Research Article　　　　　　　　　　　　　　　　　　　　　　　　　　　**Open Access**

Biodeterioration of Pipeline Concrete Coating Material by Iron Oxidizing and Sulphate Reducing Bacteria

Samson E Anichi and Gideon O Abu*

Department of Microbiology, University of Port Harcourt, P.M.B. 5323 Port Harcourt, Nigeria

Abstract

Biodeterioration/biocorrosion of concrete pipeline coating material by sulphate reducing bacteria (SRB) and Iron oxidizing bacteria was tested in the laboratory. The sulphate reducing bacteria and the Iron oxidizing bacteria were isolated from water samples collected from a swamp at a depth of 30 cm. The water samples had a viable, culturable, heterotrophic bacterial count of 1.2×10^3 cfu/ml for the sulfate reducing bacteria (SRB) and 4.0×10^3 cfu/ml for the Iron oxidizing bacteria. A loop-full of discrete colonies of the isolates of the two groups were used to initiate the biodeterioration tests. The pH of the set ups with the SRB showed a change in acidity from 8.24 in the fresh medium to 6.79 after 7 days and moved down to 5.23 in 28 days of incubation. In contrast, the pH of the set ups with Iron oxidizing bacteria showed an increase in alkalinity. The pH of the fresh Winogradsky medium changed from 6.60 to 7.80 in 7 days of incubation and to 9.02 after 28 days. The dissolved Iron II ion in the two set ups showed a gradual decrease in concentration as the time of incubation increased. The conductivity in the SRB set up increased from 0.41 s/m to 1.56 s/m in 28 days of incubation. On the other hand, the Iron oxidizing bacteria set up showed a decrease in conductivity from 1.12 s/m to 0.35 s/m in 28 days of incubation. The surfaces of the coupons of the concrete coating material used in the biodeterioration testing were slimy on touch indicating possible biofilm formation. There were changes in the physical appearance of the materials such as colour and texture. Corrosion rates were 0.6382 mpy and 0.3469 mpy for SRB and Iron bacteria respectively. Concrete coating of pipelines is a good strategy for swamp operations. The low rate of biodeterioration/biocorrosion of the concrete coating as indicated in this study is a useful piece of information for oilfield applications.

Introduction

Biodeterioration of materials can take the form of biocorrosion or biofouling. Corrosion of metals is believed to be an electrochemical process, which results in oxidation of a metal to its oxide and hydroxide thereby distorting the structural integrity of the metal. Although the electrochemical nature of corrosion remains valid, microorganisms influence metal corrosion by modifying the metal solution interface through biofilm formation [1], hence there is microbially influenced/induced corrosion (MIC). Biological activities that stimulate the anodic reaction by acidic metabolites or the cathodic reactions by microbial production of cathodic reactants such as hydrogen sulphide (H_2S), the breakdown of protective films or the increase in conductivity of the liquid environment enhance corrosion [2].

Corrosion is a leading cause of pipeline failure and is a main component of the operating and maintenance cost of gas industry pipelines [3]. As reported by Koch et al. [3], the annual cost of all forms of corrosion in 2001 in oil and gas industries was 13.4 billion dollars. Out of this, microbial induced corrosion (MIC) accounted for about 2 billion dollars [3]. It has been estimated that 40% of all internal pipeline corrosion in the gas industry can be attributed to microbial corrosion [4].

The groups of organisms widely implicated in corrosion of oil pipeline are: Sulphate reducing bacteria [5], *Bacillus cereus* ALE4 [6]. *Cladosporium resinae* was shown to induce localized pitting corrosion of Aluminum [7].

The major corrosion prevention strategy for pipelines is by applying different forms of coating. The coating provides a barrier between the environment and the steel pipes. Common coating systems used is coal tar epoxy, polyethylene, iron ore concrete, ceramic epoxy and 100% solid polyurethane [8]. In many instances, oil pipes are laid across rivers, lakes and seas for efficient transportation. Then the need arises to ensure that the pipes remain submerged in the aquatic environment. This is achieved by increasing the weight of the pipes through concrete coating. Thus, concrete coating performs the function of physical protection in addition to increasing the weight that helps to submerge the pipes.

