STUDY ON BACTERIA ASSOCIATED WITH CORROSION OF WATER PIPELINE IN KANO, NIGERIA

Gide Anasand Dr.Shamsudeen Umar

Department of Microbiology Bayero University Kano

ABSTRACT

The study was carried out to investigate the bacteria associated with corrosion of water pipeline in Kano Metropolis. Pipe scrapings were collected from different local government area of Kano state. Physicochemical properties of the soil beneath corroded pipes revealed that the temperature range from 27.1^{o}C-33.2^{o}C, pH range from 3.10 -6.72, moisture content range from 27.1-33.2, organic matter range from 5.0%-10.0%, neutrophilic bacterial count range from 1.0X10^{2}-9.7 x 10^{3}cfu/g, acidiphilic bacterial count range from 0.25 X 10^{2} -9.0 X 10^{2}cfu/g, iron bacterial count range from 1.24 x 10^{3}-8.2 x10^{2}cfu/g, consumption amount of thiosulphate iron range from 1.51-1.58mM. The acidiphilic, neutrophilic and iron bacteria were isolated and characterized. They oxidize iron and reduce sulphur compounds like thiosolphate and tetrathionate, the bacterial oxidation of reduced sulphur compound produce sulphuric acid. These result shows that the bacteria isolated are associated with corrosion and deterioration of water pipeline studied, thus they pose health hazard and economic threat to the inhabitant of the area.

Keywords: corrosion of pipeline, physiochemical properties of soil and bacterial count

INTRODUCTION

Bacterial corrosion is the destructive attack on metals by some chemical or electrochemical mechanisms as a result of changes in the environment caused by some microorganisms. This destructive attack by microorganisms could be either directly or indirectly, single or in combination (1). Some microorganisms such as sulphate reducing bacteria (SRB) cause and accelerate corrosion because of their ability to effects the changes in the environment. Growth of a bacterial biofilm on a pipe wall serve as a barrier to corrosion, but biofilms can also produce a differential aeration cell, leading to localized changes in oxygen concentration and electrical potential. The biopolymers in the biofilm may also uptake soluble metals; various bacteria can effect iron specification by reducing Fe^{+3} or oxidizing Fe^{+2}.Biofilms are a primary tool used by bacteria to adhere to a substrate's surface and facilitate metabolism or respiration optimization. In the case of minimum inhibitory concentration (MIC), biofilms are an essential part of the degredation process. Bacteria create biofilms by producing extracellular polymeric substances (EPS) such as lipids, polysaccharides, nucleic acids and proteins. Fungal species can also create biofilms, and are often found within close

vicinity of bacterial biofilms. The ability of a biofilm to bind to a metal substrate is dependent on the bonding affinity of the metallic ions and the anionic functional groups. Biofilms can be very diverse and within the one small area of biofilm, multiple species can be found and in very close proximity. Therefore, the actions of some species can affect the others. Scientific evidence suggest that within biofilms certain bacterial species might actually inhibit corrosion, instead of physically blocking adhesion to the metal substrate, as originally hypothesized; the removal of nutrient sources and the metabolites necessary for the functioning of other corrosive species has been observed. The close proximity of biofilms to metal substrate establishes electrochemical gradients that allow the generation flow at anodic sites (site where metal is dissociated by bacteria). The catodic sites are found where oxygen is present and where the corrosion is visualized (rusting, fouling etc). Bacteria also may consumed oxygen, cause localized pH gradient and produce corrosive metabolites such as H_2S or iron sulphate, but is generally considered to be detrimental to most aspects of iron corrosion. In case where such activity is dominant, it is not surprising that biocides such as chlorine effectively reduce overall corrosion problems despite their oxidative properties (2).

Microorganism can play a part in the corrosion of pipe materials; bacteria have the ability to form microzones of high concentrations of corrosive ions in a pipe. The most common bacteria involved in the corrosion reaction are sulphate producers, methane producers, nitrate reducers, sulphur bacteria and iron bacteria. The greatest possibility for this type of reaction occurs in dead ends where the water becomes stagnant. The conditions favourable to bacterial growth could be a decline of the chlorine residual and a lack of scouring velocities in the pipe. This is more common where the pitting action started, resulting in additional areas for the organism to become attached to the pipe; this corrosion could cause an increase of the number of the main breaks (3).

