CORROSION OF HIGH CARBON STEEL BY BACTERIA UNDER AEROBIC AND ANAEROBIC CONDITIONS

BY

NWOKORIE, ROMANUS CHUKWUDI(B. Tech/Microbiology)
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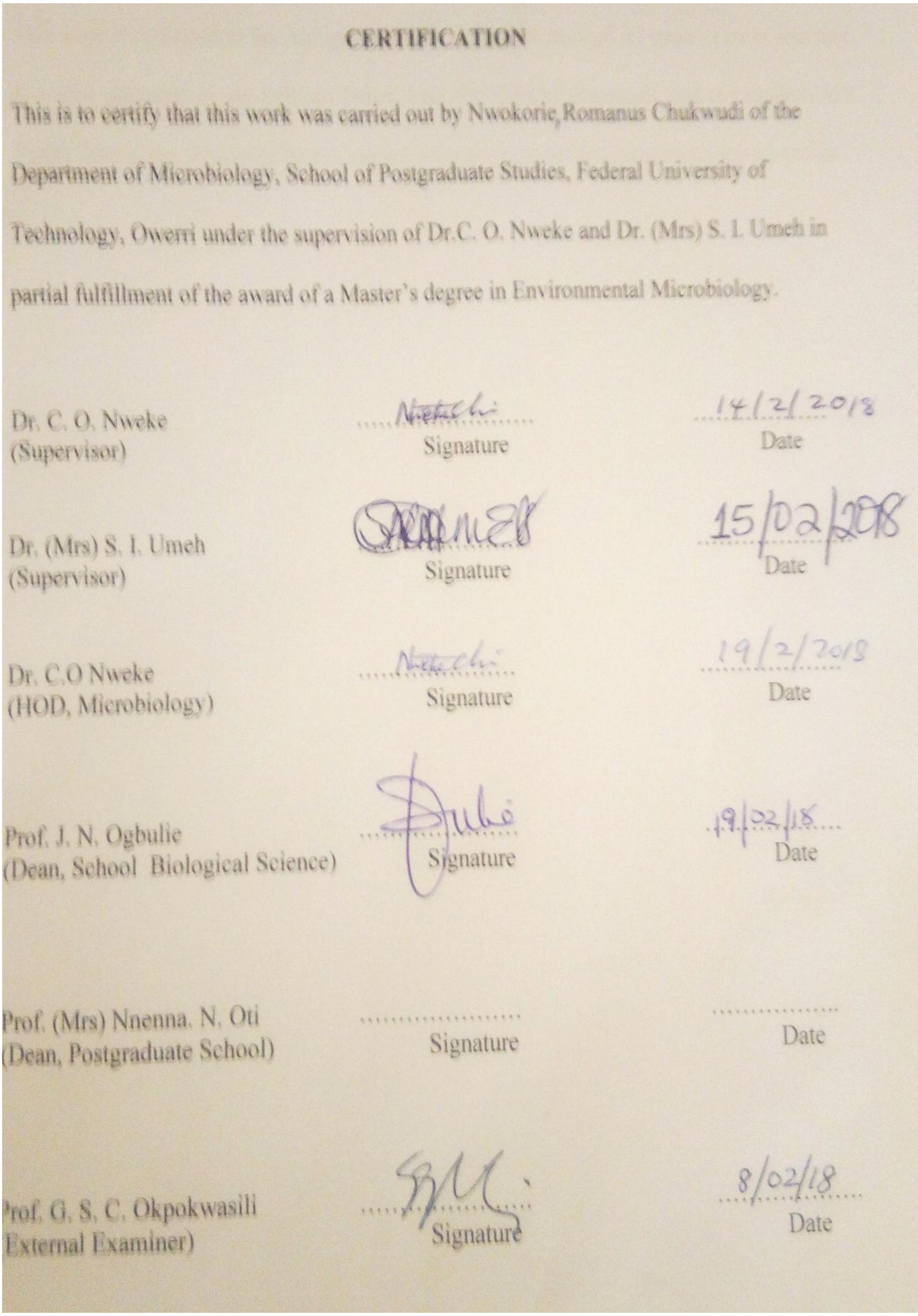

CERTIFICATION

This is to certify that this work was carried out by Nwokorie,Romanus Chukwudi of the Department of Microbiology, School of Postgraduate Studies, Federal University of Technology, Owerri under the supervision of Dr.C. O. Nweke and Dr. (Mrs) S. I. Umeh in partial fulfillment of the award of a Master's degree in Environmental Microbiology.

Dr. C. O. Nweke (Supervisor)	…………………… Signature	14/2/2018 Date
Dr. (Mrs) S. I. Umeh (Supervisor)	…………………… Signature	15/02/2018 Date
Dr. C.O Nweke (HOD, Microbiology)	…………………… Signature	19/2/2018 Date
Prof. J. N. Ogbulie (Dean, School Biological Science)	…………………… Signature	19/02/18 Date
Prof. (Mrs) Nnenna. N. Oti (Dean, Postgraduate School)	…………………… Signature	…………… Date
Prof. G. S. C. Okpokwasili (External Examiner)	…………………… Signature	8/02/18 Date

DEDICATION

This work is dedicated to the Almighty God for seeing me through all these years of learning. It is also dedicated to my beloved father, Late Mr Maurice Nwokorie and my mother, Mrs Eunice Nwokorie, especially for my mother’s immense contributions towards my education.

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ABSTRACT

The role of bacteria in the corrosion of high carbon steel under aerobic and anaerobic conditions was investigated using sulphate reducing bacteria and other aerobes. The microbes isolated from the soil included; aerobes (*Bacillus* species, *Pseudomonas* species*)* and anaerobes (*Desulfovibrio*species, *Thiobacillus*species). The role of bacteria in the corrosion of high carbon steel was estimated using the weight loss technique and the electrochemical potential measurement technique. Eighteen samples of a high carbon steel of known compositions were exposed to sulphate reducing bacteria under different environments (aerobic, anaerobic and control). The physical and chemical changes were examined and monitored on weekly basis for six weeks. It was observed that the coupons immersed in the media with sulphate reducing bacterium underwent fast activation and numerous corrosion sites were formed on the surfaces. The average corrosion rate for six weeks (42 days) period of time as determined by the weight loss method and electrochemical testing were found to be 0.0004595mm/year, -0.712mV in aerobic environment, 0.0005646mm/year, -0728mV in anaerobic environment and 0.0004458mm/year, -0702mV in the control or reference environment (distilled water environment). High carbon steel was found to corrode more only in the anaerobic environment. In view of this, high carbon steels were found to be relatively more corrosion resistant than mild and medium carbon steels and may be suitable for application in industries such as the petrochemical industry.

Keywords: *Bacillus* species, *Pseudomonas* species, *Desulfovibrio*species, *Thiobacillus*species

CHAPTER ONE

1.0 INTRODUCTION

Corrosion may be defined as the deterioration of a material resulting from chemical attack by its environment (Smith, 1990). Metals actually corrode because they succumb to environmental interaction. The environment in this regard may refer to the service conditions where the metal may find application. Steel, due to their desirable mechanical properties, in practice find application in many service conditions including the aquatic environment where the activity of microbes such as bacteria is evident. The role of microbes in the corrosion of metals is due to the chemical activities (metabolism) associated with the microbial growth and reproduction (Shera *et al.*, 2011). In the metabolic and growth process, bacteria can reproduce up to $2.8x10^{14}$ organisms every 10-60 minutes, which is responsible for the corrosion and biodeterioration of materials exposed to such environment (Borode, 1999). Microbial influenced corrosion (MIC) is due to the presence and activities of micro organisms including bacteria and fungi, and affect a wide range of industries resulting in severe economic loss (Koch *et al.*, 2002). Carbon steel, although susceptible to corrosion, is frequently employed in industries and infrastructure because of its strength, availability, relatively low cost and fire resistance (Koch *et al.*, 2002). Infrastructure affected by MIC (buried pipelines) is often operated under anaerobic or micro aerobic conditions (Kozlova *et al.*, 2010). Most studies on anaerobic – micro aerobic MIC use sulphate reducing bacteria (SRBs) because they are sulphide producers and promoters of the cathodic depolarization process in steel (Javaherdashti, 2011: Zhang *et al.*, 2011). According to von Wolzogen and Van der Vlugt (von Wolzogen *et al.,* 1934), under anaerobic conditions, electrons from the metal surface (cathode) reduce protons to form hydrogen, which form film that prevents further proton reduction, thus producing electrostatic isolation (passivation). Different bacteria that colonized the metal surface can consume the hydrogen film, resulting in Fe^{2+} release from the metal surface. Sulphide produced by SRBs combines with and Fe^{2+} to form ferrous sulphide and generate an adhesion film. This mineral film acts as a cathode for hydrogen evolution, thus increasing the corrosion rate (King *et al.,* 1971), but can also have a protective passivation effect depending on the crystalline mineral composition (Halin *et al.*, 2012: McNeil *et al.,* 1990). The differences in SRBs – related corrosive effect are also caused at least in part, by the SRB species themselves (Paisse *et al.,* 2013), in addition to other factors. A natural environment, the reaction leading to steel corrosion intensify with temperature (Benmoussa *et al.,* 2006), and high – temperature environment are characterized by diverse bacteria communities (Urios *et al.,* 2013), many of which may have corrosive effect (Almeida *et al.,* 1999: Rozanova *et al.,* 2003). The corrosive effects of bacteria on metals can be attributed to the removal of electrons from the metal and formation of corrosive products by:

a) Direct chemical action of metabolic products such as sulphuric acid, inorganic and organic sulphides and chelating agents such as organic acids.
b) Cathodic depolarization associated with anaerobic growth.
c) Changes in oxygen potential, salt concentration, pH, etc. which establish local electrochemical cells.
d) Removal of corrosion inhibitors (oxidation of nitrite or amines).
e) The presence of the biomass itself (Shera *et al.,* 2011).

These bacteria therefore are classified into two: those that require oxygen in their metabolic and growth processes referred to as aerobic bacteria and those that carry out their activities in the absence of oxygen referred to as anaerobic bacteria. These bacteria are active in stagnant water mainly at the bottom of tanks, the soil, freshwater, seawater and air.

Sulphur reducing bacteria (SRB) are anaerobic and heterotrophic as they obtain their energy from organic nutrients. SRB utilize sulphate in their respiration and often results in the formation of sulphide according to the following reaction:

$4H_2+S^{2-}O_4 \longrightarrow S^{2-}+4H_2O$…………1

The sulphide is corrosive to most engineering materials. The most commonly encountered SRB is *Desulfovibrio desulfuricans*. The petroleum industry has been plagued by the activities of SRB because it handles large volumes of deaerated water from underground reservoirs. Anaerobic bacteria such as *Thiobacillus* spp. which are autotrophs or chemolithotrophs as they utilize energy derived from the oxidation of sulphur, thiocynate, tri and tetra-athionate for their growth. The *Thiobacillus* spp. oxidize the elemental sulphur to produce sulphuric acid as shown in the reaction (Smith, 1990) below:

$2S+3O_2+2H_2O \xrightarrow{\qquad} 2H_2SO_4$ …………….. 2

Corrosion is an ever-present degradation mechanism in wetted components and systems. There are many forms of corrosion in metals, they include: pitting, stress corrosion, general corrosion, galvanic corrosion and others (Virtanen *et al.*, 2008). When a system first encounters microbial corrosion (MC), such event usually occurs during the system's initial exposure to an aqueous environment, such as hydrotest, wet lay-up, or moist soil. The presence of certain bacteria in an environment will lead to the production of microbial corrosions. This mode of corrosion can be accelerated by microorganisms, either because they produce aggressive species, such as protons or sulphide ions, or because they calalyze the electrochemical reaction themselves (Eckert *et al.*, 2003). Microbial corrosion also requires the presence of nutrients and water to ensure the survival and growth of micro-organisms. However, the ability of microorganisms to sense and rapidly respond to harsh environmental changes is also vital for their survival and to microbial corrosion capabilities (Javaherdashtic, 2011). Microbes can grow and derive energy from organic or inorganic materials in fluids with pH values ranging from 1 to 10, where 1 is the most acidic concentration, Moreover, microbes can survive temperatures which range from -4 to 210^{O}F (-20 to 99^{O}C) (Faisal *et al.*, 2013). The majority of the active organisms involved in corrosion are bacteria, about 1-5 micrometers long which either oxidize or reduce sulphur compounds as some part of their life processes. Although many culturable bacterial types with known corrosion effects have been identified (Lane, 2005) in both aquatic and terrestrial environments, the primary corrosion – causing bacteria are the sulphate – reducing and iron-oxidizing bacteria.

Desulfovibrio spp. are members of sulphate reducers, which are found to exist in all soil and water types, as well as lives symbiotically with facultative anaerobic bacteria (Jin *et al.*, 2012). Sulphate-reducing bacteria (SRB) are a group of anaerobic diverse organisms which have varied morphological and nutritional characteristics. They utilize organic matter to produce sulphide by either reducing or oxidizing sulphate compounds (Gibson, 1990), as a source of energy. Therefore, sulphate ($S0_4^{2-}$) can be reduced to sulphide (S^{2-}) by SRB leading to the generation of hydrogen sulphide as a metabolic by-product. Both physical and chemical processes transfer hydrogen sulphide (H_2S) across the air and water boundaries to environment where chemoautotrophic bacteria oxidize the sulphide to sulphuric acid (Wiener *et al.*, 2006). The corrosion process will hence occur by the reaction of the biogenic sulphuric acid with the metallic surfaces (Magot *et al.*, 1997).

In order to evaluate the significance of microbial corrosions, a look at the economical perspective of such effects is essential. In 2001, the cost of microbial-influenced corrosion on oil and gas industries accounted for about two billion dollars annually in the United States of America (Koch *et al.*, 2001).