David [9] reported that microbes caused deterioration of reinforced concrete structures located in aggressive environments. It has been found that biodeterioration of concrete structures may increase concrete porosity and accelerate the diffusion of materials and increase corrosion processes, causing a significant loss of capacity. Microbial deterioration of concrete can be due to production of biogenic sulphuric acid by microorganisms especially sulphur oxidizing bacteria. Biogenic sulphuric acid has been found to be responsible for failure of concrete pipes in waste water collection systems in United States [10]. This suggests that sulphate reducing bacteria (SRB) play an important role in deterioration of concrete buried in an anoxic environment. This is because the SRB produce H_2S which can then be oxidized to sulphuric acid.

This study was carried out to provide information about the corrosion of concrete pipeline coating material, made of iron ore, under conditions that approximate the anoxic environments where these industrial materials are often deployed. It should be a pollution prevention strategy.

Materials and Methods

Concrete coating materials

The pipeline coating material used in this study is the concrete made of 60% iron ore, 20% cement, 14% sand and water. Components of the concrete coating material were mixed in correct proportion and poured in a constructed mould to form 2 cm x 1 cm x 1 cm coupons of the concrete material. They were allowed to set for 24 hours. The coupons were weighed and wrapped with a sterile foil until they were used.

Isolation of SRB and Iron Oxidizing bacteria

Water samples were collected from a swamp at a depth of 30 cm with sterile bottles. The swamp is covered with ferns and raphia palms. The 30 cm represents a typical depth to which the weighted pipelines are usually placed. The samples were subjected to ten fold serial dilutions with deionized sterile water.

Iron oxidizing bacteria were enumerated from the samples by inoculating 0.1 ml of each dilution on Winogradsky agar medium using the pour plate method. Each dilution was plated in duplicate. Dry plates were incubated aerobically at 30°C for 7 days. Isolated colonies were counted and the titers expressed in cfu/ml.

Sulphate reducing bacteria were enumerated from the samples by inoculating 0.1ml of each dilution on Postgate agar medium containing 0.03 g sodium thioglycollate, using the pour plate method. Each dilution was plated in duplicate. Dry plates were incubated in an anaerobic jar containing gas pack, to produce anaerobic condition, at 30°C, for 14 days. Isolated colonies were counted and the titers expressed in cfu/ml.

Identification of the isolates

Identification of isolates was based on (i) use of selective media (ii) colonial forms including color

(iii) Cell morphology (iv) Gram reaction (v) spore stain and (vi) motility.

Biodeterioration testing

A suspension of Iron oxidizing bacteria was prepared by dispensing a loop full of discrete colonies of iron bacterial isolates, in freshly prepared Winogradsky broth. The broth contained all the components of Winogradsky medium except agar. Eight pre-weighed coupons of the concrete material labeled B_1-B_8 were placed, in pairs, inside Bijou bottles containing sterile Winogradsky broth; the set ups were incubated aerobically at 30°C. Two coupons were withdrawn with a sterile forceps after 7 days. They were washed, dried and reweighed. This was repeated after 14 days, 21 days and 28 days. Changes in weight were recorded. This procedure was repeated with Postgate broth inoculated with a loop full of discrete black colonies of SRB isolates. Coupons used in the SRB set up were labeled C_1-C_8. The Bijou bottles containing SBR suspension and concrete coupons were incubated in an anaerobic jar containing gas pack, to create anaerobic conditions, at 30°C. Controls were comprised of uninoculated sterile media.

The rates of corrosion in mils per year (mpy) were calculated using the formula of Davis [11] as follows:

R= KW/ATD

Where

K = constant equal to 3.45 x 10⁶,

W = weight loss in gram,

A = area to the nearest 0.01 cm²,

T = time of exposure in hours and

D = density.