MATERIALS AND METHODS

Sample collection site

The research work was conducted at Microbiology Postgraduate Research Laboratory, Department of Microbiology, Bayero University Kano. The soil samples and pipe surface scrappings (Rust) were collected from different local government areas of Kano Metropolis **Gwale** (FCE, BUK old site, Dorayi, Sharada phase I and Red bricks), **Ungogo** (BUK new site, Kurna, RijiyarZaki, Rijiyarlemu and Bachirawa), **Fagge** (IDH, France road, CAS, Sabongari and Wapa), **Kumbotso** (Reka, industrial estate, Bakinkasuwa, Panshekara, and Police quarters), and **Dala**(Koki, NOHK, Kofarruwa, Jakara and GwauronDutse).

Sample collection

Fifty (50) samples of pipe surfaces scrapings were collected in a sterile McCartney bottles with the aid of sterile lancet from each location and were taken to the laboratory for microbiological analysis. Soil samples beneath each of the corroded pipes were also collected in a clean polythene bags using sterile pan and were taken to the laboratory for physicochemical analysis.

Serial dilution of the samples was carried out by suspending one gram (1g) of pipe scrapings into a test tube containing 9ml of sterile distilled water and the tube was labeled 10^{-1}, from the 10^{-1} tube, 1ml quantity was transferred to another test tube containing 9ml of sterile distilled water. The procedure was repeated up to the 10^{-5} dilution for all the samples.

Media Preparation

A new efficient (Gelrite-FeSO4) solid medium was developed and successfully employed for isolation and enumeration of sulfur oxidizing *Acidithiobacillusferrooxidans*, microbial leached solutions and solid samples. Gelrite-FeSO4 solid medium was routinely used in the present studies.

For the growth of bacterial strains, iron Liquid medium (9kFe2+) were used.

Isolation and Enumeration of iron oxidizing Bacteria

One millilitre (1.0ml) of the 10^{-5} dilution samples (pipe scrapings) was inoculated into iron medium ($9k^{2+}$) and was incubated at 30^{o}C for 1 to 7 days. The presence of iron oxidizing bacteria in the medium was indicated by the formation of ferric iron and the medium becoming brick red in color due to the bacterial oxidation of Fe^{2+} to Fe^{3+} (4).

For the isolation of acidophilic bacteria, the pH of the medium was adjusted to 3.5 with 0.05m H_2S0_4. Also for the isolation of neutrophilic bacteria, the pH of the medium was adjusted to 0.1m Na0H solution. (5).The agar plates were cultured at 30^{0}C for 1-2 weeks.

Isolation and Enumeration of Sulfur oxidizing Bacteria

One millilitre (1.0ml) of each 10^{-5} dilution from the sample of pipe scrapings was inoculated and spread on a Gelrite $FeSO_4$ solid media which contain the above mention chemical components. After static cultivation at 30^{o}C for two weeks (2 weeks), the colonies formed were checked to see whether or not they were of sulfur oxidizing bacteria by the consumption amount of thiosulfate ions estimated by manual titration using 5mM iodine solution to a starch end point, and the final pH of the medium was measured using ion meter fitted by glass electrode (6).

CELL MORPHOLOGY AND MICROSCOPY

The microscopic observation of the isolated strains revealed that these strains were Gram negative motile and rod shaped bacteria. Iron bacteria oxidizes Fe^{2+} to Fe^{3+}, sulfur bacteria reduced sulfur compounds and produce sulphuric acid which followed a drop in the initial pH value of the medium (7).

BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

The extracellular activities was determined by starch hydrolysis and gelatin hydrolysis

While the intracellular activities was determined by hydrogen sulfide production, catalase reaction, urease test, indole production test, methyl red test, vogesproskauer test, citrate utilization and triple sugar iron test as described by (8)and (9).

PHYSICOCHEMICAL ANALYSIS OF THE SOIL SAMPLES

Temperature, pH, moisture contents, organic carbon, organic matter and sulphate contents were determined using the standard methods.

RESULT

At the end of the study, a total number of seven sulfate reducing bacteria (*Acidithiobacillusthiooxidans, Thiobacillusthioparus, Bacillus megatarium, Desulfovibriodesulfuricans, Desulfovibrio vulgaris, Deusolfotomaculumnigrificans*and *Thiobacillusthiooxidans*) and five iron oxidizing bacteria (*Acidithiobacillusferrooxidans, Leptospirulumferrooxidans, Ferrobacillusferrooxidans, Bacillus laterosporous*and *Alcaligenedenitrificans*) were isolated and identified. The following results were recorded and presented in tables as follows:

Table 1

This shows the physicochemical properties of various soil samples collected, Acidic pH range is within 6.72 – 3.10, Bayero University Kano has the least pH (3.10) while Panshekarahas the highest pH of 6.72 which correspond with low organic matter in the former and high organic matter in the later (Tayor*et al.,* 1998).

