# Isolation and Identification of Bacteria Associated with Metal Biocorrosion

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***Abstract-*** This research was carried out in Birnin kudu water treatment plant and some distribution channels within the town of Birnin Kudu on Bacterial that causes metal corrosion , deteriorate and brought pipes failure . The water treatment plant is located in Birnin kudu local government area of Jigawa state, Nigeria. Bacterial counts showed the presence of large bacterial cells in the various samples collected. The enumeration of sample from Birnin kudu treatment plant , Audu Bako way showed higher colonies among all the samples, Various metals associated with microbiological influence corrosion harbor large microbial population, which is estimated to be around 103 to 109 CFU/ml. This research confirms the association of metal bio corrosion with microbiological entities – especially bacteria. Two species of bacteria namely Bacillus subtilis and Bacillus cereus associated with corroded metals have been isolated and indentified. The effective prevention and control of MIC can be achieved through proper characterization of the microorganisms and understanding their specific role in corrosion processes. Involvement of microorganisms and microbial metabolites accelerate the deterioration of industrial water system producing considerable damages, and finally destruction of the industrial water system.

***Index Terms***- Bacteria, Bacillus Subtilis,Bacillus Cereus, Bio Corrosion, Metals

## I. INTRODUCTION

Microbiological influenced corrosion (MIC) is type of corrosion that is caused by microbes. In industrial world, especially Oil and Gas industry, thought to play an important role in this process. The forms of degradation are commonly localized at several areas, which have more contact with microbial activity (Lee and Newman, 2003; Zou, 2007).

Microbiologically influenced corrosion (MIC) attacks industrial world sections from water distribution in cast iron mains and sewers to transport of natural gas in steel pipelines. It has been estimated that for the oil industry, MIC cause approximately 40% of damage (Fontana, 1986). With that fact, it can be concluded that MIC is a serious industrial problem and yet the basic mechanism of MIC has remained unclear.

There are two types of microbes that are often related to MIC. They include: sulphate-reducing bacteria and iron-oxidizing bacteria (ASM2004) . The damages caused by microbes are frequently related with the deposits formed around the corrosion or because of their metabolism. Microbes perform oxidation and reduction reactions that simply affect the stability of metals in the environment. Sulphate-reducing bacteria reduce sulphate into sulphite and then form hydrogen sulphide. On the other hand, iron-oxidizing bacteria will consume the ferrous ionic around the metal for their metabolism.

Due to their opportunistic behavior, microorganisms are known to influence the energy yielding corrosion reaction, often enhancing corrosion in order to harvest the energy released (Fang *et al.* 2002) known as microbiologically influence corrosion (MIC). It has been postulated that microorganisms can accelerate the corrosion process by as much as 1000 to 10,000 times, as abiotic corrosion is often a relatively slow process (Fang *et al.* 2002).