Aim of the Study

The aim of the study is to determine the corrosion of high carbon steel by bacteria under aerobic and anaerobic environments.

Justification of the Study

This work is justified because a lot of work has already been done on mild and medium carbon steel biocorrosion without much achievement on the prevention of biocorrosion of steel. From the literature, biocorrosion of high carbon steel showed that high carbon steel is more corrosion - resistant than both the mild and medium carbon steel and should be applied more in some Industries like the petrochemical industries to avoid losses due to biocorrosion. Microbial induced corrosion in the US economy has reached $350 billion annually as of 2010 (Rob, 2010).

Problem Statement

A lot of researches has shown that enormous loss of resources and even lives has been experienced in many countries of the world due to corrosion (Jin *et al.,* 2012). There is therefore need to device a means of combating this menace so as to save different countries of the world including Nigeria the cost encured due to corrosion.

This study considers the corrosion rate of high carbon steel of specific and known composition in two media namely aerobic and anaerobic environments with respect to the reference or control environment of distilled water. To this end, the investigation of the bacterial corrosion of high carbon steel is supposed to achieve the following:

Objectives of the Study

a) To determine the high carbon steel corrosion by bacteria in aerobic and anaerobic environments.

b) To determine the high carbon steel corrosion rates in aerobic environment.
c) To determine the high carbon steel corrosion rates in anaerobic environment.
d) To predict the service condition or environments to which high carbon steel may be preferred.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 What is Corrosion?

Corrosion is the process of degradation of materials as the material interacts with various types of environment. Every material tends to revert to its original form, which to a reasonable extent is thermodynamically favourable. Mild steel is used widely in piping systems, storage tanks, cooling towers and aquatic structures and is the most readily corroded metals (Akpabio *et al.*, 2011).

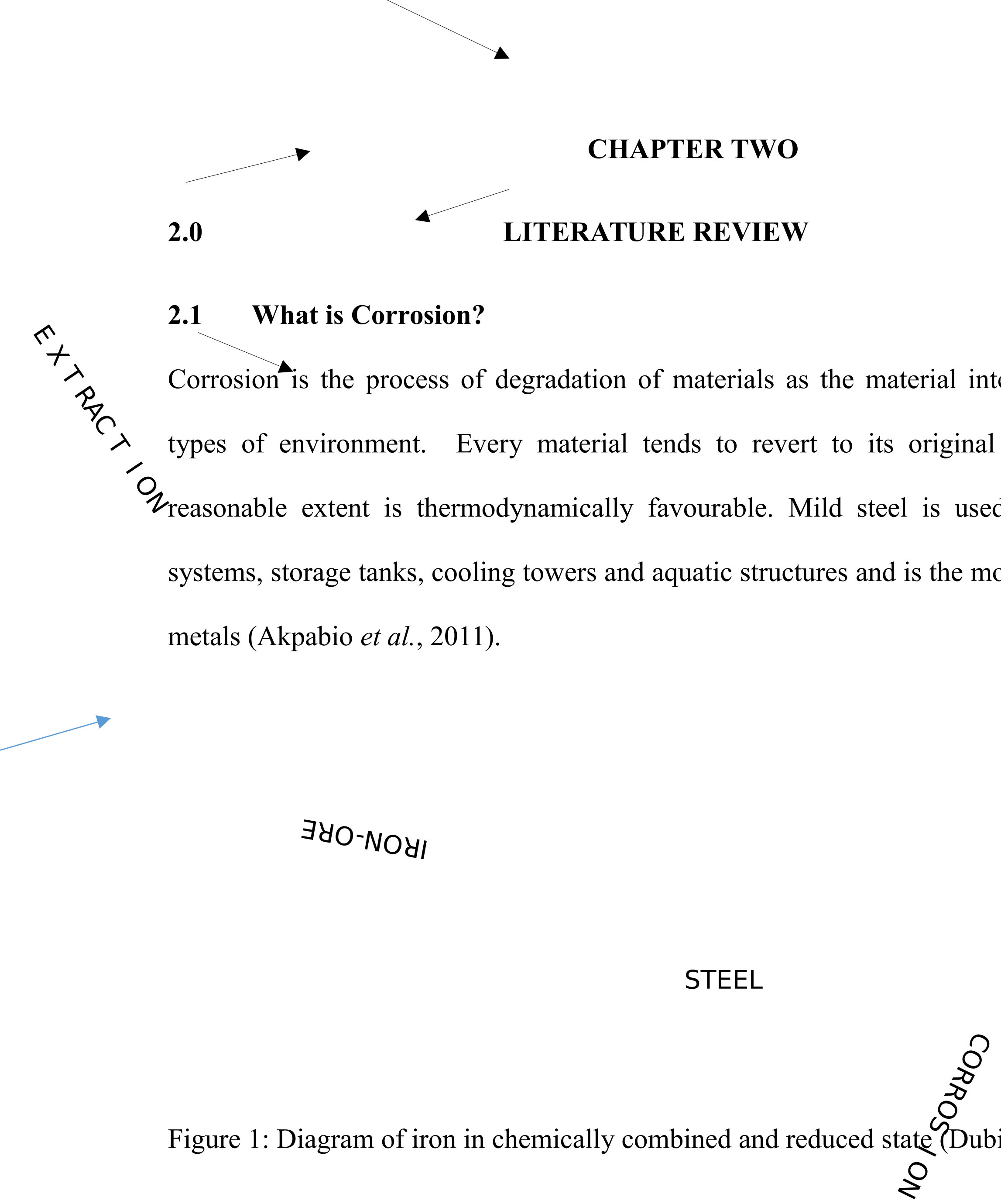

Figure 1: Diagram of iron in chemically combined and reduced state (Dubiel *et al.*, 2002)

The bacteria cells can colonize the metal surfaces causing their damage (Bachmann *et al.,* 2006). During the colonization of the solid surfaces the population of microbes increases rapidly forming a kind of biofilm which covers the attacked surface (L-ch.Xu *et al.,* 2002). This process is usually defined as biocorrosion, that is, the microbially induced corrosion (MIC) (Beech *et al.,* 2004). If the microbes are in the aqueous solution, the first attach to the surface and then grow, replicate and produce exopolymers (EPS), forming a cohesive structure known as a biofilm (Diosi *et al., 2003).* This process depends on the surface characteristics of substrate, including metal surface free energy, roughness and metallurgical features (Fang *et al.,* 2002). In other words, the biocorrosion is the result of the synergetic interaction of the metal surface, abiotic corrosion product, bacteria cells and cells metabolites

(Beech *et al.,*2004). In general, the metal surfaces are mostly affected by bacteria existing in land and water as the suphate reducing bacteria (SRB), iron oxidizing bacteria, manganese oxidizing bacteria and bacteria that secretes mucus and organic acidics (Beech *et al.,* 1999). Moreover, the investigations have shown that mentioned bacteria species coexist in biofilm forming complex structures on the corrosive metal surfaces (Beech *et al.,*2004). In addition, the literature result show that the combination of SRB and iron Oxidizing bacteria produces a higher level of corrosion than the SRB or iron oxidizing bacteria acting separately (Xu *et al.,*2008, Rao *et al.,* 2000). The deterioration of steel structure in fresh water and marine environment is crucially dependent on the biocorrosion processes. In industrial practice, due to their importance and effect, these processes attract special attention (Steel *et al.,* 1994). Studies have shown the complexity of microbiologically induced corrosion in different environment. Related to this, each particular case of biocorrosion requires development of the unique approach (Starosvetsky *et al.,* 2007).

2.2 Corrosion of Steel Caused by Microrganisms

The wide application of various types of steel in industries is a consequence of their ability to create passive thin films resistant to electro chemical corrosion. The stainless steels are defined as iron alloys containing at least 16.5% Chromium and up to 13.5% Nickel. In addition, Molybdenum may be added to increase the resistant to pitting and crevice corrosion of steel (Bachmann *et al.,* 2006). These stainless steels are largely used in sugar and Agricultural industries. Analysis of the localized corrosion mechanism of steel in the presence of iron oxidizing bacteria, *Sphaerotilus* spp. Is isolated from rust deposit in clogged carbon steel heater exchanger from an oil refinery plant (Haifa, Isreal) showed that stainless steel is characterized by passivity and therefore with more improved resistance to pitting attach by the iron bacteria in comparison with some other steel (Starosvetsky *et al.,* 2008). Xu *et al.* (2008) showed that the combination of sulphate reducing bacteria (SRB) *Desulfovibrio spp.* and iron oxidizing (IOB) of the *Leptothrix* spp. which were isolated from the system for cooling water in the refinery, induced a higher degree of corrosion of stainless steel that each of mentioned bacteria species separately. By adding 0.01M NaCl, as the result of synergy between constituent 0.01M NaCl + SRB + IOB, the highest level of corrosion has been obtained. This indicated that the chloride anions accelerated the pitting effect (Xu *et al.,* 2008). In sea waters, the corrosion potential of stainless steel can be increased to several hundreds millivolts. The corrosion potential changes only to a small extent in the filter or heat – sterile pasteurized sea water which is used for experimental control (Wanh *et al.,* 2006). Although, there are several possible explanations for increasing of the corrosion potential of stainless steel in natural sea water, all authors agreed that the main cause are changers in the cathodic reaction on the metal surface due to the microbial activity (Videla, 1994). The investigation of the MIC on the steel with marine aerobic *Pseudomonas* spp. have shown that the thickness of the passive films on steel samples is lowered by mutual attack of these bacteria and anions in sea water. Therefore, it is proposed that the Pseudomonas are involved in the oxidation/reduction reactions of iron (Yuan *et al.,* 2007). Mild steels are steels with the low carbon content. The mild steels and carbon steels are also broadly used in industry. The main triggers of corrosion of these steel are suphate reducing bacteria. The SRB types show considerable adaptability to extreme conditions. Among them, the SRB, the *Desulfovibrio* spp. can be relatively easily isolated and purified (Sani *et al.,* 2001). SRB is metabolized in the following way $4H_2 + SO_4^{2-} + 2H^+ \longleftrightarrow 4H_2O + H_2S$.

The activity of enzymes involved in the metabolism of S and H is one of the main factors which determine the activity of the cells, *Desulfovibro.* It was found that the level of H_2S production is directly proportional to the specific activity of the enzyme and that the degree of microbiologically induced corrosion of carbon steel is directly proportional to the bacterial resistant to the metal ions (Dzieriewicz *et al.,* 1997). The structure of the medium affects the corrosion of the steel by suphate reducing bacteria. Results obtained in sterile and inoculated media without sulphate were similar. On the other hand, in the presence of sulphate it is indicated that the reduction of SO_4^{2-} and S^{2-} and/ or H_2S are key processes in the SRB induced corrosion (Fonseca *et al.,* 1998). The presence of thiosulphate increased biocorrosion, which was probably the main factor in the SRB corrosion process (Miranda *et al.,* 2006). Under nutritionally rich and oligotrophic growing conditions of *Desulfovibrio alaskensis,* a risk of localized corrosion of steel charcoal process is latent. However, oligotrophic conditions can cause more serious and harmful localized corrosion processes (Viveros *et al.,* 2006). Iihan – Sungur *et al* (2007) found that the concentration of Zn of 27.8 +_ 0.046mg/cm^2 in the biofilm was toxic to the pure species *Desulfovibrio* spp. On the other hand, it has been found that mixed SRB can survive in the biofilm with very high concentration of Zn. It seems that the effect of Zn on the SRB varies depending on the type of SRB and their growth conditions. Therefore, this appears as a main reason for corrosion of galvanized steel, although originally, it was thought that the galvanized steel is resistant to biocorrosion (Sungur *et al.,* 2010). Circuit cooling systems of nuclear reactor at Kalpakkama had a problem which include pipeline blockage due to iron oxidation, sulphate reducing bacteria and exopolymer produced by *Pseudomonas aeruginosa.*

In industrialized nations, approximately 5% of the nation's annual budget is on corrosion control, prevention and the maintenance or replacement of products lost or contaminated as a result of corrosion. Corrosion occurs in various forms: an electrochemical process (that is gain or loss of electron), erosion (that is mechanical wearing away of materials, abrasion as a consequence of fluid flow robbing of surfaces) (Yuzwa and Eng. 1991). Erosion corrosion combination occurs in piping at bends, elbows and abrupt changes in pipe diameter. Propeller turbine blades, valves and pumps are susceptible to this form of corrosion. All the definitions of corrosion point to the fact that it is a natural occurring phenomenon which degrades our materials. Corrosion has been defined as the destruction, degradation and deterioration of materials due to the reaction of materials with the environment. It is the destructive attack upon a metal by agents such as polluted air, rain, seawater or aggressive chemicals. Corrosion is the natural deterioration of materials as a result of its interaction with its environment regardless of whether the material is a polymer, metal, elastromeric or even ceramics. Metallic corrosion is the passage of metal into the chemically combined state (Videla *et al.*, 2009). Corrosion is the deterioration of metallic materials which in aqueous environment proceeds by simple electrochemical mechanisms. Thus, corrosion is electrochemical in nature and it involves the oxidation of metal and concomitant reduction of species in its environment. In the electrochemical process of corrosion, electricity passes from a negative chamber called cathode to a positive chamber called the anode, and it leads to the corrosion of the positive chamber. In recent years, corrosion has been extended to include the reaction of metals, glasses, asbestos, polymeric solids, ionic solids and composites with its environment. The word corrosion has also been used more loosely to include the loss in resilience of non-metallic materials such as plastics. Metals corrode because of their inherent tendency to revert to their original combined form as oxides and compounds after they have been extracted. Therefore, this tendency is the driving force behind corrosion (Schlegel, 1992). Since corrosion is a natural phenomenon, almost all metals corrode or are expected to deteriorate with time. For instance, aluminium acquires a whitish hydrated oxide film when exposed to the atmosphere, iron rusts when exposed to moist air and copper tarnishes; thus, there is a variation in practical examples of corrosion damage.

