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INFLUENCE OF BACTERIA ASSOCIATED WITH CORROSION OF METALS

[1]Manga, S.S., [2]Oyeleke, S.B, [3]Ibrahim, A.D., [1]Aliero, A.A. and [1]Bagudo, A.I.
[1]Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria
[2]Department of Microbiology, Federal University of Technology, Minna, Nigeria
[3]Department of Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

The physicochemical characteristics of the soil beneath and adjacent to corroded petrol tanks were examined. The result revealed that the moisture content of the soil beneath the tanks was higher (3.2%) than the adjacent soil (0.4%). There was no significant difference in the temperature of the soil beneath and the adjacent one. The pH of the soil beneath the tanks was acidic (5.9). The organic matter of the adjacent soil was higher (1.8%) than the soil beneath the tanks. Nitrate and sulphate contents of the adjacent soil were higher (1.36 and 153.5mg/kg) than soil beneath the tanks which was 0.7 and 87.2mg/kg respectively. The bacteria isolated from scrapings of the tanks included *Desulfovibrio vulgaris, Desulfotomaculum nigrificans, Desulfovibrio desulfuricans, Thiobacillus thiooxidans, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus cereus, Clostridium perfringes, Bacillus firmus, Staphylococcus cohnii, Bacillus laterosporus and Micrcococcus sedentarius. P. aeruginosa* was the most frequently observed (12.5%), *while the least was B. laterosporus* (4.5%*).* The corroding ability of bacteria isolated was also examined and the result revealed that bacteria isolated have showed the corroding ability on metal strips and the average percentage weight loss of metal strips was 3.8%, thus indicating that these bacteria have contributed in the deterioration of the petrol tanks studied. The corrosion of storage petrol tanks could lead to seepages of petroleum products in the area, contaminating soil surface and ground water sources which could result in destruction of crops and aquaculture which consequently affect the ecosystem. The use of metals/plastic that can resist corrosion especially alloys to construct storage fuel tanks should be encouraged.

KEYWORDS: Corrosion, Bacteria, Petrol tanks, Deterioration, Metal strips

INTRODUCTION

The electrochemical nature of corrosion remains valid for microbiologically influenced corrosion (MIC), participation of microorganisms in the process nonetheless induces several unique features, the most significant being the modification of the metal-solution interface by biofilm formation (Videla and Herrera, 2004). Biofilms affect interactions between metal surfaces and the environment in the biodeterioration process such as MIC (Videla and Characklis, 1992). Thus, the key to the alteration of conditions at a metal surface, and hence the enhancement of corrosion is the formation of biolfilm (Videla, 1996). It is well known that the metabolic activity of clusters of biofilm organisms can change the pH value for more than three units locally (Beech, *et al*., 2002). The main types of bacteria associated with corrosion of metallic materials are sulphate-reducing bacteria (SRB), iron-oxidizing bacteria (IOB), as well as bacteria secreting organic acid and extracellular polymeric substances (EPS) or slime. The participation of microorganisms in a corrosion process was ignored in the past, but is now acknowledged and remains the focus of present and future research work.

The activities of microorganisms in natural and man-made environment are of great concern to many different industrial operations. In particular, water pipelines, oil, gas and shipping industries are seriously affected by the metabolic activities of microorganisms. For instance, biogenic sulphide production leads to health and safety problems, environmental hazards and economic losses due to reservoir souring and corrosion of equipment (Geesey *et al.*, 2000; Heitz *et al.,* 1996). The negative effect of microbial corrosion of metals on the environment and economy of man makes the identification of microorganisms involved in corrosion and understanding of the new microbial corrosion phenomena becomes a paramount issue. This will lead to the new research outlook and possible solution to the problem of corrosion. In view of this, the study is aimed at evaluating the role of bacteria in deterioration of petrol tanks in the study area. The specific objectives of the study are:

1. To examine the physicochemical characteristics of the soil samples beneath and adjacent to corroded petrol tanks.
2. To enumerate and identify the bacteria associated with corrosion of petrol tanks.
3. To examine the corroding ability of each bacterium isolated.

MATERIALS AND METHODS

Sample collection

Surface scrapings of four (4) corroded petrol tanks of IMAD filling station, Ahmad Bello way, Sokoto were obtained using sterile lancets. Five (5) samples were collected from each tank and were collected in sterile McCartney bottles for microbial analysis. Likewise, the soil samples beneath each of these corroded tanks were collected in clean polythene bags and taken to the soil science laboratory for analysis. Five samples of soil adjacent to the tanks were also collected for both physicochemical and microbial analysis.