Physico-chemical analysis

Measurements of pH were carried out, on the water sample from where the test organisms were isolated. The pH of the experimental set ups was also taken. All pH measurements were done with the Hanna pH meter model Hl 8314, USA. The conductivity of the water sample and the experimental set ups was measured with the HAC conductivity meter, model 2845500, USA. The iron II ion content of the experimental set ups was analyzed using an atomic absorption spectrometer (GBC Avanta PM AAS model A6600, Australia).

Results

The coupons moulded had different weights ranging from 8.46 g to 10.64 g and densities from 4.23 g/cm³ to 5.32 g/cm³.

The water samples gave an average Iron oxidizing bacterial count of 4.0 x 10³ cfu/ml. The colonial morphology of the iron bacterial isolates revealed convex, concave, smooth and serrated edged colonies with a rusty brown colour. The Gram and spore stains revealed non spore forming rods that were Gram negative. The motility test revealed motile and none motile rods. The non motile rods with a sheath were identified as *Leptothrix* species. The motile straight rods were identified as *Sphaerotilus* sp and the motile bean-shaped rods were identified as *Gallionella* species.

The water samples gave an average SRB count of 1.2 x 10³ cfu/ml. The colonial morphology of the SRB isolates was mostly circular, convex and black in colour; the isolates showed motility in test media. There were spiral to vibroid shaped, Gram negative, non spore forming and motile cells; these were identified as *Desulfovibrio* species. There were singly curved rods, Gram positive, spore forming and motile cells; these were identified as *Desulfotomaculum* species. There were singly spiral shaped, Gram negative, none spore forming and motile cells; these were identified as *Desulfovibrio* sp.

Identifications were based on the schemes of [12]. The coupons showed changes in physical appearance. The coupons in the iron bacterial culture appeared rusty in colour and rough on touch. The surface was slimy on touch before vigorous washing, suggesting biofilm development. Coupons in SRB became black which may be due to the deposition of iron II sulphide compounds. The black colour disappeared after exposure to air. This may be due to the oxidation of sulphide to sulphate. Change in the weight of the coupons were recorded after the test period (Tables 1and 2).

The conductivity of the original water sample was 0.004 s/m. The conductivity of the set ups with the iron bacteria increased from 0.81

Time In days*	Identification	Intial Weight at day 0	Final weight after test period	Changes in Weight (g)	Rate of corrosion in mil per year (mpy)
7	B1	9.8742	9.8742	0.0000	0.0000
7	B2	9.8742	10.2671	0.0000	0.0000
14	B3	10.0115	10.0115	0.0000	0.0000
14	B4	10.5622	10.5622	0.0000	0.0000
21	B5	10.3222	10.3221	0.0001	0.0663
21	B6	10.3824	10.3824	0.0000	0.0000
28	B7	10.4450	10.4440	0.0010	0.4915
28	B8	10.6323	10.6313	0.0010	0.4829

*presentation represents duplicate set ups.

Table 1: Change in weight of concrete coating material coupons placed in Iron bacterial culture for 28 days.

Time In Days	Identification	Initial Weight at day 0	Final weight after test period	Change in Weight (g)	Rate of corrosion in mil per year (mpy)
7	C	9.4621	9.4621	0.0000	0.0000
7	C	9.7832	9.7832	0.0000	0.0000
14	C	8.7731	8.7731	0.0000	0.0000
14	C	9.0702	9.0700	0.0002	0.2264
21	C	9.7422	9.7422	0.0000	0.0000
21	C	8.8013	8.8013	0.0000	0.0000
28	C	9.6213	9.6193	0.0020	1.0672
28	C	9.0922	9.0911	0.0011	0.6211

*presentation represents duplicate set ups.

Table 2: Change in weight of concrete coating material coupons placed in sulphate reducing bacterial set up after 28 days.

s/m in fresh medium to 1.12 s/m after 7 days of incubation and then decreased to 0.35 s/m after 28 days. On the other hand the conductivity of the set up with the SRB fell from 0.96 s/m in the fresh medium to 0.41 s/m after 7 days and then rose to 1.56 s/m after 28 days (Figure 1). Of the two systems, the SRB with its lower pH and higher conductivity shows more tendencies to corrode the concrete material.