2.3 Activities of Microorganisms as the Driving Force for Biocorrosion

Microbes implicated in biocorrosion of metals such as iron, copper and aluminum and their alloyed are physiologically diverse. Their ability to influence the corrosion of many metals are normally considered corrosion resistant in a variety of environments makes microbes a real treat to the stability of those metals. The main types of bacteria associated with corrosion failure of cast iron, mild and stainless steel structures are sulphate reducing bacteria (Hamilton, 1985), sulphate oxidizing bacteria (Cregnolino *et al.,* 1984), iron oxidizing/reducing bacteria (Obuekwe *et al.,* 1981), manganese oxidizing bacteria (Dickinson *et al.,* 1996), and bacteria secreting organic acids and exopolymers or slim (Cragnolino *et al.,* 1984). These organisms can co-exist in naturally occurring biofilm often forming synergistic communities (consortia) that are able to affect electrochemical processes through co-operative metabolism not seen in the individual species. (Dowling *et al.,* 1991). Much resent research activity has centered on the role of quorum sensing molecules such as acylhomoserine lactones in control of microbial activities in biofilms (Davis *et al* 1998: Marton *et al.,* 1998), with the aim of using this knowledge to reduce problematic biofilm formation in industry (Wethem *et al.,* 1999).

2.4 Sulphate Reducing Bacteria (SRB)

SRB are group of diverse anaerobes which carry out dissimilatory reduction of sulphur compounds such as sulphate, sulphite, thiosulphate and even sulphur itself to sulphide (Bak *et al.,* 1987, Lovley *et al.,*1994). Although SRB are often considered to be strictly anaerobic, some genera tolerate oxygen (Abdollahi *et al.,* 1990, Hardly *et al.,* 1981), and at low dissolved oxygen concentration certain SRB are able to respire with Fe^{3+} or even oxygen with hydrogen acting as electron donor (Dilling *et al.,* 1990). Oil, gas and shipping industries are seriously affected by the sulphides generated by SRB (Hamilton, 1994). Since the beginning of investigations into the effect of SRB on corrosion of cast iron in 1930s, the role of these bacteria in the pitting corrosion of various metals and their alloys in both aquatic and terrestrial environment, under anoxic as well as oxygenated condition had been confirmed.

However, there are few practical benefits of corrosion of metals, though they are insignificant when compared to the harmful effect of corrosion. They include; the lead-acid battery which depends on corrosion of zinc in primary cell to produce electrical energy. Anodic oxidation is the basis of electrolytic polishing of metals. The recovery and purification of certain metals by electrolysis is also a corrosion related phenomenon (Hannachi *et al.*, 2008).

2.5 Common Forms of Corrosion

It is pertinent to recapitulate the common forms of corrosion to which materials used in design of structures and equipment are most subjected. This should enable the designer to correct his records of past failures with the demands of future design to secure effective prevention of corrosion. Premature cracking of metals produced by the continued action of corrosion and surface tensile stresses residual or applied. It is caused by a combination of high tensile stress and a corrosive environment e.g. seawater, perspiration, welding fluxes, lubricants and organic solvents. Corrosion action concentrates stresses causing them to exceed the yielding point of the metal. Under continued exposure with the metal corroding and local high stress concentration building up, the metal will eventually fail (Anyakwo, 1993). Non-metals are subjected to similar phenomenon. Corrosion manifestation in iron and steel is viewed in so many forms, though it depends on the environment.

2.6 General Corrosion

This is also known as uniform corrosion. General corrosion is the most common type of corrosion and is caused by a chemical or electrochemical reaction that results in the deterioration of the entire exposed surface of a metal. General corrosion accounts for the greatest amount of metal destruction by corrosion, but is considered as a safe form of corrosion, due to the fact that it is predictable, manageable and often preventable. This is the most common form of corrosion which account for more metal wastage than any other form. It is predictable and usually obvious, less likely to cause unexpected dramatic failure. It occurs where there is no surface feature that localizes anode or cathode reaction. Typical examples include: steel in aerated seawater, steel in dilute sulphuric acid and zinc in an industrial atmosphere.

2.7 Pitting Corrosion

This results when a small hole or cavity forms in the metal, usually as a result of passivation of a small area. This area becomes anodic, while part of the remaining metal becomes cathodic producing a localized galvanic reaction. The deterioration of this small area penetrates the metal and can lead to failure. This form of corrosion is often difficult to detect due to the fact that it is usually relatively small and may be covered and hidden by corrosion produced compounds. This form occurs on normally passive metals. Local failure of passive film results in restricted area of intensely localized corrosion often surrounded by unattacked metals. Pitting is promoted by aggressive ions especially chloride. Aggressive ions also interfere with re-establishment of passive film which promotes development of pit. It is impossible to predict which environment is most likely to cause pitting in a given metal. It is not possible to be sure that pit will develop or identify where or when pits will form. Typical examples include some stainless steel in slow moving aerated seawater, aluminum structure in marine environment (Mataqi *et al.,* 2013).

2.8 Microbial Corrosion

Sulphate reducing bacteria known as *Desulfovibrio desulfuricans* in the scientific community is also referred to as SRB. These bacteria are non-pathogenic (that is, they are not capable of causing diseases) and anaerobic but, they are capable of causing severe corrosion of iron material in water system because they produce enzymes which have the power to accelerate the reduction of sulphate compounds to the corrosive hydrogen sulphide, thus SRB act as a catalyst in the reduction reaction. However, in order for this reduction to occur, four components must be present. These components include; SRB, sulphate, an external energy source in the form of free electron must be present, and the temperature of the water must be less than 65^0C (Iwona *et al.*, 2000).

2.9 Erosion Corrosion

This occurs when high velocity flowing fluids disrupt passive films or prevent their formation. Damage to passive film may be due to the erosive effects of fluids. Fluids are corrosive towards the unprotected metal. Turbulent flow in pipe work may initiate erosion corrosion. Flow disturbance produced at bends and tees, at heat exchange tube inlets, in pumps and valves or by orifice plates or badly made welds can initiate erosion corrosion problems. Examples are damaged copper based heat exchange tubes at inlets end, and vessel wall damage near badly directed inlet tube.

2.10 Galvanic Corrosion

This is also called dissimilar metal corrosion, it occurs when two different metals are located together in a corrosive electrolyte. A galvanic couple forms between the two metals, where one metal becomes the anode and the other the cathode. The anode corrodes and deteriorates faster than it would alone while the cathode deteriorates more slowly. This form of corrosion is associated with the current resulting from the coupling of dissimilar electrodes in an electrolyte. Galvanic corrosion results when two dissimilar metals exposed to an electrically conducive environment are in direct contact.

2.11 Mechanisms of Biocorrosion

MIC does not invoke any new electrochemical mechanisms of corrosion, rather it is the result of a microbiologically influenced change that promotes the establishment or maintenance of physico-chemical reaction not normally favoured under otherwise similar conditions. Various mechanisms of biocorossion which reflects the variety of physiological activities carried out by different types of microorganisms have been identified: however, it must be remembered that in nature, these microbial processes do not act in isolation, but in concert with the chemical and electrochemical forces in the particular environment.

A water system naturally contains sulphate based compounds, but when sulphide is added to a closed water system as an oxygen scavenger and corrosion inhibitor, the sodium sulphide is oxidized to sodium sulphate as indicated in reaction 3 below:

$2Na^{+}+SO_3^{-1}+1/2O_2^{-2} \longrightarrow 2Na^{+1} + SO_4^{-2}$ ……..(3)

Excess electrons occur in a water system as a result of iron corrosion at the anode and cathode cells as shown in reactions 4 and 5 below:

$4Fe \longrightarrow 4Fe^{2+}+8e^{-1}$ (Anode reaction) ……. (4)

$8H^{+1} + 8e^{-1} \longrightarrow 8H$ (Cathode reaction)….. (5)

The resultant accelerated corrosion mechanism of iron by the sulphate reducing bacteria is illustrated in equations 6,7,8,9, and 10 below: (Liu *et al.*, 2007)

$SO_4^{-2}+8H \longrightarrow S^{-2}+4H_20$……… 6 (Cathode depolarized by SRB)

$8H_20 \longrightarrow 80H^{-1} + 8H^{+1}$ ….7 (dissociation of water)

$2H^{+1}+S^{-2} \leftrightarrows H_2S$ ….8 (reversible reaction)

$Fe^{+2}+S^{-2} \longrightarrow FeS$ ….9 (anode corrosion product)

$3Fe^{+2}+6(0H)^{-1} \longrightarrow 3Fe\ (OH)_2$ ….10 (cathode corrosion product)

2.12 Factors Affecting Corrosion of Iron and Steel

Corrosion can affect metals in a variety of ways depending on certain metallurgical, biological, engineering and other prevailing environmental conditions. Metallurgical factors such as chemical composition, structure, crystal orientation, heat treatment, impurities as well as the type of phase present, affect the incidence of corrosion. A metal surface is a complex of crystal faces, edges, corners, boundaries, disturbed layers, oxide, impurities and inclusions, formed during manufacturing. Notably, sulphur and phosphorus in plain carbon steel are detrimental to its corrosion resistance, unfortunately, oxides are usually cathode with respect to steel. These cause severe corrosion on steel when oxide scale breaks off (Choudhary, 1998). It has been observed that corrosion proceed in a relatively faster pace in certain media. It has shown that rusting can be very severe in environments polluted with oxides of sulphur, nitrogen and carbon dioxide. Common experience has shown that corrosion rate is affected by engineering factors such as design approach and presence of residual stresses. Certain design approach tends to enhance rusting. Electrical contact between dissimilar metals induce corrosion of the more active metal. Localized shear, torque and stress are known to provide sites for stress corrosion cracking and should be minimized in design and construction. Relative movement of the media facilitates materials transport and hence increase the rate of corrosion.

2.13 Rusting in Polluted Atmosphere

With increase in the consumption of fossil fuels and industrial activities, certain aggressive gasses have been released into the atmosphere. Some of these substances are oxidized into acid radicals like sulphur dioxide, nitrogen dioxide and carbon dioxide. These gases under conducive atmospheric condition form weak solution of acid-like sulphuric acid, nitric acid and carbonic acid with moisture in the air. These can be represented as follows:

$SO_2+{}^1/_2 0_2+H_20 \longrightarrow H_2S0_4$ ……………………(11)

$2N0_2+{}^1/_2 0_2+H_20 \longrightarrow HN0_3$ ………………………..(12)

$C0+{}^1/_2 0_2+H_20 \longrightarrow H_2CO_3$ …………………….(13)

2.14 Rusting in Marine Environment

Sodium chloride is a major component of seawater. Also magnesium, calcium, potassium and ammonium salts are found in seawater in variable concentrations. Thus corrosion behavior of iron and steel structures exposed to marine environment depend on the concentration of other components as may be determined by the level of pollution in the sea. This is why 0.1mol sodium chloride solution has been chosen to stimulate generalized marine environment. Corrosion in seawater is aggravated by the relative movement of water and steel as well as the activities of some sea microbes. Iron and steel structures serving in marine environment often have some of their parts above sea level. Thus their corrosion behavior is comparable to that of specimen partially immersed in a corrosive medium. Here rusting occurs more rapidly in the position below the water line. This is as a result of differential aeration. Oxygen is readily available to the portion at the meniscus. Under this condition, the oxygen starved region inside the water undergoes severe oxidation (Liu *et al.*, 2007)

2.15 Reasons for Combating Corrosion

The phenomenon of corrosion has been of great concern to mankind since the Iron Age. The need to combat corrosion calls for no emphasis especially in the era of modern technology. Corrosion control as a maintenance exercise is very vital mainly because of economic reasons, to forestall premature failure of equipment and industrial accident, for conservation of material and also for the safety of personnel and environmental protection (Fontana, 2006). Corrosion control is justifiable in view of the huge economic losses resulting from corrosion related problems. The continuous painting of steel bridges, ships, tanks etc., illustrates that protection against corrosion is an ever present problem. From studies carried out in developed countries, between 3% and 4% of their gross national income is spent on corrosion related problems. Annual losses due to corrosion of iron and steel have been variously estimated at nearly seventy billion dollars in the United States. In Nigeria, there are no statistics showing losses caused by corrosion. As a result, we hardly quantify the losses due to corrosion in this country. This should be a challenge to the authorities and professional bodies like Nigerian Society for Microbiology (NSM), Nigerian Corrosion Association (NCA) and Nigerian Metallurgical Society (NMS).

Corrosion in a plant can lead to a shutdown of course, maintenance can be cost effective. The repair or replacement of a piece of equipment may cost relatively little but while the repairs are going on, the whole plant may be shot down for weeks or months. Under this circumstance, it becomes essential to reduce the periods when the plant has to shut down for maintenance (down tool time) to a minimum. The indirect cost of corrosion can hardly be effectively calculated. For instance, the cost of shutting down a Nigeria refinery as a result of corrosion problem could not be fully assessed without considering its effect on the transport sector. The economic consequences of corrosion are numerous especially as it concerns the sea, air, rail and road transportation, building and construction, petroleum and chemical industries as well as agricultural and domestic implements (Jin *et al.*, 2012). Corrosion control is also vital in order to forestall premature failure of equipment and industrial accident. Documentation cases abound of premature accidents. Accumulation of corrosion products can reduce the efficiency of operating system. For instance, extra pressure is required for conveying petroleum products through pipelines whenever the interior of the pipe becomes rough as a result of corrosion. Conservation of engineering materials is another reason for combating corrosion. Since corrosion devours our materials, if the materials are not adequately protected to give them long life, we will soon deplete our natural reserves. This is because we will always have to extract new materials for production. Corrosion control also saves the problem of over design. The principle of over design is the use of much thicker section than would normally require for good mechanical strength in order not to allow for the ravages of corrosion. This approach of design result in extra consumption of materials and extra cost too. Corrosion control is important not only for the benefit of industries, but also for the safety of personnel and environmental protection. An incident which readily comes to mind is the tragic accident of Bhopal, India caused by corrosion of tank containing some poisonous gases. This led to the death of many people in the neighbourhood. Corrosion control is very vital in the petrochemical industry, otherwise failure in oil pipeline will result in oil spillage and its resultant problems, such could cause waste of products, wide spread of fire, destruction of agricultural resources, ravages in natural habitat and other kinds of environmental disasters.