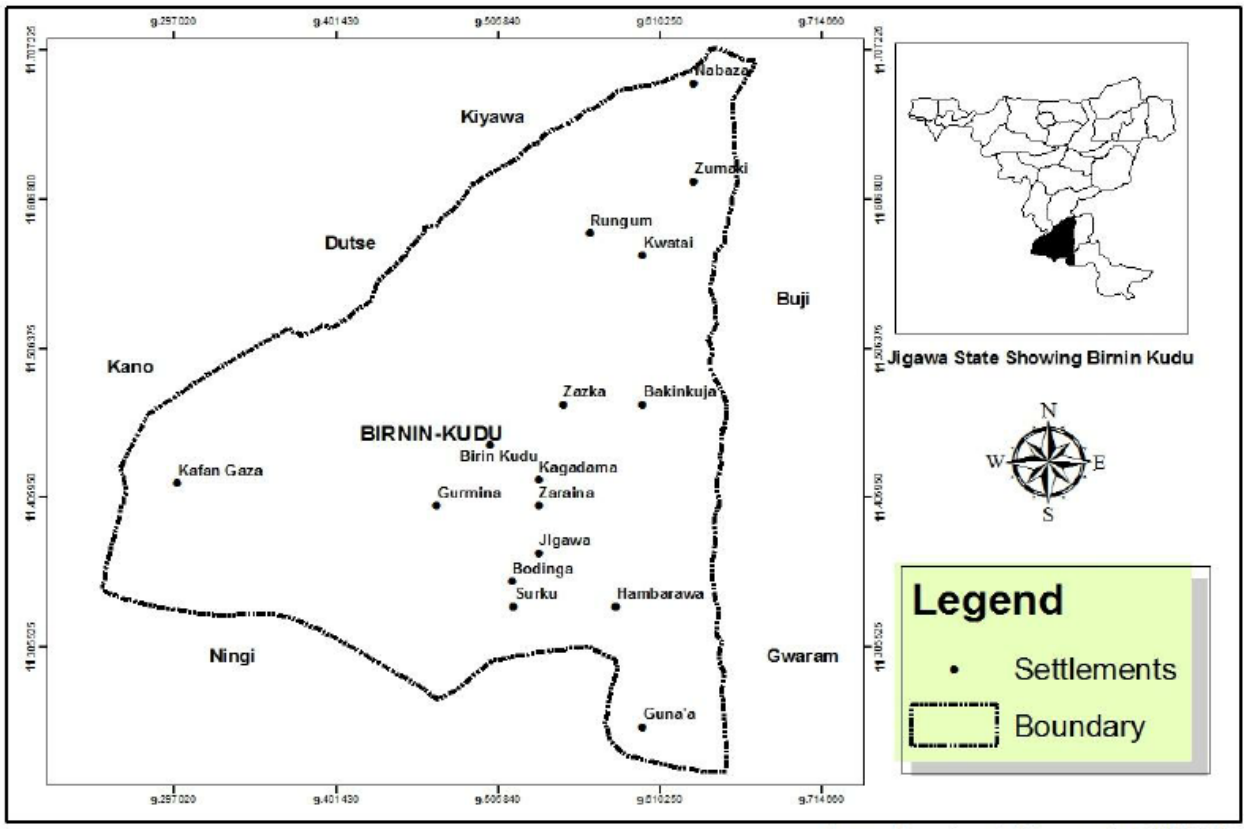
Various metals are submitted to MIC in natural waters where the microbial population is estimated to be around $10^3$ to $10^9$ CFU/ML. Within a relatively short time depending on the environment, extracellular polymeric substance (EPS) production and microbial growth will result in a mature biofilm. The biofilm facilitates MIC by altering the chemistry such as pH, pressure, oxygen levels and nutrients at the interface between the metal and the bulk solution (Beech and Sunner, 2004; Videla and Herrera, 2005). This leads to major changes in the concentration and type of ions, redox potentials, pH and Oxygen levels, resulting in an alteration of the active or passive properties of the metal as well as the corrosion products formed (Videla and Herrera, 2005). The main reason of carrying out these research work is to isolate and identify the bacterial associated with metal corrosion in Birnin kudu water treatment plant in order reduce the deterioration of bacterial corrosion on metal pipes and to seek for alternative solutions.

## II. MATERIAL AND METHODS

### The study Area

Birnin Kudu town, Jigawa state, northern Nigeria . It lies at the intersection of roads from Kano city, Gwaram, and Ningi. Coordinate UTM: geographical coordinates in decimal degree (WGS84) Latitude 11.450, Longitude 9.500 geographical coordinates in degree minutes seconds (WGS84)Latitude 1127,00 Longitude 930,00.It is best known as the site of Dutsen Habude, a cave containing Neolithic paintings of cattle (which bear strong resemblance to some found in the central Sahara) and rock gongs believed to be more than 2,000 years old. It is a collecting point for peanuts (groundnuts), which are sent to Kano city 76 miles (122 km) west-northwest for export by rail, and is a market centre for millet, sorghum, peanuts, cowpeas, cotton, and livestock.