The physicochemical properties of the soil was analysed immediately after sample collection (within 1-2 hours of collection), while samples for microbial analysis were kept in the refrigerator at $4^{o}C$ in microbiology laboratory of Usmanu Danfodiyo University, Sokoto and were later analysed.

Physicochemical properties of soil samples

Temperature, pH, moisture content, organic carbon, organic matter, sulphate and nitrate contents of the soil were determined using the methods described by International Institute of Tropical Agriculture, Ibadan (IITA, 1979).

Microbial analysis

Enumeration of bacteria

The total bacterial count was determined by standard plate count method. Serial dilution of the sample was aseptically carried out first before inoculation. The plates were then incubated at $37^{o}C$ for 24-48 hours and colonies were counted as described by Fawole and Oso, 1998. Another nutrient agar plates were also inoculated using the same dilution factor. These plates were incubated anaerobically at $37^{O}C$ for 24-48 hours using anaerobic jars. Iron oxidizing bacteria medium, and postgate medium were also inoculated with one of the dilutions for the selective enumeration of iron oxidizing bacteria (aerobic) and sulphate reducing bacteria (anaerobic) respectively.

Isolation of bacteria

Different colonies observed from different culture plates were selected and further sub cultured on respective medium using streak plate techniques after which they were maintained on agar slants for further analysis.

Characterization of bacteria

The bacterial isolates were identified based on colonial morphology, cultural characteristics and biochemical tests. These biochemical tests include gram staining, spore staining, MR-VP, catalase, coagulase, indole, motility, starch hydrolysis, nitrate reduction, citrate, sulphide indole formation and carbohydrate fermentation as described by Postgate, (1984); Fawole and Oso (1998); Steve and Dannis (2001); Warren *et al*., (2005); and Oyeleke and Manga (2008).

Measurement of corroding ability of each organism

The corroding ability of each bacterium was determined by the method described by Mara and William (1982) in which the active growing stages of pure cultures of the bacteria were inoculated into sterile universal bottles containing appropriate medium and the weight of iron strips was weighed and dipped in the cultures. The medium containing anaerobes was filled to the brim and coated with candle wax to maintain anaerobic condition. This was monitored for a period of 30 days, after which the iron strips were washed, dried and reweighed to check for weight loss.

Statistical analysis

The result obtained were subjected to statistical analysis using one way analysis of variance (ANOVA).

RESULTS

Table 1 represents the means of physicochemical characteristics of soil samples beneath and adjacent to corroded petrol tanks. The soil beneath the tanks had higher moisture content (3.2%) than the adjacent soil

(0.42%). Statistically, there was no significant difference in the temperature of the two locations. The pH of the soil beneath the tanks was acidic (5.9), whereas the adjacent soil had pH value of 7.4. The organic matter of the adjacent soil was higher (1.81%), even though, there was no difference statistically. The nitrate and sulphate at adjacent soil were higher (1.336 and 153.5mg/kg) than the beneath soil (0.7 and 87.2mg/kg) respectively.

Table 1: Means of physicochemical characteristics of soil samples beneath and adjacent to corroded petrol tanks

	Moisture (%)	Temp (^oC)	pH	Org. C (%)	Org. M (%)	Nitrate (mg/kg)	Sulphate (mg/kg)
Beneath	3.2^a ±0.4	26.3^a ±0.2	5.9^b ±0.1	0.8^a ±0.1	1.4^a ±0.1	0.7^b ±0.1	87.2^b ±5.6
Adjacent	0.42^b ±0.17	27^a ±0.49	7.4^a ±0.23	1.04^a ±0.49	1.81^a ±0.85	1.36^a ±0.58	153.5^a ±23.95

The mean bacterial counts of soil samples beneath and adjacent to corroded petrol tanks are presented in Table 2. There was significant difference ($p \leq 0.05$) in the aerobic, SRB and IOB counts, the higher aerobic counts ($1.0x10^7$cfu/g) been recorded in adjacent soil, while the higher SRB counts (1.9 $x10^6$cfu/g) and IOB counts ($2.4x10^6$cfu/g) been recorded in the soil beneath the tanks. There was no significant difference ($p > 0.05$) in the anaerobic count of soil beneath and adjacent to the tanks.