Another reason for corrosion control is to avoid contamination of products. Notably, the chemical and food processing industries cannot tolerate the pickup of even small quantities of metal ions as corrosion products. We cannot afford to ignore corrosion especially here in the tropics where the combination of heat and high moisture content in the air aggravates and hastens the pace. Corrosion control operations are aimed at detecting the occurrence of corrosion in good time, apply remedial measures and hence save cost by preventing equipment failure and ensuring safety of personnel and environmental protection (United States Environmental Protection Agency, 2001).

2.16 Corrosion Control

Since corrosion is an inevitable industrial process, corrosion control is an area in which the scientists and engineers are expected to use their ingenuity to enable man contain the challenges of corrosion in the ever increasing industrial world. The best man can do is to monitor it and minimize its effects as much as possible. Corrosion monitoring is aimed at understanding at all times the corrosion situation in plants. Although the mechanism of corrosion is complex, it can be controlled by the application of concepts based on the knowledge of corrosion science. On this basis, corrosion control methods fall under the following categories:

a) Material selection and design considerations
b) Modification of environment.
c) Use of coating materials.

2.17 Materials Selection and Design Consideration

Foundation material science and engineering exposes the engineer/scientist to structure and properties of scientific engineering materials, interdependence of production techniques and properties in material design and selection of materials with unique property combinations for specific engineering/scientific applications. To select the right material, the scientist should:

- Know desirable or optimum properties or combinations of properties expected of the component to perform optimally at the cheapest cost possible.
- Decide the constraints of the material selection to enable the engineer/scientist to not only select the material but also to maximize the performance of the component.
- Know exactly the function of the component to be made from the material.
- Develop a unified equation combining all the relevant parameters for the optimum performance of the component.
- Recognize the parameters that should be optimize with respect to mass and relative to the material cost.
- Refer to the performance chart if available or develop the chart from the unified equation.
- Finally, select the right material for the particular application.

Apart from proper selection of materials, the design stage is an important stage in taking care of corrosion. Since corrosion is a penetrating action, it is necessary to make allowance for the reduction in thickness using the principle of over design. Some design guidelines that should be observed include: avoid crevices, avoid deposit, permit free drainage, create access for maintenance, avoid condensation of aggressive fluids, and pay attention to flow rate and stresses (United States Patent, 2009).

2.18 Modification of Environment

In gaseous environment we make use of air conditioners to cut down on relative humidity of storage rooms where metallic wares are stored. For every small object, protection is achieved by addition of solid inhibitors which volatilizes to modify the environment and such are known as vapour phase inhibitors. In aqueous environment, corrosion is reduced by the removal of stimulants also known as depolarizers. Inhibitors are organic or inorganic substances which when added (in small amount) to the corrosive environment, effectively decreases corrosion rate. Most paint primers contain inhibitors of partial soluble pigments such as zinc chromates. Some pigments passivate by contributing alkalinity thereby slowing down the chemical attack on steel. Also some inhibitors work by adsorbing on the metal surface forming a barrier between the metal and its environment.

2.19 Modification of Materials

It is not always convenient or and possible to modify the environment especially in the chemical and food processing plants. This is to avoid contaminating the products. However, it is possible to modify the properties of the metal by alloying. For instance, nickel and chromium improve the corrosion resistance of steel. Certain heat treatment and chemical treatment are known for increasing the corrosion of plain carbon steels. Electroplating is also an important method of corrosion control (Fortin, 2009).

2.20 Use of Coating Materials

Protective coatings and linings serve to control corrosion by providing one of the following: an inert or highly corrosive resistance between the metal and the environment, examples include the vitreous enamel coating, nickel alloy cladding, chromium coating etc. It provides a sacrificial barrier between the metal and the environment, examples are zinc (galvanized, flamed spray), cadmium coating. It also provides a high resistance electrolyte path between the anode and the cathode reaction sites, examples are: most polymeric coatings, paints, rubbers, plastics. Also a reservoir of corrosion inhibitors adjacent to the metal surface, example is the primer paints with anticorrosive pigments.

The population growth of the micro-organisms is inevitable in living conditions (Sand et al., 2006). Therefore the biocorrosion of metal surfaces becomes significant problem in industrial environment. It can irreversibly damage metal surfaces highly increasing the cost of production processes. Bearing in mind this fact, the prevention of the bacterial growth on surfaces should be in the focus of research interest. One possibility is the selection of materials which are insensitive to the biocorrosion. Also the proper technical design of the production set-up can help to avoid situations that favour microbial growth and contamination in the system as the stationary conditions, cracks, or lack of adequate drainage. The regular cleaning in association with previously mentioned preventive measures can additionally optimize production process (Videla, 2002). Physical and chemical methods can be used for fixing antimicrobials on solid surfaces (Hequet *et al*., 2011).

Generally, the methods commonly used to prevent and control microbial corrosion can be divided into several categories:

i. Cleaning procedures
ii. Biocides (Rakotonirainy *et al*., 2007)
iii. Coating and
iv. Cathode protection (Videla, 2002).

However, micro-organisms themselves can lead to corrosion inhibition. Micro-organisms can contribute to corrosion inhibition by neutralizing the action of corrosive substances present in the environment, forming protective films or stabilizing a pre-existing protective film on a metal and inducing a decrease in the medium corrosiveness. The proper understanding of the identity and role of microbial contaminants present on the metal surface can be exploited to induce corrosion inhibition by bacteria to prevent microbial induced corrosion effect (Videla et al., 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection

A sample of high carbon steel grade of chemical composition: 96% iron (Fe), 1.08% carbon (C), 0.349% silicon (Si), 0.841% manganese (Mn), 0.005% phosphorus (P) and 0.005% sulphur (S), was obtained in the form of steel plate. The chemical analysis of the sample was done using the spectroscopy method at Testing Research and Certification Engineering Services, Port Harcourt, Nigeria in accordance with American Society for Testing Materials (ASTM 2009).

3.2 Material/Sample Preparation

The high carbon steel plate was cut to a dimension of 28 x 28 x 2mm using hack saw blade according to the standard of American Society for Testing Materials (ASTM 2009). The size and shape (flat surface) were chosen for ease of measurement and convenience. Eighteen (18) samples of the high carbon steel were prepared.

3.3 Surface Preparation

The cut surfaces were cleaned and polished to obtain fine surface using abrasive papers ranging from coarse to fine grades according to American Society for Testing Materials (ASTM 2009). The need to suspend the sample in the sulphate reducing bacteria environment necessitated drilling a hole of 4 mm of the sample near the edge of each steel sample. The initial dimensions of each sample and their weights were measured and recorded.

3.4 Treatment of the Environment

Twenty samples of soil were collected from different locations in Ebeocha in Rivers State and Orji mechanic village in Owerri, Imo State for the isolation of the microorganisms. The soil samples were collected at a depth of 2 cm as described under National Association of Corrosion Engineers (NACE, 2004) standard. Small portion of the soil was polluted with 20 g of sulphur powder and allowed for the period of two weeks. The soil sample was collected and 1 g of the polluted soil was suspended in 9 ml of sterile distilled water. This constituted the 10^{-1} dilution. The test tube was shaken and a sterile 10 ml pipette was used to transfer 1ml of the 10^{-1} dilution into a test tube containing 9 ml sterile distilled water to achieve the 10^{-2} dilution. The content of 10^{-2} dilution was shaken and the dilution continued (always using a fresh sterile pipette for each transfer) until the 10^{-6} dilution was obtained according to American Public Health Association (APHA, 2r005) standard. After the serial dilution was completed, an aliquot (0.1ml) of the 10^{-2} dilution was inoculated onto a mineral salt agar plates using spread plate technique and allowed for 24 hours at 32°C. The mineral salts agar composed of 10g of NaCl, 0.42g of $MgSO_4$. $7H_2O$, 0.29g of KCl, 0.83g of $K_2H_2PO_4$, 0.42g of $NaNO_3$ and 12.5g of agar per litre. Some of the organisms isolated from the soil samples after the inoculation on a mineral salt agar were aerobes which include *Bacillus* spp., *Pseudomonas* spp. An aliquot 0.1ml of the 10^{-2} dilution was inoculated unto a nutrient-rich Baar's medium using spread plate technique and allowed for 24 hours at 32°C. The medium consisted of sodium lactate (6.0g/l), sodium sulphate (4.5g/l), ammonium chloride (1.0g/l), yeast extract (1.0g/l), potassium phosphate (0.5g/l), sodium citrate (0.3g.l), calcium chloride (0.06g/l), magnesium sulphate (0.06g/l) and iron sulphate (0.004g/l). The bacterial strains obtained from the above exercise were then tested using an SRB kit and an iron reducing bacteria kit. The anaerobes isolated include *Desulfovibrio* spp. and *Thiobacillus* spp.. The isolates were purified and sulphate reducing bacteria were inoculated onto the Baar's medium in the anaerobic container. *Bacillus* species was inoculated onto the mineral salt medium in the aerobic container. SRB growth was observed as strong black precipitate formation on the medium due to intensive FeS production. The pH and concentration of the growth medium in each environment was measured and recorded.

3.5 Preparation of the Environment

a. Control (Distilled Water) environment

This is distilled water that contained no microbe. It is poured into a sterile 2.5 litres container for the experiment.

b. Aerobic environment

This is the mineral salts medium that contained the test organism in a 2.5 - litre container. This container was not closed rather it was exposed to air.

c. Anaerobic environment

This environment is prepared in an anaerobic jar made of thick glass with a capacity of 2.5 litres. The jar is equipped with a secured gas-poof lid that is easily removed and replaced. After putting the high carbon steel coupons into the jar, an anaerobic atmosphere is generated by introducing a disposable anaerobiosis-generating device and closing the lid. The device used here is the candle stick. This activates the binding of oxygen and the release of carbon dioxide.

3.6 Corrosion Measurement

The steel coupons (samples) were cleaned and dried before exposure to their respective environment. The initial weight and dimensions of the specimens were measured and recorded. The samples were then suspended with the aid of a rubber thread which passed through the drilled hole in each sample into the environment (aerobic and anaerobic), with respect to the control environment (distilled water) as described by Oparaodu *et al* (2004). After a duration of one week, a sample was removed from each medium or environment and the electrode potential reading of the sample was recorded accordingly. The oxide film formed on the surface of the sample was visually examined, brushed with a sponge in water in order to remove corrosion products and then dried in acetone. The cleaned sample was weighed. The loss in weight in each case was evaluated and tabulated accordingly. This procedure was repeated until the end of the sixth week when the last set of the samples were removed for corrosion inspection and measurement. The rate of corrosion of the samples were monitored and determined by the weight loss technique and electrode potential measurement technique as described by Kumar *et al* (2012).

3.7 Visual Inspection

Visual examination carried out revealed the presence of corrosion products and oxide scales somewhat brownish in colour on the surface of the sample. The corrosion products such as the oxide films were scraped out, ground and mixed with few drops of sodium hydroxide solution ($NaOH_{aq}$) in a test tube.

3.8 The Weight Loss Technique

The bacterial corrosion of the high carbon steel in aerobic and anaerobic environments with respect to a distilled water- control environment was studied and evaluated by the weight loss of the samples at periodic intervals of one week. A weighing balance Mettle Toledo (New classic ML 204 Switzerland) was used to determine the weight loss of the steel coupon. The initial weight of the samples before introducing them into the different environments were determined and recorded. The weight of the samples, that is the final weight after corrosion must have taken place were also determined and recorded. The loss in weight after each exposure time was calculated from the equation below:

$$W1 = Wo - Wi \text{ - - - - - - } (14)$$

Where W1 = weight loss (g), Wo = initial weight(g) before each exposure time (T days) and Wi = final weight in grams after each exposure time (T days).

$$\text{Percentage weight loss} = \frac{Wo - Wi}{Wo} \times \frac{100}{1} \text{ ....} (15)$$

3.9 Electrical Potential Measurement Method

This method as described by (ASTM, 2009) standard indicates if the metal specimen is passive (non-corroding) or active (corroding). The method is simple, only needing an appropriate electrode (copper electrode with copper sulphate electrolyte) and voltage measuring instrument (Ovri, 2010). An electrical potential measurement was conducted using a copper electrode as a reference electrode and a high carbon steel as the working electrode in a standard electrochemical glass cell with copper sulphate as the electrolyte.

The test were operated using the PARSTAT 2273 electrochemical measurement system manufactured by EG&G. The instrument was used to read the electrochemical potential values of the steel coupons before and after the exposure time.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of SRB on High Carbon Steel in Different Environments

The results of the weight loss and corrosion rate of the steel coupons in aerobic, anaerobic and distilled water (control) environments are shown in Tables 4.1, 4.2, and 4.3. Figures 4.1 – 4.3 show the plots of weight loss versus exposure time in the different environment. The lowest weight loss of 0.0201 g in aerobic, 0.0247 g in anaerobic and 0.0195 g in distilled water environment were observed in day 7. The highest weight loss 0.0714 g in aerobic, 0.0939 g in anaerobic and 0.0669 g in distilled water environments were observed in day 28.