Birnin Kudu is a town and a Local Government Area in the south of Jigawa State , Nigeria. It is 120 kilometres south-east of Kano. The town of Birnin Kudu had an estimated population of 27,000 (Census,2006).




Source: Department of Geography BUK (2014)

**Topography and Soil**

Southern part are underlain by granites ,schists nd gneisses of the basement complex. However , the basement complex rocks have undergone weathering to give rise to fairly deep soils which are covered by a sheet of laterite which has been exposed by denudation in some places.

***Occupation***

Agricultural activities is there main occupation in Birnin kudu which includes cultivation of crops such as millet, sorghum, peanuts, cowpeas, cotton and rearing of animals which includes goats, sheeps, and cows (livestock) and few traders.

**Relief and drainage**

The relief is generally undulating ,but the rock outcrops .Birnin kudu the southern part of the state , the relief is about 500-600 meters above the sea level.

***Climate***

The climate of the Birnin kudu is semi-arid , which is characterized by a long dry season and short wet season .The temperature out regime is warm to hot. The mean annual temperature is about 25$^{o}$c but the mean monthly values range between 21$^{o}$c in the coldest month and 31$^{o}$c in the hottest month.

Vegetation

Guinea savannah vegetation are found in the Birnin kudu which is the southern part of the state. Extensive open grasslands, with few scattered stunted trees, are characteristics of the vegetation.

**Sample collection**

Samples of pipe scrape were collected from five areas, these includes:

1. First sample is located at Audu Bako ways, and the area is situated at the middle of the city, most of the pipe linkages that supplies water to Birnin kudu passes through the area.
2. Second sample is located at Gangare area, the pipe scrape is found within distribution channels in Gangare Area.
3. Third sample is located at Zango quarters water treatment plant branch Birnin kudu. The branch supplies water to most parts of the Zango quarters
4. Fourth and fifth sample were found from the service tank pipes in Birnin kudu water treatment plant ,which were the main pipes that discharge the water to the storage tank for the distribution pipes within the town.

Samples of pipe scrape were collected in sterile bottles with the aid of sterile lancet from each location and were taken to the laboratory for microbiological analysis at controlled temperature.

Serial dilution of the samples were carried out by suspending 1gram of pipe scrapings into a test tube containing 9ml of peptone water and diluted up to six folds dilutions.

**Methods**

**Samples and sampling Technique**

Samples of pipe scrape were collected randomly from Birnin kudu Water Treatment plant and other distribution channels within Birnin kudu town Jigawa state

**Sampling preparation**

Samples of pipe scrape collected were taken to the laboratory for microbiological analysis at controlled temperature 37°c. Serial dilution of the samples were carried out by suspending 1gram of pipe scrapings into a test tube containing 9ml of peptone water and dilute up to six folds dilutions.

*Experimental Techniques*

**Enumeration and Isolation of Bacteria**

Aliquots (1ml) of the $10^{-6}$ dilution samples (Pipe scrapings) were inoculated using spreader (spread method) into Winogradski medium and were incubated at $37^0$C for 1-3days. Viable colonies were counted using colony counter and expressed as CFU/ml/Sub-culture of the colonies were carried out using wire loop (streak method) and have been incubated at $37^0$C for 1-3 days. Distinct colonies were selected and cultured on slants as pure colonies for further investigation.

**Characterization of Isolates**

Isolates were characterize based on the following characteristic

**Morphology: Gram Stain**

A looful of organisms were placed on a clean microscope slide. A thin smear were also prepared on a clean microscopic slide. Heat fixation were carried out showing the slide in flame. The slide were flooded with crystal violet for immunities, the slide were rinsed up with water . A few drops of gram's loading were added to cover the smear and allowed to reacted for 30 seconds. The slide were decolorized with 95% ethanol and rinsed water. The slide were flooded with safranine and allowed to reacted for one minutes and rinsed with tap water, the slide were bloated dry and observed under oil immersion objective.