The pH of the original water sample was 6.2. The pH of the set up with the iron bacterial changed from 6.60 in the fresh medium to 9.02 after 28 days. The pH of the set up with the SRB changed from 8.24 in the fresh medium to 5.23 after 28 days (Figure 2).

There was a general decrease in the amount of ferrous ion (Fe^{2+}) in

Figure 2: Change in pH in the presence of concrete coating material coupons in suspension of Iron Oxidizing and Sulphate reducing bacteria.

the set ups with both the iron bacteria and SRB, (Figure 3).

Discussion

The biodeterioration potential of the concrete pipeline coating material was evaluated. The coating material coupons used in the study had high densities due to the iron ore Figure 4. The magnetic property of the coupons was due to the ferromagnetism of the iron. The result of the microbiological analysis showed that sulphate reducing bacteria can be isolated from commonly occurring stagnant aquatic environments such as swamps. The low counts of the SRB may be due to their strict anaerobic demands. Iron oxidizing bacterial counts was relatively higher. This is because the environmental conditions for their growth were easier to simulate in the laboratory.

The result of the biodeterioration testing revealed that there was colonization of the coupons by the organisms. The defacement and slimy nature of the surfaces of the coupons could be attributable to biofilm formation and deposition of bacterial cells or their metabolites.
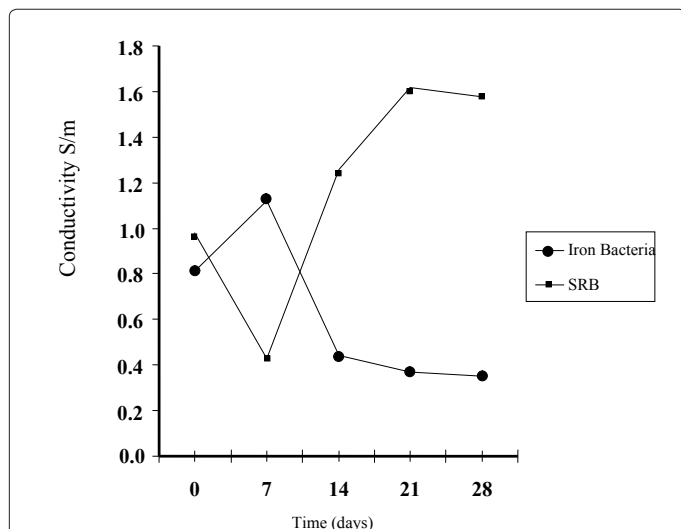
Figure 1: A profile of conductivity measurement of laboratory experiments in the presence of Iron oxidizing and Sulphate reducing bacteria with concrete coating material coupons.
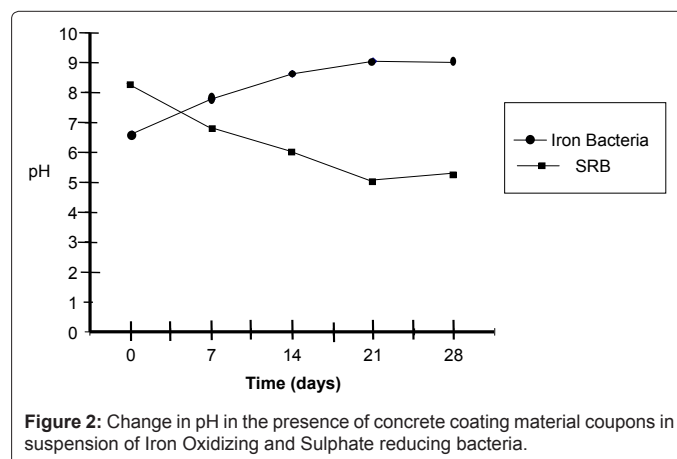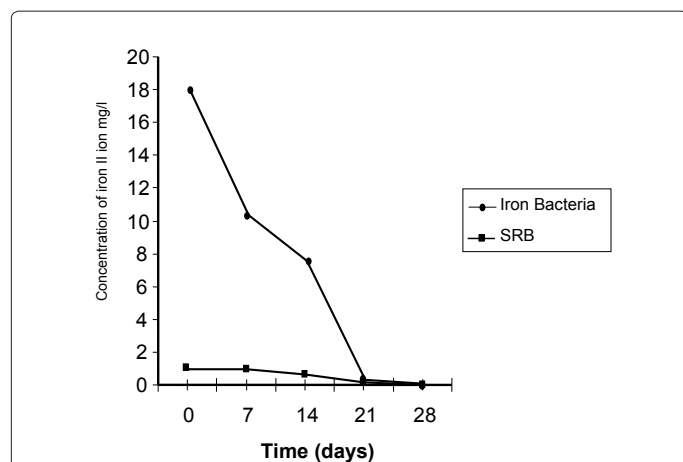
Figure 3: Change in iron II ion concentration in laboratory experiments involving iron oxidizing and sulphate reducing bacteria in the presence of concrete coating material coupons.
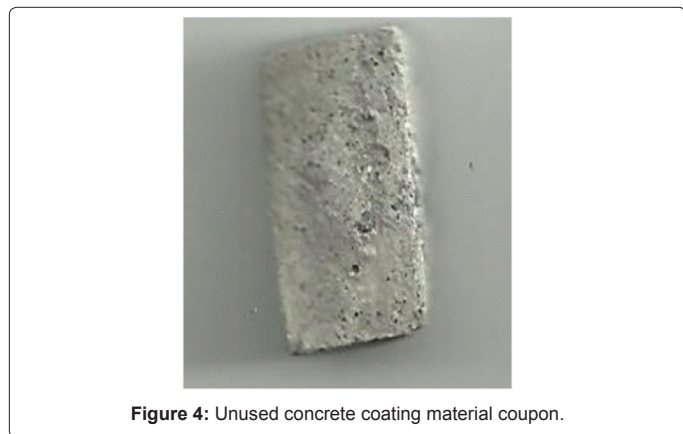