Figures 4.4 – 4.6 show the plots of the corrosion rate versus exposure time in the different environment. The lowest corrosion rate of 0.0002667 mm/yr in aerobic, 0.0003067 mm/yr in anaerobic and 0.0002290 mm/yr in distilled water environment were observed on day 42. The highest corrosion rate of 0.0004595 mm/yr in aerobic, 0.0005646 mm/yr in anaerobic and 0.0004458 mm/yr in distilled water environments were observed in day 7.

4.2 Electrode Potentials of the Steel Coupons and Concentrations of the Growth Medium in Different Environments.

Tables 4.4 – 4.6 show the results of the electrode potentials of the samples and concentrations of the medium in different environments.

Figures 4.7 – 4.7 show the plots of electrode potentials versus exposure time in the different environments. The lowest electrode potentials of -0.712 mV in aerobic and -0.717 mV in anaerobic were observed on day 35 and -0.072 mV in distilled water was observed on day 28. The highest electrode potential of -0.550 mV in aerobic, -0.554 mV in anaerobic and -0.550 mV in distilled water environment were observed on day zero.

Figures 4.10 – 4.12 show the plots of the concentration of the media versus the exposure time in different environments. The lowest concentrations of 500 ml/l was observed on day zero for all the environments. The highest concentrations of 535 ml/l in aerobic, 560 ml/l in anaerobic and 525 ml/l in distilled water environment were observed on day 42.

4.3: Initial Conditions of Specimen and Environments

Average initial electrode potential value of the specimen = -0.553 mV

Initial pH reading of the aerobic, anaerobic and control environments are 7.0, 7.0 and 7.0 respectively.

Initial concentration of the aerobic, anaerobic and control environment are 500 m/litre, 500 m/litre and 500 m/litre respectively.

TABLE 4.1: Weight losses and corrosion rates of steel coupons in aerobic environment

Exposure Time(T) day	Initial weight (W_o)g	Final weight (W_i)g	Weight loss (W_1)g	Corrosion rate (CR) mm/yr.	% Weight loss
0	-	-	-	-	-
7	9.9378	9.9177	0.0201	0.0004595	0.2022
14	11.7102	11.6745	0.0357	0.0004080	0.3048
21	14.8781	14.8258	0.0523	0.0003985	0.3515
28	9.9467	9.8753	0.0714	0.0004080	0.7178
35	15.3923	15.3221	0.0702	0.0003209	0.4560
42	13.0048	12.9348	0.0700	0.0002667	0.5382

TABLE 4.2: Weight losses and corrosion rates of steel coupons in anaerobic environment

Exposure Time(T)	Initial weight	Final weight	Weight loss	Corrosion rate (C.R)	% Weight loss
day	(W_o)g	(W_i)g	(W_1)g	mm/yr.	
0	-	-	-	-	-
7	11.0777	11.0530	0.0247	0.0005646	0.2229
14	11.9859	11.9448	0.0411	0.0004698	0.3429
21	9.2557	9.1932	0.0625	0.0004762	0.6752
28	11.0338	10.9399	0.0939	0.0005366	0.8510
35	9.0682	8.9853	0.0830	0.0003795	0.9152
42	11.8944	11.8139	0.0805	0.0003067	0.6767

TABLE 4.3: Weight losses and corrosion rates of steel coupons in control environment (distilled water)

Exposure Time(T)	Initial weight	Final weight	Weight loss	Corrosion rate (CR)	% Weight loss
day	(W_o)g	(W_i)g	(W_1)g	mm/yr.	
0	-	-	-	-	-

7	16.4389	16.4194	0.0195	0.0004458	0.1186
14	11.5512	11.5295	0.0217	0.0002480	0.1878
21	12.5035	12.4633	0.0402	0.0003063	0.3215
28	14.9969	14.9300	0.0669	0.0003823	0.4460
35	10.7760	10.7135	0.0625	0.0002857	0.5799
42	9.0040	8.9439	0.0601	0.0002290	0.6674

4.3.1: Weight losses and exposure times in aerobic environment

The weight loss (in grams) against exposure time (in days) in aerobic environment was shown in Figure 4.1. it is observed that the weight lost increased with the exposure time reaching the highest at day 28 which is 0.0714 g. Further exposure beyond 28 days showed no significant weight loss.

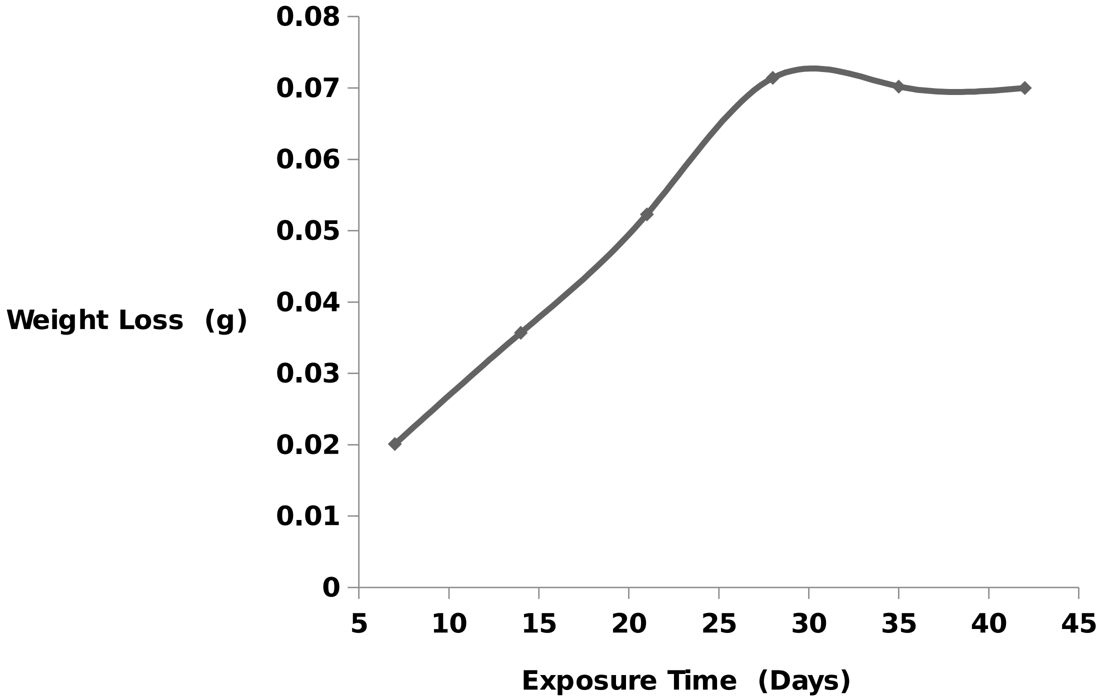

Fig.4. 1: Weight loss (grams) against the exposure time (days) in the aerobic environment.

4.3.2 Weight losses and exposure times in anaerobic environment

Figure 4.2 shows the weight loss against exposure time in anaerobic environment. From Figure 4.2, it is seen that the greatest weight loss of 0.0939 g was recorded at day 28. After 28 days it was observed that weight loss slightly decreased with exposure time from day 35 – 42.

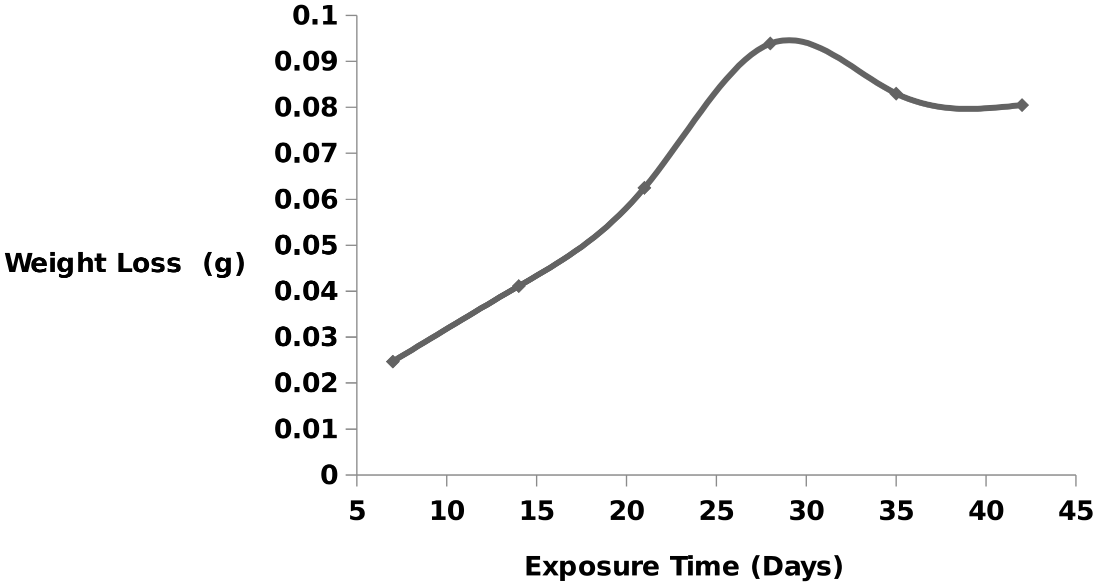

Fig.4. 2: Weight loss (grams) against the exposure time (days) in the anaerobic environment.

4.3.3: Weight losses and exposure times in distilled water (control) environment.

The weight loss (in grams) against the exposure time (in days) for the control environment was shown in Figure 4.3. There was no significant weight loss between days 7 and 14. On prolonged exposure time, weight loss was observed to increase from 14 – 28 days. Further exposure beyond 28 days showed no significant loss of weight. The highest weight loss of 0.0669 g was observed at day 28 and this is however the least among the three media.

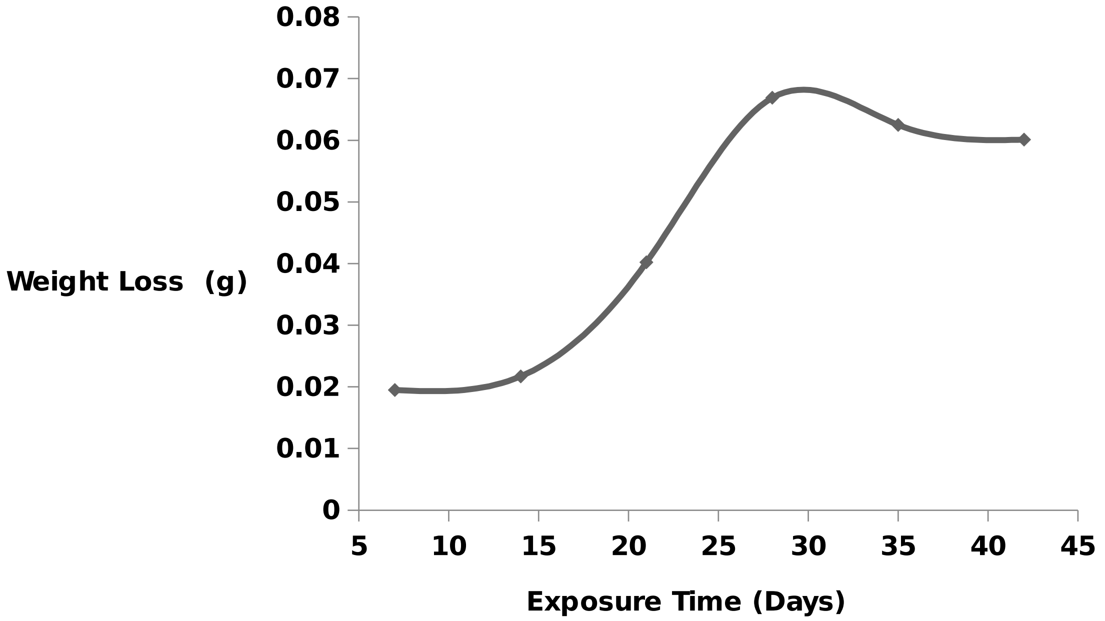

Fig . 4.3: Weight loss (grams) against the exposure time (days) in the control environment

4.4.1: Corrosion rates and exposure times in aerobic environment

The corrosion rate (in milimeter per year) against exposure time (in days) in aerobic environment was shown in Figure 4.4. The corrosion rate was observed to be highest at day 7 which is 0.0004595 mm/yr. corrosion rate was observed to decrease irregularly upon prolonged exposure time reaching the least of 0.0002667 mm/yr at day 42.

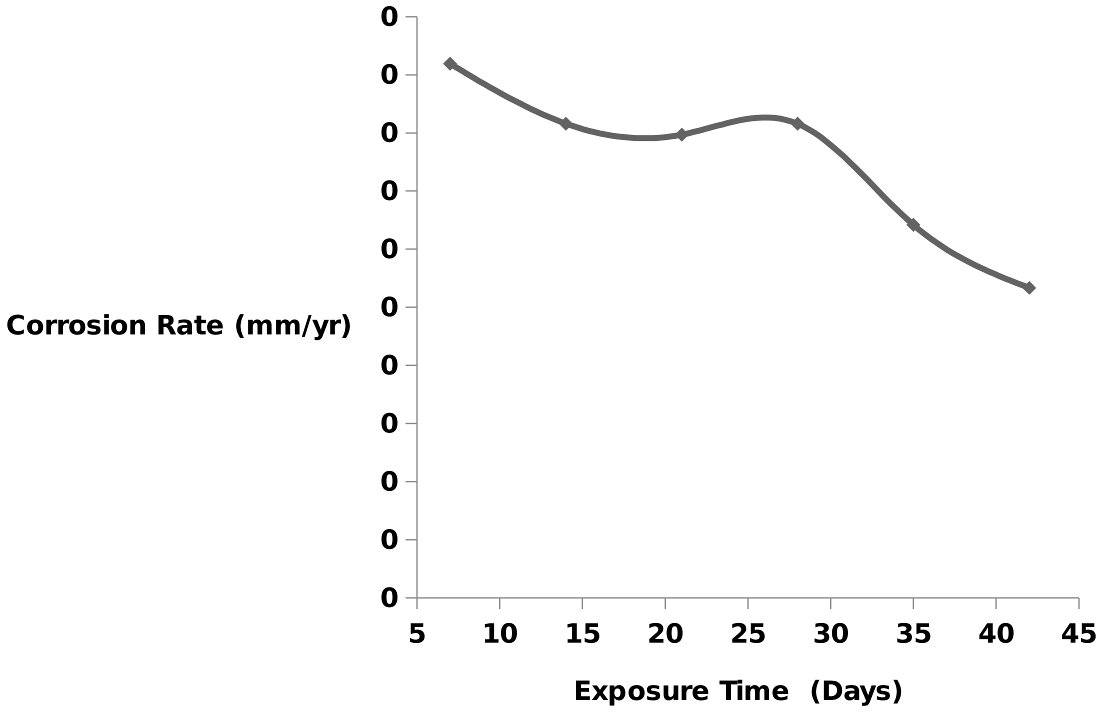

Fig. 4.4: Corrosion rate (mm/yr) against the exposure time (days) in the aerobic environment.