**Identification of Bacteria**

Isolated colonies were identified based on morphology and biochemical tests. Fawole and Oso, 1988

**Laboratory Analysis**

**Winogradski media**

Winogradski media were prepared, Composition of Winogradski medium is (g/L);

| | |
|---|---|
| $K_2HPO_4$ | 0.5 |
| $MgSO_4.7H_2O$ | 0.5 |
| $CaCl_2.2H_2O$ | 0.2 |
| $NaNO_3$ | 0.5 |
| $NH_4NO_3$ | 0.5 |
| ammonium iron citrate | 6.0 |

The pHs of the medium were adjusted to 7.2 and autoclaved at 121°C for 15 mins.

**Biochemical Test**

**Catalase test**

Small amount of bacterial colony were transferred to a surface of clean, glass slide which were allowed to dry using a loop, a drop of 3% $H_2O_2$ were on the slide and were mixed thoroughly. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling, while a negative result is no bubbles or only a few scattered bubbles.

**Citrate test**

To a sterile simon's citrate medium, a loopful of 24 hours old culture were incubated aseptically. Incubation was done at $37^0$C for 24 hours after which they were examined daily for turbidity for a period of 3 days. Turbidity indicates citrate utilization.

**Indole production test:**

A looful of the isolate were incubated in the sterile nutrient broth. Incubation were done at $37^0$C for 48 hours. After incubation, 0.5ml of Kovac's reagent were added and shake, this were examined after one minute. A red colour in the reagent layer indicates indole production.

## III. RESULT

Table 1. LEVEL OF BACTERIAL COUNT

| S/N | Sampling Area | No. of samples | Range count (cfu/g) | Average count (cfu/g) |
|---|---|---|---|---|
| 1 | Audu Bako way | 1 | $5.8\times10^6$cfu/g-$3.7\times10^6$cfu/g | $4.6\times10^6$cfu/g |
| 2 | Gangare Area | 1 | $4.7\times10^6$cfu/g-$2.9\times10^6$cfu/g | $3.9\times10^6$cfu/g |
| 3 | Zango quarters | 1 | $4.2\times10^6$cfu/g-$2.7\times10^6$cfu/g | $3.4\times10^6$cfu/g |
| 4 | Service tank pipe 1 | 1 | $3.9\times10^6$cfu/g-$2.2\times10^6$cfu/g | $3.1\times10^6$cfu/g |
| 5 | service tank pipe 2 | 1 | $4.0\times10^6$cfu/g-$2.5\times10^6$cfu/g | $3.3\times10^6$cfu/g |

**Table 2. Morphological and biochemical characteristics of the Isolate**

| Sample E | Gs | Mor | Cat | Ind | Cit | Organism |
|---|---|---|---|---|---|---|
| $E_1$ | P | R | + | - | + | B subtilis |
| $E_2$ | P | R | + | - | + | B subtilis |
| $E_3$ | P | R | + | - | + | B cereus |
| $E_4$ | P | R | + | - | + | B cereus |
| $E_5$ | P | R | + | - | + | B subtilis |

**KEY:** GS-gram stain CAT- catalase IND-indole MOR-morphology CIT-citrate

**Table 3 Morphological and biochemical characteristics of the Isolate**

| Sample F | Gs | Mor | Cat | Ind | Cit | Organism |
|---|---|---|---|---|---|---|
| $F_1$ | P | R | + | - | + | B subtilis |
| $F_2$ | P | R | + | - | + | B cereus |
| $F_3$ | P | R | + | - | + | B subtilis |
| $F_4$ | P | R | + | - | + | B cereus |
| $F_5$ | P | R | + | - | + | B cereus |