Figure 4: Unused concrete coating material coupon.

The experimental set up with the Iron oxidizing bacteria showed a significant change in pH from 6.60 to 9.02 after 28 days of incubation. The alkalinity drift of the set ups with the Iron oxidizing bacteria can be attributed to the formation of oxides and hydroxides of iron. This is more or less the classic corrosion by oxide formation and not as a result of acidic environment. The bacteria seem to be able to make the concrete alkaline. This is usually the condition of freshly cured cement. On the other hand the pH of the set up with the SRB fell from 8.24 to 5.23 after 28 days of incubation. The acidic nature of the set up with the SRB may be due to the production of acidic hydrogen sulphide [10]. The increase in conductivity of the set ups with the SRB could be attributed to hydrogen sulphide (H_2S, a weak acid) formation. The conductivity of the set ups with the Iron oxidizing bacteria decreased. This may be due to the presence of non ionic species such as oxides. The decrease in the amount of ferrous ion in both set ups suggests transformation of ferrous ion to other forms of iron that were not identifiable by the spectrometric analysis or deposition of the iron II ion as insoluble oxides. Of the two systems, the SRB with its lower pH and higher conductivity shows more tendencies to corrode the concrete material. A direct proportionality has been shown between concrete conductivity and corrosion rate [13].

The role of microorganisms in concrete deterioration has been recognized [9]. Among the genera that cause microbial corrosion, iron oxidizing and sulphate reducing bacteria are of great importance due to their wide distribution in the environment, specific metabolic activities and the prevailing anoxic conditions where the pipes are laid. Apart from producing hydrogen sulphide (H_2S) that stimulates the absorption of hydrogen into the structural metals leading to embrittlement, SRB can induce corrosion by formation of biofilms on the surface of metals and concretes. They form the biofilms deriving energy through their fermentative activities. Oil pipelines are protected by several means of coating but the efficiency of the coating will depend on the resistance of the coating material to microbial attack. Concrete coating material actually offers good protection to pipes and adds weight to reduce buoyancy of the pipes in aquatic environments but its lifespan depends on its resistance to microbial biodeterioration. Corrosion rates were 0.6382mpy and 0.3469mpy for SRB and Iron bacteria respectively. Concrete coating of pipelines is a good strategy for swamp operations. The low rate of biodeterioration of the concrete coating as indicated in this study is a useful piece of information for oilfield applications.

