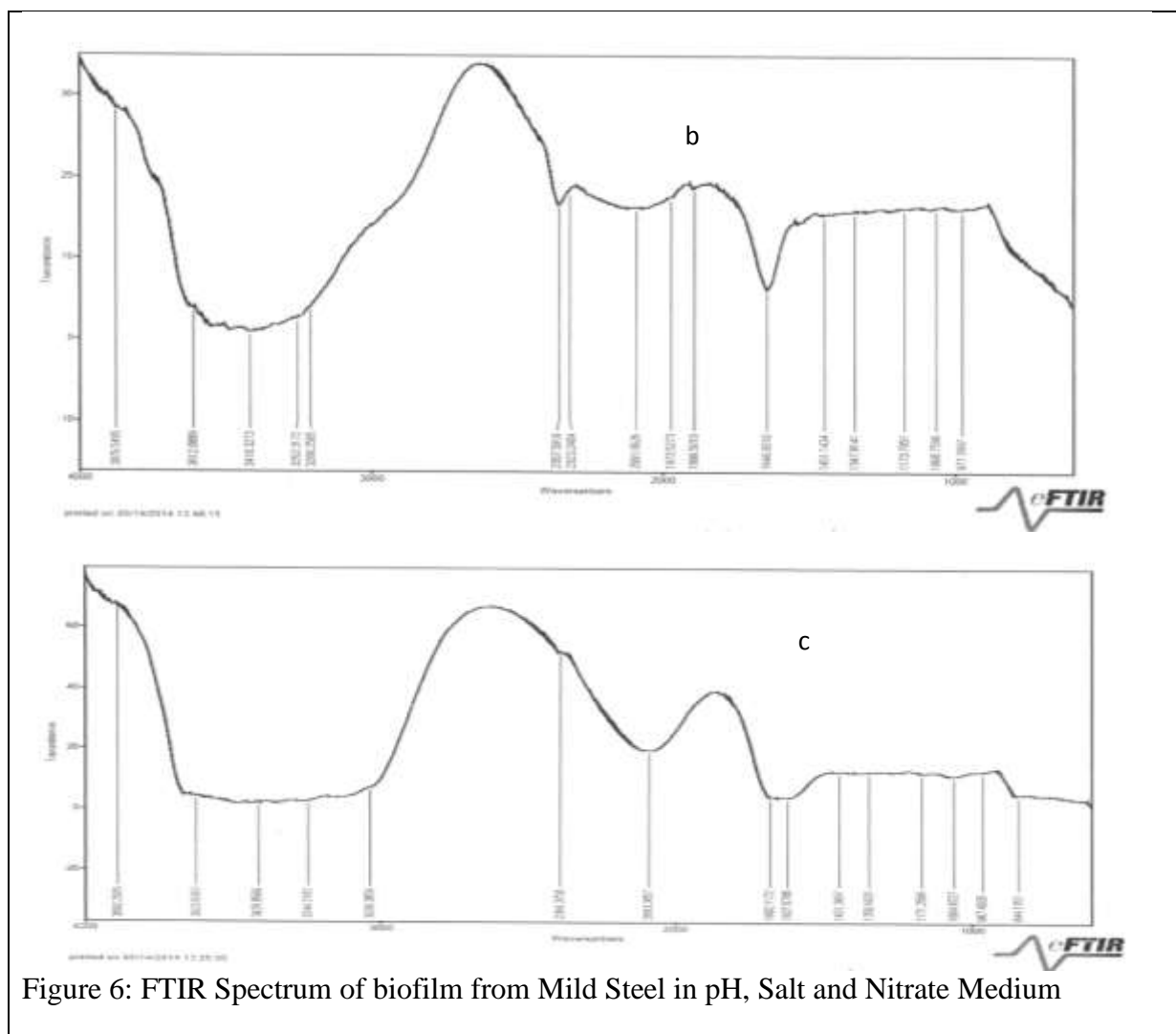


Figure 6: FTIR Spectrum of biofilm from Mild Steel in pH, Salt and Nitrate Medium

As investigated by Lin and Ballim (2012), extracellular polymeric substances (EPS), which are the major building blocks of biofilms, are composed of polysaccharides, lipids, DNA and proteins containing functional groups including carboxylic acid (COOH) and amino acid groups (NH<sub>2</sub>). Kumar *et al.* (2011) also established that the presence of hydroxyl group (OH) and COOH in biofilms indicate the presence of EPS which are able to bind metallic ions. Therefore, the OH, COOH and NH<sub>2</sub> functional groups identified depict the presence of EPS which are capable of inciting the corrosion observed on the mild steel coupons exposed to crude oil environments.

## CONCLUSION

This study considered the influence of three operating conditions of crude oil environments: pH, salinity and nitrate concentration on the growth and activities of microorganisms in the corrosion of mild steel in crude oil environments. It can be concluded from the physicochemical and biological characteristics of the formation water that crude oil environments have all the necessary properties capable of promoting the growth of microorganisms into biofilms which have a great impact on corrosion of mild steel. It can be deduced that increase in salinity and nitrate concentrations of crude oil environments do not enhance corrosion rate. The pH and salinity variation analyses confirm the dual attribute of biofilms either in promoting or inhibiting corrosion of metals. The corrosion studies and

microstructure examination showed that the pH of crude oil environments has more influence on microbial growth and hence corrosion rate, notably acidic and alkaline environments with  $\text{pH} < 6$  and  $\text{pH} > 8$ . Microbial characterization further alludes to the fact that bacteria, more specifically Gram negative bacteria, are more actively involved in the corrosion of mild steel in crude oil environments. The identification of OH, COOH and  $\text{NH}_2$  functional groups from FTIR analysis of the isolated biofilms indicates the presence of EPS, which are the major building blocks of biofilms, thus validating the role of microbial biofilms in the corrosion processes of metals. This study will aid oil industries in material selection as well as maintenance of oilfield equipment and machines against MIC.

## RECOMMENDATIONS

This study potentially identifies the influence of some factors on the MIC of mild steel in crude oil environments, but the influence of different combinations of these factors can also be a menace to oilfield facilities. It is therefore recommended that the combined effects of these factors be investigated and probably modelled for easy quantification and interpretation.

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