Table 2: Bacterial counts (cfu/g) of soil samples beneath and adjacent to corroded petrol tanks

Soil Sample	Aerobic Count	Anaerobic Count	SRB count	IOB count
Beneath	1.8 $x10^{6\,b}$	2.7 $x10^{6\,a}$	1.9 $x10^{6\,a}$	2.4 $x10^{6a}$
SD±	6.9 $x10^5$	7.5 $x10^5$	7.6 $x10^5$	6.8 $x10^5$
Adjacent	1.0 $x10^{7a}$	2.2 $x10^{6\,a}$	6.8 $x10^{5\,b}$	2.2 $x10^{5b}$
SD±	1.9 $x10^6$	8.6 $x10^5$	3.8 $x10^5$	1.2 $x10^5$

KEY:
SRB= Sulphate Reducing Bacteria
IB= Iron oxidizing Bacteria
SD= Standard Deviation

Table 3 represents the bacteria isolated and their occurrence from scrapings of corroded petrol tanks. The bacteria isolated included *Desulfovibrio vulgaris, Desulfotomaculum nigrificans, Desulfovibrio desulfuricans, Thiobacillus thiooxidans, Pseudomonas aeruginosa, Listeria monocytogenes, Bacillus cereus, Clostridium perfringes, Bacillus firmus, Staphylococcus cohnii, Bacillus laterosporus and Micrcococcus sedentarius. P. aeruginosa* was the most frequently observed (12.5%)*, while the least was B. laterospurus* (4.5%*).*

Table 3: Bacteria isolated and their occurrence from scrapings of corroded petrol tanks

Bacteria	Frequency of occurrence	Percentage occurrence
Pseudomonas aeruginosa	14	12.5
Thiobacillus thiooxidans	13	11.6
Desulfovibrio vulgaris	12	10.7
Bacillus cereus	11	9.8
Clostridium perfringes	11	9.8
Desulfoto.nigrificans	10	8.9
Desulfovibrio desulfuricans	9	8.0
Micrococcus sedentarius	9	8.0
Bacillus firmus	6	5.4
Staphylococcus cohnii	6	5.4
Listeria monocytogenes	6	5.4
Bacillus laterosporus	5	4.5

Bacteria isolated from soil samples adjacent to the corroded petrol tanks are presented in Table 4. These included *Micrococcus sedentarius, Bacillus firmus, Bacillus cerius, Desulfotomaculum nigrificans, Pseudomonas aeruginosa, Thiobacillus thiooxidans, Staphylococcus aureus, Desulfovibrio desulfuricans, Bacillus laterosporus, Desulfovibrio vulgaris* and *Listeria monocytogenes. M. sedentarius* was the most frequently encountered (16.3%), while the least was *Listeria monocytogenes* and *D. vulgaris* (3.5%).

Table 4: Bacteria isolated and their occurrence from soil samples adjacent to Petrol tanks

Bacteria	Frequency of occurrence	% occurrence
Micrococcus sedentarius	14	16.3
Bacillus firmus	12	14.0
Bacillus cerius	11	12.8
Desulfotomaculum nigrificans	10	11.6
Pseudomonas aeruginosa	9	10.5
Thiobacillus thiooxidans	8	9.3
Staphylococcus aureus	6	7.0
Desulfovibrio desulfuricans	5	5.8
Bacillus laterosporus	5	5.8
Desulfovibrio vulgaris	3	3.5
Listeria monocytogenes	3	3.5

Measurement of corrosion ability of bacteria by weight loss of metal strips over a period of 30 days was carried out and the result is presented in Table 5. The average percentage weight loss was 3.8%.

Table 5: Measurement of corroding ability of microorganisms by weight loss of metal strips

Bacteria	Original Wt (g)	Final Wt (g)	Wt Loss (g)	% Wt Loss
Thiobacillus thiooxidans	2.68	2.51	0.17	6.3
Desulfovibrio vulgaris	3.54	3.33	0.21	5.9
Desulfovibrio desulfuricans	3.71	3.51	0.20	5.4
Desulfotomaculum nigrificans	4.35	4.15	0.21	4.8
Pseudomonas aeruginosa	1.66	1.59	0.07	4.2
Listeria monocytogene	1.66	1.59	0.07	4.2
Clostridium perfringes	1.69	1.64	0.05	3.0
Bacillus laterosporus	2.12	2.06	0.06	2.8
Bacillus firmus	2.35	2.30	0.05	2.1
Bacillus cereus	2.56	2.51	0.05	1.9
Staphylococcus aureus	2.45	2.42	0.03	1.2

KEY:
Wt= Weight
% weight loss= (Initial wt-Final wt) divide by Initial wt x 100

DISCUSSION

Temperature and pH are among the parameters that governs the bacterial growth. The temperature of the soil around corroded petrol ranged between 26.3 and 27.0^{o}C. There was no significant difference in the temperature between the soil beneath and adjacent one. The pH of the soil beneath the tanks was acidic (5.9) compared to adjacent soil which was 7.4 (Table 1). The low pH of the soil beneath corroded petrol tanks could be attributed to the acid produced by the iron bacteria as the low pH corresponded to the higher population of iron bacteria and hence could accelerate corrosion. This could also be as a result of seepage of petrol from the petrol storage tanks. Similar observation was made by Water Quality and Health Council U.S., (2010); and USEPA, (1997) who reported that the acidic nature of the soil contributes to corrosion and eventually causes metallic material failure. The mean organic matter of the soil beneath was lower (1.4%) compared to the adjacent soil (1.81%); even though, statistically there was no significant difference at 95% confidence limit. The higher sulphate recorded at the soil adjacent to the petrol tanks compared to the beneath soil could probably be as the result of refuse dumping and some grasses found within the adjacent soil area.