Conclusion

This study showed evidence of low rate of biodeterioration in form of pitting corrosion of the concrete pipeline coating material by bacteria. To prevent or reduce the rate of deterioration and improve their lifespan, expertise of microbiologists should be part of the process of the formulation and testing of the concrete coating materials. Use of biocides, possibly green biocides, which should not impact negatively on the environment [14], should be incorporated in the formulation of the concrete materials. There should be routine checks on the laid pipelines to dislodge the biofilms on the existing pipes.

References

1. Videla HA, Herrera LK (2004) Biocorrosion, In Vazquez Dahalt R, Quintero Ramivez R (eds) *Petroleum Biotechnology Development and Perspectives*. Elsevier Amsterdam, the Natherland, 193–218.

2. Videla H A, Liz KH (2005) Microbiologically influenced corrosion: looking into the future. International microbiology 8: 169–180.

3. Koch GH, Brougers MPH, Thompson NG, Virmani YP, Payer JH (2001) Corrosion cost and preventive strategies in USA FHWA- RD- 01- 156. Federal Highway Administration Washington.

4. Graves JW, Sullivan EH (1996) Internal corrosion in gas gathering system and transmission lines. Matter prot 5: 33–37.

5. Ilhan-sungur E, Cansever N, Cotuk A (2006) Microbial Corrosion of galvanized steel by a freshwater strain of sulphate reducing bacteria (Desulfovibrio sp). Corrosion Science 49: 1097–1109.

6. Rajasekar A, Ganesh BT, Karutha SP, Maruthumuthu S, Palan Swamy N, et al. (2007) Biodegradation and corrosion behaviour of manganese oxidizer Bacillus cereus ACE4 in Diesel transporting pipeline. Corrosion Science 49: 2694–2710.

7. Salvarezza RC, Videla HA (1986) Electrochemical behavoiour of Aluminium in Cladosporium resinae culture. In Biodeterioration VI C.A.B, International Far ham House, UK, 212–213.

8. Shiwei G (2008) Synergistic protection against microbiologically influenced corrosion using a 100% solids polyurethane incorporated with antimicrobial agent. Madison chemical Industrial Inc 490, McGeachic Drive Milton Ontario, L9T 3Y5 Canada.

9. David T (2008) Analysis and Assessment of microbial biofilm-mediated concrete deterioration. A project by Texas department of transportation.

10. Elke V, Verstichel S, Monteny J, Verstraete W (2000) A new test procedure for biogenic sulfuric acid corrosion of concrete. Biodegradation 10: 421-428.

11. Davis JR (2000) Corrosion: Understanding the basics. ASM International 47.

12. David HB, John GH (1994) Bergey's manual of determinative bacteriology. 335-340.

13. Feliu S, Gonzalez JA, Andrede C (1989) Relationship between conductivity of concrete and corrosion of reinforcing bars. British Corrosion Journal 24: 195-198.

14. Wen J, Gu T (2006) Green biocide enhancers enhanced the biocide inhibition of the growth of sulphate reducing bacteria. Green biotechnology poster session.

15. Videla HA (1989) Metal dissolution/redox in biofilms. In Characklis WG, Wilderer PA (eds) *Structure and Function of Biofilm*. John Wiley Chichester, UK, 301–320.

16. Videla HA, Characklis WC (1992) Biofouling and microbial influenced corrosion. International Biodeterioration & Biodegradation 29: 195 – 212.

17. Videla HA (1996) Corrosion inhibition in the presence of microbial corrosion. NACE international, Houston, TX.