4.4.2: Corrosion rates and exposure times in anaerobic environment

The corrosion rate against exposure time in anaerobic environment was shown in figure 4.5. From the Figure, it was observed that the corrosion rate was highest at day 7 which is 0.0005646 mm/yr after which there was decrease in the corrosion rate. It was equally observed that the corrosion rate was highest in anaerobic environment when compared to that of aerobic and distilled water (control) environments which are 0.0004595 mm/yr and 0.0004458 mm/yr respectively.

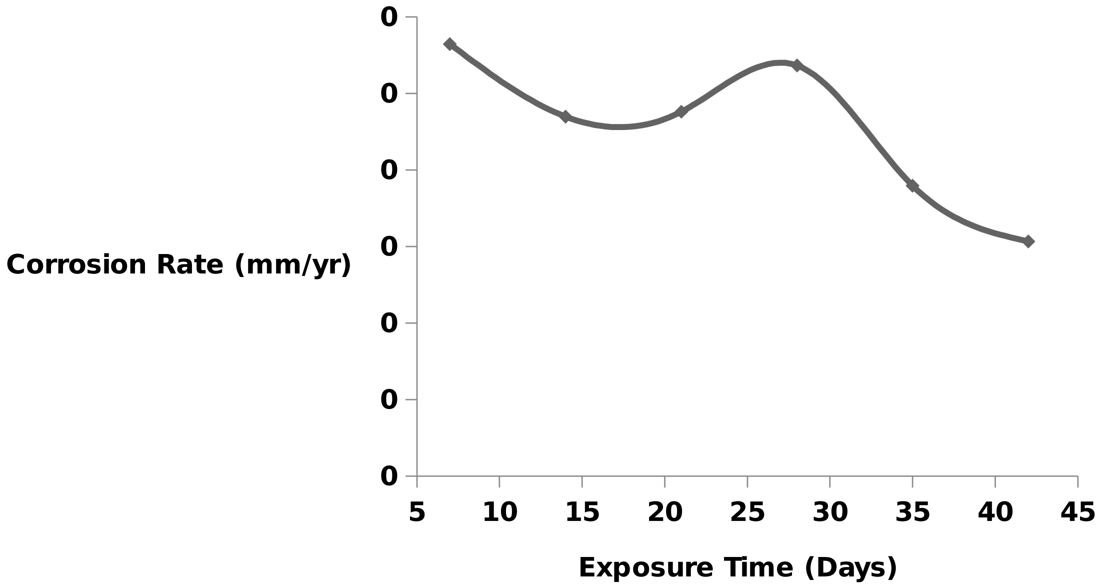

Fig.4. 5: Corrosion rate (mm/yr) against the exposure time (days) in the anaerobic environment.

4.4.3: Corrosion rates and exposure times in distilled water (Control) environment

The effect of exposure time on the corrosion rate in the control environment was shown in Figure 4.6. The corrosion rate was highest at day 7 at 0.0004458 mm/yr. The corrosion rate decreased irregularly with exposure time, reaching the least rate of 0.0002290 mm/yr at day 42.

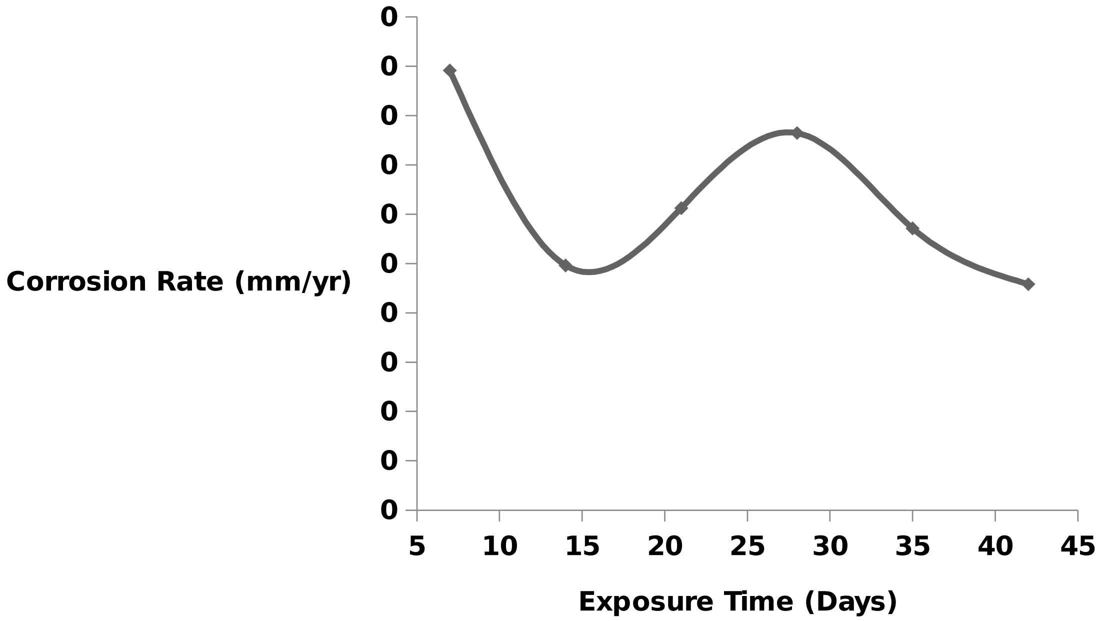

Fig.4. 6: Corrosion rate (mm/yr) against the exposure time (days) in the control environment.

TABLE 4.4: Electrode Potential and Concentration in Aerobic Environment

Exposure Time (days)	Electrode Potential (mV)	pH of Environment	Concentration of mineral salt medium in mil/litre
0	-0.550	7.0	500
7	-0.661	6.8	510
14	-0.672	5.0	520
21	-0.710	5.0	525
28	-0.710	5.0	530
35	-0.712	4.0	530
42	-0.710	6.0	535

TABLE 4.5: Electrode Potential and Concentration in Anaerobic Environment

Exposure Time (days)	Electrode Potential (mV)	pH of Environment	Concentration of the Baar's medium

			in mil/litre
0	-0.554	7.0	500
7	-0.670	6.8	512
14	-0.676	6.0	524
21	-0.726	5.0	536
28	-0.728	5.0	548
35	-0.717	4.0	548
42	-0.712	6.0	560

TABLE 4.6: Electrode Potential and Concentration in Control Environment

Exposure Time (days)	Electrode Potential (mV)	pH of Environment	Concentration of the distilled water in mil/litre
0	-0.550	7.0	500
7	-0.563	6.8	505
14	-0.631	6.8	510
21	-0.647	6.8	515
28	-0.702	6.8	520
35	-0.700	6.8	520

42	-0.701	7.0	525

4.5.1: Electrode potentials and exposure times in aerobic environment.

The electrode potential (in millivolt) against the exposure time (in days) was shown in Figure 4.7. The electrode potential was highest at day zero which is -0.550 mV after which it started decreasing in the subsequent days reaching a stable state at days 21, 28, 42 which are -0.710 mV, -0.710 mV and -0.710 mV respectively. The least electrode potential was -0.712 mV at day 35.

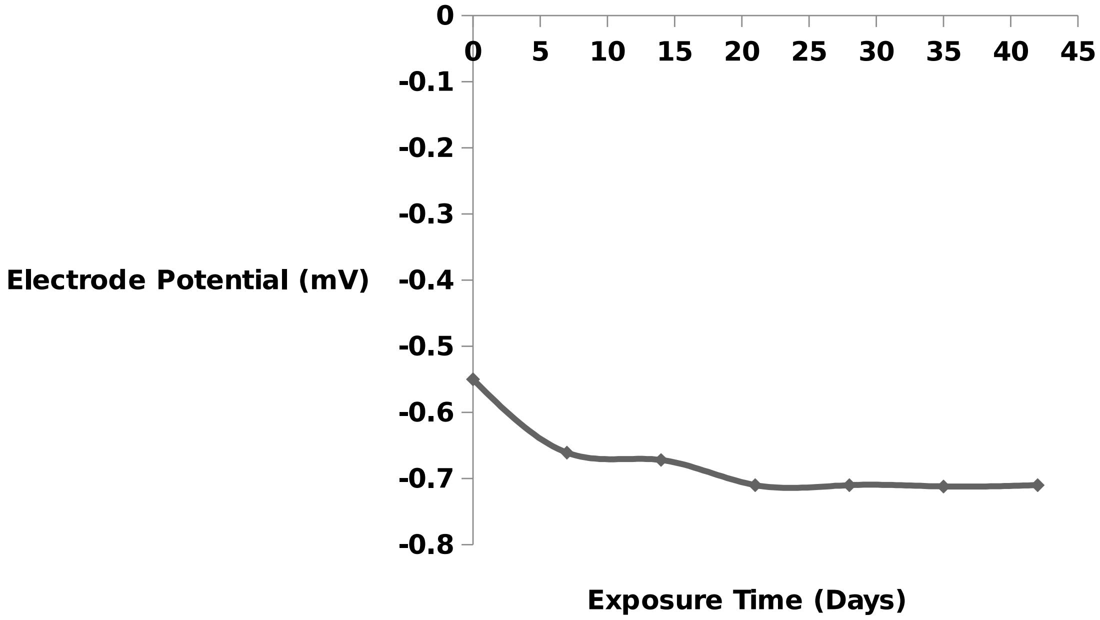

Fig.4. 7: Electrode potential (millivolt) against exposure time (days) in aerobic environment.

4.5.2: Electrode potentials and exposure times in anaerobic environment.

The electrode potential against exposure time in anaerobic environment was shown in Figure 4.8. The electrode potential was highest at day zero which is -0.554 mV. The electrode potential was seen to decrease as day goes by and was lowest at day 28 which is -0.728 mV.

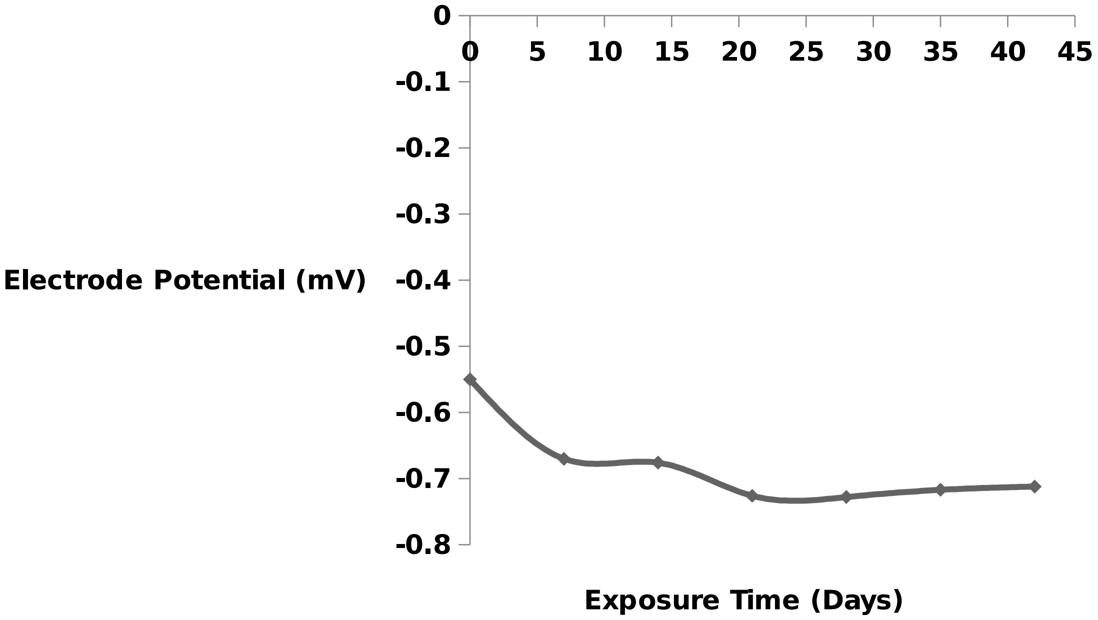

Fig.4. 8: Electrode potential (millivolts) against exposure time (days) in anaerobic environment

4.5.3: Electrode potentials and exposure times in distilled water (control) environment.

The electrode potential against exposure tine in distilled water (control) environment was shown in Figure 4.9. The electrode potential was highest at day zero which -0.550 mV, after which it continues to decrease in subsequent days reaching the least at day 28 which is -0.702 mV.