Jakara recorded the highest moisture content while RijiyarLemu/Zaki recorded the least soil moisture content. The temperature ranges from 33.2^{o}C – 27.1^{o}C, Panshekara has the highest temperature. The organic matter content of the soil ranges from 15cm^3 – 5cm^3, while the clay particles and stone particles ranges from 285cm^3 – 250cm^3 and 55cm^3 – 40cm^3 respectively (10).

Table 1. PHYSICOCHEMICAL CHARACTERIZATION OF SOIL SAMPLE BENEATH CORRODED PIPES

Location	Moisture content	Temperature	pH	Organic matter	Clay particles	Stone particles
FCE	29.87	27.1	3.34	5	285	50
BUKO	65.21	27.1	3.1	4	275	45
DRYI	102.6	28.7	4.6	5	280	50
SRD	11.25	27.2	5.2	10	282	40
RB	79.45	27.3	3.5	5	273	50
BUKN	94.8	31.1	3.3	10	265	45
KNA	26.7	31.1	4.7	5	282	50
RZK	111	30.1	5.2	5	275	50
RLM	10.8	27.5	5.6	5	250	40
BRW	107.8	31.3	3.7	5	286	40
IDH	16.8	28.2	3.7	5	256	40
FRD	99.1	27.1	4.2	5	273	50
CAS	110.1	28.2	5.25	10	264	50
SGR	94.8	27.1	3.2	5	282	40
WPA	105.8	27.3	5.26	5	255	45
RKA	146.6	32.1	4.32	5	281	45
IE	93.2	31	5.73	5	250	50
BKW	106.3	28.1	5.2	5	260	40
PSKR	110.7	33.2	6.72	15	265	40
PQS	106	31.1	5.43	10	280	45
KKI	111	31	6.25	5	263	55
NOHK	102.3	28.1	5.41	5	272	50
KRW	332	30.1	4.31	5	285	50
JKR	122.3	32.1	5.32	8	282	55
GDS	103.2	31.1	5.21	10	271	50

FCE = Federal College Of Education Kano, BUKO = Bayero University Old Site, BUKN = Bayero University New Site,

DRY = Dorayi, RDB = Red Bricks, RKZ = RijiyarZaki, RLM = RijiyarLemu, BRW = Bachirawa, IDH = Infectious Diseases Hospital, CAS = College of Art And Remedial Studies, SGR = SabonGari, IE = Industrial Estate, PSKR = Panshekara, PQS = Police Quarters, KKI = Koki, NOHK = National

Othorpedic Hospital Kano, KRW = KofarRuwa

Table 2

This shows the consumption amount of thiosulphate ion and the bacterial count of scrapings from corroded water pipes (cf/g), both neutrophilic and acidophilic sulphur and iron oxidizing bacterial counts are indicated in the table. Sharada has the highest neutrophilic bacterial count (9.7×10^3), while Bayero University Kano has the least (1.00×10^2). Acidophilic bacterial count ranges from $9.0\times10^2 – 3.0\times10^2$, while the consumption amount of t

hiosulphate by acidophilic and neutrophilic bacteria ranges from 1.58 – 1.51 respectively (11)

Table2. BACTERIAL COUNT OF SCRAPINGS FROM CORRODED WATER PIPES (cfu/g) AND CONSUMPTION AMOUNT OF THIOSULPHATE ION (mM)