The SRB count ($1.9x10^6$cfu/g) was higher in the scrapings of the tanks compared to the adjacent soil. The sulphate-reducing bacteria (SRB) isolated from the corroded petrol tanks included *D. nigrificans* (8.9%), *D. desulfuricans* (8.0%) *and D. vulgaris* (10.7%). These counts are significant to aid corrosion of metallic materials. The corroding ability of the sulphate reducing bacteria observed in this study (Table 5) may be attributed to the fact that the organism is capable of producing enzymes such as hydrogenases which are known to stay active within biofilm matrix, irrespective of the absence of living cells, and can play a significant role in the biocorrosion of iron and ferrous alloys as reported by Beech (2002). In addition to hydrogenases, the activity of enzymes such as catalases, phosphatases, lipases and esterases, can be readily detected in aqueous oxygenated solutions of the dried SRB exopolymer as reported by Beech and Coutinho (2003). This study agrees with the observation made by Videla and Herrera (2005) who reported that the removal of hydrogen from the cathodic area on the iron surface by the hydrogenases of the bacteria, coupled with the reduction of sulphate, to sulphide could account for the severe corrosion of iron. Thus, the corrosion reaction would be indirectly accelerated by depolarization of the cathodic reaction. Sulphide accumulation due to bacterial sulphate reduction is responsible for a number of serious problems in the oil industries (Ibiene *et al*., 2006).

T. thiooxidans had the frequency of occurrence of 11.6% (Table 3) and was observed to have the corroding ability on metal strips (Table 5). This is most probably because the organism is capable of generating sulphate and hydrogen ions which lowers the pH, often resulting in a highly acidic environment (Table 1) that cause pit and gouge metallic substances. The result is similar to the study conducted by Ibiene *et al*. (2006), that iron-oxidizing bacteria such as *Thiobacillus* spp may convert hydrogen sulphide to sulphuric acid which is corrosive to metals. Also, similar observation was made by USEPA, (2004) who reported the health risk associated with IOB and biofilm formation in drinking water pipelines.

SRB and IOB counts were higher in the scrapings than in the soil adjacent to the tanks (Table 2). The higher counts in SRB in scrapings could be attributed to the anaerobic condition established in the biofilm which favours the growth of SRB. Whereas the higher counts of IOB in the scrapings compared to the adjacent soil could be attributed to the metallic material which serve as substrate to the organism; also the sulphate generated by the SRB serve as a substrate to IOB and is converted to sulphuric acid by the organism and this acidic medium in turn favours its replication.

Other bacteria isolated and their frequency of occurrences included *P. aeruginosa* (12.5%), *C. perfringes* (9.8%), *M. sedentarius* (8.0%) *S. cohnii* (5.4%), *B. laterosporus* (4.5%), *B. firmus* (5.4%), *B. cereus* (9.8%) *and L. monocytogenes* (5.4%). These bacteria have also showed the ability to corrode metallic materials (Table 5). The participation of *P. aeruginosa* in biocorrosion is justified because the organism is capable of producing some organic acids such as alginic acid which corrode metallic materials. *P. aeruginosa* and other bacteria such as *Bacillaceae* can form slime and set up a concentration cells which create an anaerobic condition and thereby allow proliferation of sulphate reducers, which accelerate corrosion as reported by Zhu *et al*., (2003); Strictland *et al*., (1996) as well as Terzenbach and Blaut, (1994).

CONCLUSION

Generally, the determination of corroding ability of each bacterium isolated by weight loss method of iron strips carried out revealed an average percentage weight loss of 3.8%. Thus, the study has indicated that the bacteria isolated have contributed in the deterioration of petrol tanks studied. The corrosion of storage petrol tanks could lead to seepages of petroleum products in the area, contaminating soil surface and ground water sources which may result in destruction of crops and aquaculture. The use of metals/plastic that can resist corrosion especially alloys to construct storage fuel tanks should be encouraged.

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Corresponding Author
Manga, S.S.,
Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria
Email: ssmanga2000@yahoo.com