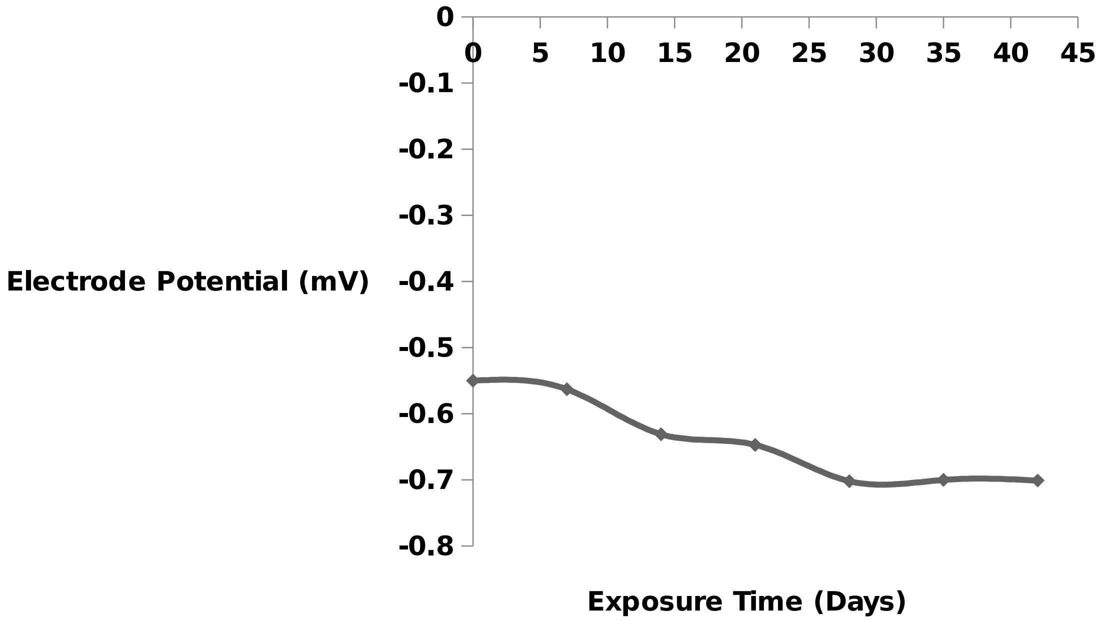

Fig.4. 9: Electrode potential (millivolts) against exposure time (days) in control environment

4.6.1: Concentrations and exposure times in aerobic environment.

The concentration (in mil/liter) against exposure time (in days) was shown in Figure 4.10. The concentration increases with the exposure time starting from day zero which is 500 ml/liter. The concentration reached a stable state at days 28 and 35 which are 530 ml/liter and 530 ml/liter respectively before reaching the highest at day 42 which is 535 ml/liter.

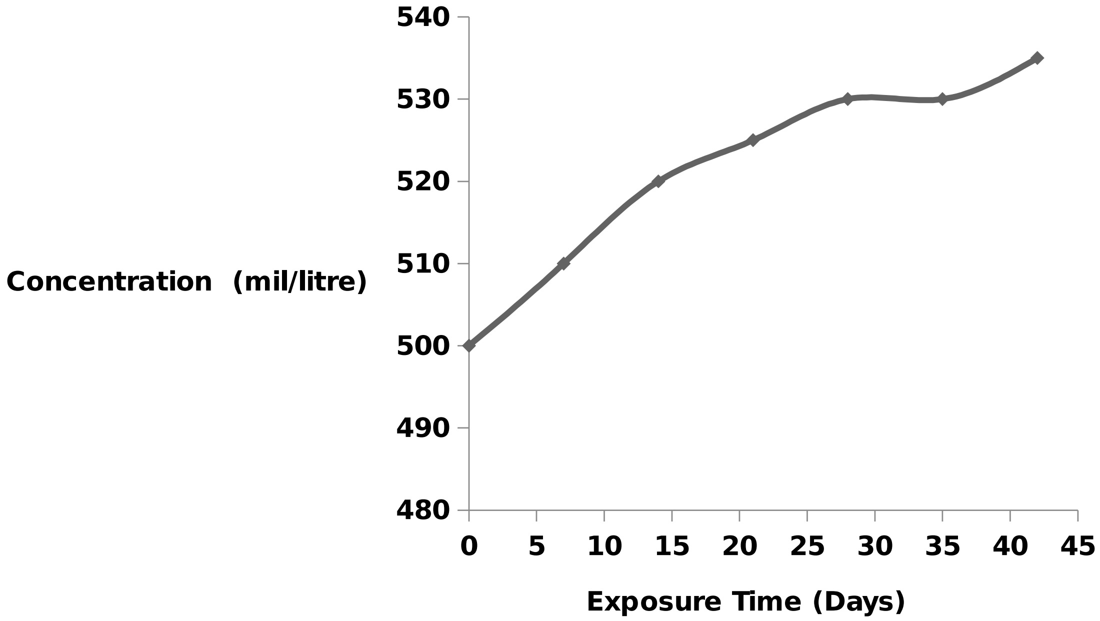

Fig. 4.10: Concentration (ml/l) against exposure time (days) in aerobic environment.

4.7: Concentrations and exposure times in anaerobic environment.

The concentration in (in mils/liter against exposure time (in days) anaerobic environment was show in Figure 4.11. The concentration increases with the exposure time starting from day zero at 500 ml/liter and in subsequent days. The concentration reached a stable state at days 28 and 35 which are 548 mills/liter and 548 mils/liter respectively. It reached the highest at day 42 which is 560 mils/liter.

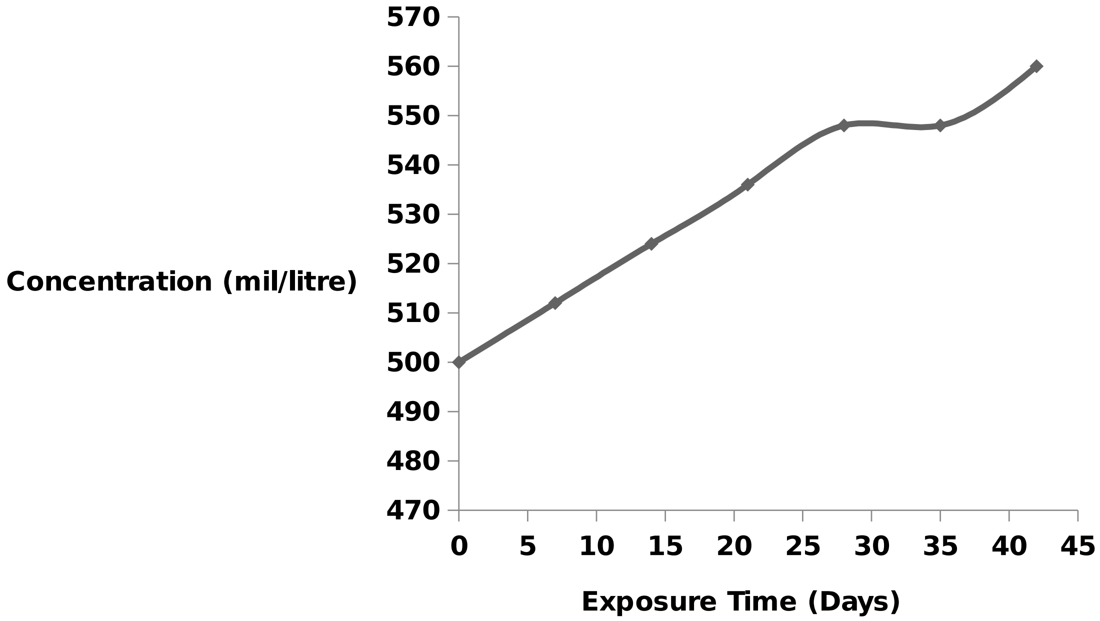

Fig. 4.11: Concentration (ml/l) against exposure time (days) in anaerobic environment.

4.7.1: Concentrations and exposure times in distilled water (control) environment.

The concentration versus exposure time in control environment was shown in Figure 4.12. The concentration increases with the exposure time beginning from day zero which is 500 mls/liter. There was a stable state of the concentration at 520 mls/liter and 520 mils/liters respectively at day 28 and 35. The highest concentration was equally recorded at day 42 which is 525 mils/liter.

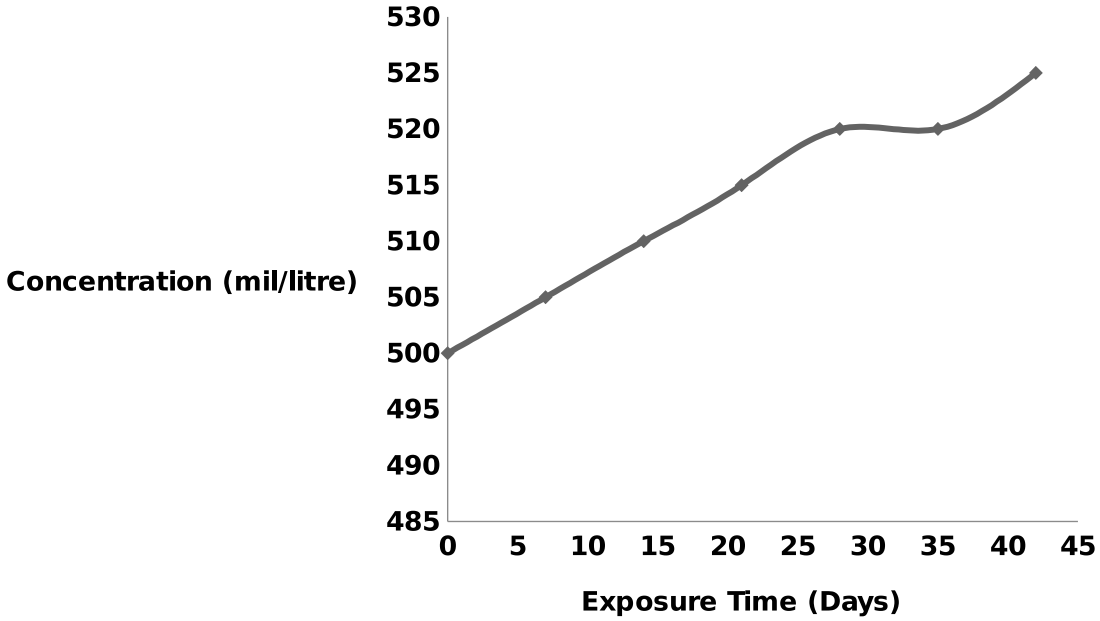

Fig. 4.12: Concentration (ml/l) against exposure time (days) in control environment.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study, the high carbon steel appeared to corrode faster in anaerobic environment than in aerobic environment. The corrosion rate was observed to be highest in the first 7 days (0.0005646 mm/yr) and then decreased with exposure time in each environment. The formation of a passive oxide film on the surface of the specimen is believed to be responsible for the observation. Moreover, by observing the pattern of corrosion rate in all the three environment, it is apparent that the highest corrosion rate was measured in the first seven days before the rate started to decrease. These reflect the power law pattern of corrosion growth. This is in agreement with Yuzwa *et al.*(1991) work on sulphate reducing bacteria corrosion. The formation of rust and the depletion of food supply in a medium has restricted the progress of corrosion, hence the decrease in metal loss volume. The corrosion rate tends to be higher in anaerobic environment. This is in agreement with Carpen *et al.* (2012) who demonstrated that corrosion rate might be as high as 10 – 63 micrometer per annum in anaerobic environment. Such high corrosion rate in anoxic ground water cannot occur without the acceleration of the corrosion process by microbial activities (Carpen *et al.*, 2012).

Owate (2008) reported that the corrosion rates obtained for mild steel supported the fact that carbon content in itself has little effect if any on the general corrosion resistance of the steel.

Ovri (2010) found that mild steel corroded more than medium carbon steel in the aerobic as well as in the anaerobic environment.

The corrosion inducing agents predominant in the environment were OH^- and SO_4^{2-} radicals according to Ovri (2010)

$4Fe_{(s)} + SO_4^{2-}{}_{(aq)} + 4H_2O \longrightarrow FeS(s) + 3Fe(OH)_2 + 2OH^-$ - - (17)

The electrode potential decreased as resistance increases due to corrosion products formed on the steel surface. The corrosion product formed with the exposure time raises the resistance of the sample thereby decreasing the electrode potential values (Figures 4.7 – 4.9). The result obtained on weekly basis showed that the electrode potential value of the exposed samples decreases with exposure time to a certain extent corresponding to 28 days. This is believed to be due to formation of corrosion products which leads to resistance polarization of the samples (Ovri *et al.,* 1992).

This implies that the electrode potential values decrease with exposure time. This result is in agreement with the findings of Jin *et al.* (2012) and also confirmed the result of weight loss measurement. Weight loss was found to increase with time and concentration. Ovri, *et al.* (2010) in their work on microbial corrosion of mild and medium carbon steel observed that the bacteria concentration of the environment increases with the time of exposure in days. The result of the weight of the respective specimen after each exposure time was taken and recorded. In addition, weight loss increased as pH is decreased, making the environment more acidic and consequently more corrosive. This study agrees with the findings of Oparaodu *et al.* (2014) who reported that generally the weight loss of the steel increased with the exposure time whereas the corrosion rate decreased with the exposure time. This also agrees with the report of Wan *et al.* (2013) on corrosion rate. The result equally showed a general pattern of increase in percentage weight loss and a corresponding decrease in corrosion rate with respect to time (Tables 4.1 - 4.3).

Initially corrosion in the anaerobic environment was severe, this can be explained from the fact that when oxygen is not available for the removal of electrons from the metal surface, an alternative cathode reaction is necessary for corrosion to occur. This suggested that sulphate–

reducing bacteria contribute directly to the corrosion of iron by the removal and utilization of hydrogen available at the cathode areas of the metal for the reduction of sulphate to sulphide. This is in agreement with the report Ma *et al*. (2000) that corrosion of 99.99% pure iron immersed in solution was inhibited by a protective layer of ferrous sulphide (FeS) under such conditions as less than 0.04mmol/dm^3 of hydrogen sulphide (H_2S) concentration, pH value of 3 – 5 and an immersion time longer than two hours.