Location	Neutrophilic	Acidiphilic	Iron Bacteria	
FCE	7.1×10^3	2.25×10^3	3.1×10^2	1.51
BUKO	1.0×10^2	3.4×10^2	6.4×10^2	1.54
DRYI	7.3×10^3	5.0×10^2	5.3×10^3	1.52
SRD	9.7×10^2	2.05×10^2	2.8×10^3	1.57
RB	6.9×10^2	7.25×10^2	4.7×10^2	1.53
BUKN	7.12×10^3	2.1×10^2	3.8×10^2	1.53
KNA	5.8×10^3	9.0×10^2	1.7×10^3	1.55
RZK	8.3×10^3	1.65×10^2	8.2×10^2	1.53
RLM	6.5×10^3	2.10×10^2	3.4×10^3	1.52
BRW	4.8×10^3	0.25×10^2	6.2×10^2	1.53
IDH	5.9×10^3	1.45×10^2	2.5×10^3	1.53
FRD	7.3×10^3	1.55×10^2	5.7×10^2	1.51
CAS	4.2×10^3	8.0×10^2	1.8×10^3	1.52
SGR	7.0×10^3	3.0×10^2	2.10×10^3	1.52
WPA	6.4×10^3	2.3×10^2	7.3×10^3	1.57
RKA	8.1×10^2	6.0×10^2	3.2×10^2	1.56
IE	4.3×10^2	1.55×10^2	1.24×10^2	1.52
BKW	8.2×10^2	2.8×10^2	6.1×10^2	1.56
PSKR	1.05×10^2	4.6×10^2	3.4×10^2	1.53
	7.9×10^2	2.8×10^2	2.1×10^2	1.5
	9.5×10^2	3.15×10^2	1.5×10^2	1.51
NOHK	7.8×10^2	8.0×10^2	6.0×10^2	1.54
KRW	5.6×10^2	8.0×10^2	3.1×10^2	1.58
JKR	4.1×10^2	2.55×10^2	2.5×10^2	1.53
GDS	6.2×10^2	3.2×10^2	4.2×10^2	1.52

FCE = Federal College Of Education Kano, BUKO = Bayero University Old Site, BUKN = Bayero University New Site,
DRY = Dorayi, RDB = Red Bricks, RKZ = RijiyarZaki, RLM = RijiyarLemu, BRW = Bachirawa, IDH = Infectious Diseases Hospital,
CAS = College of Art And Remedial Studies, SGR = SabonGari, IE = Industrial Estate, PSKR = Panshekara,
PQS = Police Quarters, KKI = Koki, NOHK = National
Othorpedic Hospital Kano, KRW = KofarRuwa

DISCUSSION

The isolation and characterization of bacteria associated with corrosion of water pipeline in Kano Metropolis was carried out. Samples of pipe surface scrapings were collected in sterile McCatney bottles with the aid of sterile lancet; the samples were taken to the laboratory for microbiological analysis. Soil samples beneath each of the corroded pipes were also collected in clean polythene bags for physicochemical analysis.

The result obtained revealed the physicochemical properties to include a mean temperature range from 33.2^{o}C – 27.1^{o}C which is consistent with temperature range of 25 – 35^{o}C for microbial growth as reported by Lee and Newman (2003). Change in moisture content of the soil is a common factor that contributes to corrosion and eventually causes pipe failure, the high percentage of organic matter in the soil particularly at Kurna, CAS, industrial estate, Jakara and Koki may be unconnected with lots of refuse dumping in the area which might have accumulated the organic matter from continuous decomposition of the wastes. This is in agreement with the report of Fawole*et al.,* 1998 (12) and Ibien*et al.,* 2006 who reported the susceptibility of metallic materials to corrosion by sulphate reducing bacteria in oil production system in Niger Delta. The pH rage from 3.10 – 6.72 which is in consistent with pH range of 3.5 – 6.0 and also remarkably tolerant to a wide range of metal ions as reported (13).

The pipe scrapings at Sharada, Rijiyarzaki, France Road,Reka, BakinKasuwa, police Quarters recorded the highest neutrophilic counts, which corresponds to the high organic matter in the area. Microorganisms especially the neutrophilic bacteria proliferate more in the presence of high organic matter and hence more hydrogen sulphide is produce favouring corrosion process as reported by Kawo and Manga (2000). The high population of iron bacteria that was obtained at F.C.E, B.U.K old site, B.U.K new site, GoronDuste, and CAS corresponds with the low pH obtained from the areas. In the soil, hydrogen sulphide is converted to sulphuric acid (H_2S04) by iron bacteria such as *Thiobacillusthiooxidans.* This acid corrodes pipe materials including buried pipes as reported by Stainer*et al.,* 1998, Kawo and Oyeleke (2011). Also, the high population of iron bacteria (8.2×10^2 cfu/g) that was obtained in scrapings of water pipes at RijiyarZaki corresponds with the low pH obtained from the area and minimum temperature where the water becomes stagnant, resulting in additional areas for the organism to become attached to the pipe; causing an increase in the number of the main breaks as reported by Kelly *et al.,* (2000).