Similarly, the corrosion in the aerobic environment was also severe. Aerobic bacteria grow by assimilating CO_2 using energy derived from the oxidation of sulphur to sulphite for their growth. A localized pitting corrosion results from the direct anaerobic bacteria reduction of the sulphate at the metal surface. This is possible because the biofilm formed by the bacteria creates an anaerobic condition. This is in agreement with the findings of Jin *et al*. (2012) that pitting holes were observervered on the surface of the steel in the soil extract solution with sulphate reducing bacteria, but not in the absence of sulphate reducing bacteria (SRB). They showed that the susceptibility of the steel to corrosion was more in the soil extract solution with SRB than that without SRB and the pitting corrosion readily occurred on the surface of the steel in the presence of SRB. This study also agrees with the findings of Faisal *et al.* (2013) that the coupons immersed in the sulphate reducing consortium exhibited aggressive and deeper pitting. SRB inoculation inhibited the corrosion rate under aerobic condition at days 14 and 28 which are both 0.0004080mm/yr. The whole surfaces of the corroded steel coupons were covered with homogeneous ferrous sulphide (FeS). When the entire coupon surface was covered with homogeneous ferrous sulphide, corrosion of carbon steel was inhibited due to passivation. This is in agreement with a study by Herrera *et al*. (2009) which reported that when the entire coupon surface was covered with homogenous ferrous sulphide, corrosion of carbon steel was inhibited. The formation of a dirty green precipitate which is insoluble in excess sodium hydroxide confirms the presence of iron (II) oxide. It was observed that a general or uniform type of corrosion occurred on the specimen as the colour of the specimen gradually changed from grey eventually emerging dark in appearance with pale green patches in anaerobic environment while the specimen transformed from grey to reddish brown colour and eventually dark in aerobic environment. It was observed that the colour of the environments changed from colourless to brown, dark pale green and reddish brown in aerobic, anaerobic and the control environment respectively.

The observation under anaerobic condition indicated that hydrogen sulphide didn't accelerate corrosion of carbon steel. Corrosion acceleration by SRB activity such as the cathode depolarization was stopped at day 7. This is in agreement with the findings of Mataqi *et al.* (2013) using soil model where they discovered that upon the inoculation of SRB under aerobic conditions, little corrosion on the steel coupon were observed during the 14th – 28th day of incubation period. They reported that sulphate concentration for the SRB inoculated in both anaerobic and aerobic conditions stopped decreasing at day 7 suggesting that the sulphate reducing activity of SRB was high until this day as it began to decrease afterwards. They equally indicated that the heterogeneous structure of the soil led to the formation of a heterogeneous biofilm with corrosion products on the carbon steel coupons. This is in agreement with this study that the heterogeneous biofilm that resulted in the uneven distribution of dissolved oxygen on the metal surface accelerated microbial corrosion. Dubiel *et al.* (2002) reported that the corrosion of carbon steel in the culture inoculated with both sulphate oxidizing bacteria (SOB) and sulphate reducing bacteria (SRB) was accelerated compared to that in the culture inoculated with either SOB or SRB.

5.2 Conclusion

As steel undergoes corrosion in different environment, there tends to be an inverse relationship in the weight loss and the corrosion rate trends in the exposed steel. While weight loss tends to increase over time, corrosion rate tends to decrease simultaneously.

The high carbon steel experienced a general corrosion in each environment. The corrosion rate of the high carbon steel was observed to be 0.0004595 mm/yr in aerobic environment, 0.0005646 mm/yr in anaerobic environment and 0.0004458 mm/yr in distilled water (control) environment. These results were compared to those reported by Ovri *et al (*2010) in microbial corrosion of mild and medium carbon steel and Owate (2008) in corrosion behaviour of mild and high carbon steels in various acidic media. In view of this, high carbon steels were found to be relatively more corrosion resistant than the mild and medium carbon steels and may be more suitable for application in the industries such as the petrochemical industry.

5.3 Recommendations

The effect of bacteria in the corrosion of high carbon steel has been evaluated and found to be partly responsible for failures of the material exposed in such environments which favours microbial growth.

The activities of microbes may be arrested by the following methods:

a) Treating the environment with chlorine but with caution as solutions of chlorine gas in water is corrosive to steels.
b) Use of bactericides or biocides to arrest the action of microbes.
c) By use of cathodic protection
d) By dozing the material with a suitable inhibitor.
e) By design considerations based on fundamental knowledge of microbial ecology thereby maintaining unfavourable conditions for microbial growth.
f) By use of protective coatings particularly of resistance synthetic polymers or paints containing inhibiting salts such as chromium, zinc, copper etc (Shera *et al.*, 2011).

5.3.1 Contributions to Knowledge

A. It is discovered that high carbon steel corrode more only in the anaerobic environment, therefore high carbon steel should be used in many industries especially in petrochemical industries.
B. There is need to use microbes such as aerobic *Pseudomonas fragi* biofilms and aerobically grown *Escherichia coli* biofilms which are known to posses qualities which can inhibit corrosion in the prevention of corrosion. This is because other chemical methods of corrosion control are not environmentally friendly.

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APPENDIX

CORROSION RATE CALCULATION

The results of the weight loss of the high carbon steel exposed to aerobic and anaerobic bacteria with respect to control environment of distilled water were converted to corrosion rate in millimetre per year (mpy) using the standard mathematical relation. (Owate, 2008)

CR = 534 W/DAT - - - - - - - (16)

Where CR = corrosion rate in mpy

W = Weight loss in grams

D = Density of steel in g/cm^3, that is 7.87g/cm^3

T = Exposure Time in hours

A = Total surface area in cm^3

534 = Constant

For the rectangular specimen with a hole of about 2mm, the total surface area can be calculated from the equation stated below:

$$A = 2(LW + LH + WH) - \left[2\left(\pi \; d^2/4 \right) \right] \qquad - - - - - (17)$$

Where L = Length of the specimen in cm

W = Width of the specimen in cm

H = Height or thickness of the specimen in cm

D = Diameter of the hole in cm.

ILLUSTRATION

The corrosion rate CR for the exposed time of 7 days was calculated as follows:

From equation (15) above: $CR = 534\ W/DAT$

From tables 4.1, 4.2 and 4.3

W = Weight loss after 7 days = 0.020lg, 0.0247g and 0.0195g for aerobic, anaerobic and controlled environment respectively.

T = Exposure time = 7days =7x 24 hours = 168 hours

D = Density of steel = 7.87g/cm^3

From equation (16) above: The total surface area (A) can be calculated as follows:

$A = 2\,(LW + LH + WH) - 2\,(\pi\ d^2/4)$

Where L = Length of the specimen = 2.8cm

W = Width of the specimen = 2.8cm

H = Height or thickness of the specimen = 0.2cm

D = Diameter of the hole = 0.4cm.

$A = 2\ (2.8 \times 2.8) + (2.8 \times 0.2) + (2.8 \times 0.2) - 2\ (22 \times 0.4^2)/(4 \times 7)$

$A = 2\ \ 7.84 + 0.56 + 0.56\ \ -\ \ 7.04/28$

$A = 17.92 - 0.2514 = 17.6686\text{cm}^2$

FOR AEROBIC ENVIRONMENT

The corrosion rate CR (in mpy) for the exposure time of 7 days was calculated as follows:

$CR = 534\ W/DAT$

$CR = 543 \times 0.201 \ / \ 7.87 \times 17.6686 \times 168$

$CR = 10.7334 \ / \ 23360.7162$

Therefore $CR = 0.0004595$mpy

FOR ANAEROBIC ENVIRONMENT

The corrosion rate for the exposure time of 7 days is calculated using:

$CR = 534\ W/DAT$

$CR = 543 \times 0.0247 \ / \ 7.87 \times 17.6686 \times 168$

$CR = 13.1898 \ / \ 23360.7162$

Therefore $CR = 0.000564$mpy

FOR CONTROL ENVIRONMENT

The corrosion rate CR for the exposure time of 7 days is calculated using

$CR = 534\ W/DAT$

$CR = 543 \times 0.0195 \ / \ 7.87 \times 17.6686 \times 168$

$CR = 10.413 \ / \ 23360.7162$

Therefore $CR = 0.0004458$mpy.

CORROSION RATE AFTER 14 DAYS (336hrs) IN AEROBIC ENVIRONMENT, the corrosion rate (CR) in mpy =

CR = 534 W/DAT

CR = 543 x 0.0357 / 7.87 x 17.6686 x 336

CR = 19.0638 / 46721.4324

CR = 0.0004080mpy

FOR ANAEROBIC ENVIRONMENT

using:

CR = 534 W/DAT

CR = 534 x 0.0411 / 7.87 x 17.6686 x 336

CR = 21.9474 / 46721.4324

CR = 0.0004698mpy

IN CONTROL ENVIRONMENT

using

CR = 534 W/DAT

CR = 534 x 0.0217 / 7.87 x 17.6686 x 336

CR = 11.5878 / 46721.4324

CR = 0.0002480mpy.

CORROSION RATE AFTER 21 DAYS (504Hours) IN AEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0523 / 7.87 x 17.6686 x 504

CR = 72.9282 / 70082.148

CR = 0.0003985mpy.

IN ANAEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0625 / 7.87 x 17.6686 x 504

CR = 33.3750 / 70082.148

CR = 0.0004762mpy.

IN CONTROL ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0402 / 7.87 x 17.6686 x 504

CR = 21.4668 / 70082.148

CR = 0.0003063mpy.

CORROSION AFTER 28DAYS (672 (HOURS) IN AEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0714 / 7.87 x 17.6686 x 672

CR = 38.1276 / 93442.8630

CR = 0.000408mpy.

IN ANAEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0939 / 7.87 x 17.6686 x 672

CR = 50.1426 / 93442.8630

CR = 0.0005366mpy.

IN CONTROL ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0669 / 7.87 x 17.6686 x 672

CR = 35.7246 / 93442.8630

CR = 0.0003823mpy.

CORROSION RATE AFTER 35 DAYS (840 HOURS IN AEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0702 / 7.87 x 17.6686 x 840

CR = 37.4868 / 116803.5809

CR = 0.0003209mpy.

IN ANAEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0830 / 7.87 x 17.6686 x 840

CR = 44.3220 / 116803.5809

CR = 0.0003795mpy.

IN CONTROL ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0625 / 7.87 x 17.6686 x 840

CR = 33.3750 / 116803.5809

CR = 0.0002857mpy.

CORROSION RATE AFTER 42 DAYS (1008 HOURS) IN AEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0700 / 7.87 x 17.6686 x 1008

CR = 37.3800 / 140164.2971

CR = 0.0002667mpy.

IN ANAEROBIC ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0805 / 7.87 x 17.6686 x 1008

CR = 42.9870 / 140164.2971

CR = 0.0003067mpy.

IN CONTROL ENVIRONMENT

Using CR = 534 W/DAT

CR = 534 x 0.0805 / 7.87 x 17.6686 x 1008

CR = 32.0934 / 140164.2971

CR = 0.0002290mpy.

Calculation of Percentage Weight Loss of the Steel Coupons in different Environments

$$\% \text{ weight loss} = \frac{Wo - Wi}{Wo} \text{ X } \frac{100}{1}$$

For Aerobic Environment

$$\text{Day 7} = \frac{9.9378 - 9.9177}{9.9378} \text{ X } \frac{100}{1} = 0.2022\%$$

$$\text{Day 14} = \frac{11.7102 - 11.6745}{11.7102} \text{ X } \frac{100}{1} = 0.3048\%$$

$$\text{Day 21} = \frac{14.8781 - 14.8258}{14.8781} \text{ X } \frac{100}{1} = 0.3515\%$$

$$\text{Day 28} = \frac{9.9467 - 9.8753}{9.9467} \text{ X } \frac{100}{1} = 0.7178\%$$

$$\text{Day 35} = \frac{15.3923 - 15.3221}{15.3923} \text{ X } \frac{100}{1} = 0.4560\%$$

$$\text{Day 42} = \frac{13.0048 - 12.9348}{13.0048} \text{ X } \frac{100}{1} = 0.5382\%$$

Anaerobic Environment

$$\text{Day } 7 = \frac{11.0777 - 11.0530}{11.0777} \quad \text{X} \quad \frac{100}{1} \quad = 0.2229\%$$

$$\text{Day } 14 = \frac{11.9859 - 11.9448}{11.9859} \quad \text{X} \quad \frac{100}{1} \quad = 0.3429\%$$

$$\text{Day } 21 = \frac{9.2557 - 9.1932}{9.2557} \quad \text{X} \quad \frac{100}{1} \quad = 0.6752\%$$

$$\text{Day } 28 = \frac{11.0338 - 10.9399}{11.0338} \quad \text{X} \quad \frac{100}{1} \quad = 0.8510\%$$

$$\text{Day } 35 = \frac{9.0682 - 8.9853}{9.0682} \quad \text{X} \quad \frac{100}{1} \quad = 0.9152\%$$

$$\text{Day } 42 = \frac{11.8944 - 11.8139}{11.8944} \quad \text{X} \quad \frac{100}{1} \quad = 0.6767\%$$

Distilled Water (Control) Environment

$$\text{Day 7} = \frac{16.4389 - 16.4194}{16.4389} \times \frac{100}{1} = 0.1186\%$$

$$\text{Day 14} = \frac{11.5512 - 11.5295}{11.5512} \times \frac{100}{1} = 0.1878\%$$

$$\text{Day 21} = \frac{12.5035 - 12.4633}{12.5035} \times \frac{100}{1} = 0.3215\%$$

$$\text{Day 28} = \frac{14.9968 - 14.9300}{14.9968} \times \frac{100}{1} = 0.4460\%$$

$$\text{Day 35} = \frac{10.7760 - 10.7135}{10.7760} \times \frac{100}{1} = 0.5799\%$$

$$\text{Day 42} = \frac{9.0040 - 8.9439}{9.0040} \times \frac{100}{1} = 0.6674\%$$