CONCLUSION

Iron pipe corrosion is extremely complicated and is affected by practically every physical, chemical, and biological parameter in water distribution systems. In conclusion, substantial number of sulphate reducing bacteria, iron bacteria and other bacteria together with the prevailing physiocochemical conditions

(temperatute, pH, moisture contents, organic matter, nitrate and sulphate) cause the deterioration of water pipeline studied.

RECOMMENDATION

There is heightened awareness regarding the use of metallic pipes in the distribution of water systems in most communities and its impacts on human health. The complexity of the use of polyvinylchloride (PVC) pipes should be encouraged in the distribution of water systems; because metallic pipes main materials are prone to corrosion and scale build up. Vinyl pipe to some degree is flexible; this property provides a distinct advantage when pipes are laid through unstable shifting or heavy soils. Vinyl pipes are inherent inert to aggressive soil conditions and do not need the costly secondary internal protection found inside metallic pipes.

In the context of a developing country, there is a need for increased awareness for target surveillance for infection control. It is therefore recommended that the general public and the government agencies should give proper attention to the use of polyvinylchloride (PCV) pipes in water distribution systems.

REFERENCE

1. Beck, J. V 1960. A ferrous iron oxidizing bacterium isolation and some general physiological characteristics.*Journal of bacteriology.* 79: 502-509.
2. Grobelski T, Kiczma FJ, Farbiszewka T. 2007. *Bioleaching of polish black shale. Physicochemical Problems of Mineral Processing*41, 259-264.
3. Kelly DP, Wood AP. 2000. Reclassification of some species of *Thiobacillus*to the newly designated genera *Acidithiobacillus gen*. Nov., *Halothiobacillus gen*. nov.and*Thermithiobacillus gen*. *Internacional Journal of Systematic and Evolutionary Microbiology* 50, 511-516.
4. Banfield MJ (2001) Structure of HisF, a histidinebiosymthesis protein from pyrobaculumaerophilum. Actacrystallogr D Bio crystallogr 57(Pt11):1518-25
5. Sallah, N., M. Nonaka and T. Kamura 1993. Consequences of microbial pyrite oxidation in acid sulfate soils. *Bull. Jpn. Soc. Microbial. Ecol.,* 8: 27-33.
6. Takagi S, Yamaki M, Masuda K and MJ (1976). Head module control mediator interactions.Mol cell 23(3):355-64
7. Sugio T, Wakabayashi M, Kanao T, Takeuchi F. 2008. Isolation and Characterization of Acidithiobacillusferrooxidans Strain D3-2 Active in Copper Bioleaching from a Copper Mine in Chile Biosci. *Biotechnology. Biochem*72(4), 998–100.
8. Atlas M.R (1993): *Thiobacillusthiooxidans*medium*. Hand book of Microbiological media.* CRC press U.S Pp. 859.
9. Oyeleke S.B, and Manga, S.B (2008): Biochemical test and identification of fungal isolates. *Essentials of laboratory practicals in microbiology.* (1st edition) tobest publishes Minna, Nigeria. Pp. 28-62.
10. Taylor RM (1998) Molecular cloning and Functional analysis of Arabiodopsis thaliana DNA ligase I homologue. Plant *J* 14(1): 75-81
11. Sallah, N., M. Nonaka and T. Kamura 1993. Consequences of microbial pyrite oxidation in acid sulfate soils. *Bull. Jpn. Soc. Microbial. Ecol.,* 8: 27-33.

12. Fawole, M.O. and Oso, B.A (1998): Biochemical test for the identification of bacteria. *Laboratory manual of microbiology.* Spectrum books limited, Ibadan, Nigeria. Pp.16-35.
13. Dopson M, Baker-Austin C, Ram Kopponeedi, P, Bond P. 2003. Growth in sulfidic mineral environments, metal resistance mechanisms in acidophilic micro-organisms.*Microbiol;*149, 1959-1970.
14. Oyoleke S.B, A.H Kawo (2011): Observation of sulphate reducing bacteria associated with corrosion of water pipeline in Sokoto. *Biological and Environmental Science Journal for the Tropics.* 8(2), June, 2011.
15. Kelly DP, Wood AP. 2000. Reclassification of some species of *Thiobacillus*to the newly designated genera *Acidithiobacillus gen*. Nov., *Halothiobacillus gen*. nov.and*Thermithiobacillus gen*. *Internacional Journal of Systematic and Evolutionary Microbiology* 50, 511-516.
16. Drinking Water Infrastructure Needs Survey:First Report To Congress,*US Environmental Protection Agency -- Office of Ground Water and Drinking Water,* (4101), January 1997.

.