**KEY:** GS-gram stain CAT- catalase IND-indole MOR-morphology CIT-citrate

**Table 4 Morphological and biochemical characteristics of the Isolate**

| Sample G | Gs | Mor | Cat | Ind | Cit | Organism |
|---|---|---|---|---|---|---|
| $G_1$ | P | R | + | - | + | B cereus |
| $G_2$ | P | R | + | - | + | B subtilis |
| $G_3$ | P | R | + | - | + | B subtilis |
| $G_4$ | P | R | + | - | + | B subtilis |
| $G_5$ | P | R | + | - | + | B cereus |

**KEY:** GS-gram stain CAT- catalase IND-indole MOR-morphology CIT-citrate

**Table 5 Morphological and biochemical characteristics of the Isolate**

| Sample H | Gs | Mor | Cat | Ind | Cit | Organism |
|---|---|---|---|---|---|---|
| $H_1$ | P | R | + | - | + | B subtilis |
| $H_2$ | P | R | + | - | + | B subtilis |
| $H_3$ | P | R | + | - | + | B subtilis |
| $H_4$ | P | R | + | - | + | B cereus |
| $H_5$ | P | R | + | - | + | B cereus |

**KEY:** GS-gram stain CAT- catalase IND-indole MOR-morphology CIT-citrate

**Table 6 Morphological and biochemical characteristics of the Isolate**

| Sample I | Gs | Mor | Cat | Ind | Cit | Organism |
|---|---|---|---|---|---|---|
| $I_1$ | P | R | + | - | + | B subtilis |
| $I_2$ | P | R | + | - | + | B cereus |
| $I_3$ | P | R | + | - | + | B subtilis |
| $I_4$ | P | R | + | - | + | B cereus |
| $I_5$ | P | R | + | - | + | B subtilis |

**KEY:** GS-gram stain CAT- catalase IND-indole MOR-morphology CIT-citrate

**Table 7: Frequency distribution**

| Isolate | Number of appearance (%) |
|---|---|
| *B.* cereus | 11 (44) |
| *B* subtilis | 14(56) |

## IV. DISCUSSION

Result shows that from all the sample collected from Audu Bako way of Birnin kudu town $E_1$ has highest colonies ($5.8\times10^6$cfu/g) when compared with the other sample collected from the same area. Similarly, in Gangare area of the same town the sample leveled $F_1$ contain highest number of bacterial colony count (***($4.7\times10^6$cfu/g)***than the other samples collected from the same area. This shows that conditions favorable for the growth of the microbes (bacteria) were available in these areas. The conditions includes Temperature and PH as described in the work of Hamilton(1994),who said temperature and PH are among the parameters that govern the bacterial growth. Furthermore, Sample collected from Zango quarters of the same town that is Birnin Kudu town the sample leveled $G_1$ recorded the highest number of bacterial colony in comparism to the rest of the samples collected from the same area. The areas have recorded large number of bacterial colonies indicating the presence of the microbes in the area which are potential to be causing corrosion in water pipes which may finally cause considerable damage and deterioration of the system at large. This look similar to the statement made by Allison (2003), who says involvement of microorganism and microbial metabolite accelerate the deterioration of industrial water system producing considerable damages, and destruction of the industrial water system.

In the same vein the result obtain from the samples collected at Birnin kudu water treatment plant from service tank levelled $H_1$($3.9\times10^6$cfu/g) and service tank pipe 2 levelled $I_1$ ($4.0\times10^6$cfu/g) which served as the main pipes that discharge water to storage tank for consumers distribution within the town had recorded highest colonies of bacteria than the rest of the pipes sample from the treatment plant in the study area. Bacteria was

detected in the sample this may because microorganism are said to be found everywhere provided there are conditions favourable for their growth as it was revealed by Fawole et al (1998), who said Microorganism are ubiquitous in nature and grow at rapid rates in soil, water and air. They show extreme tolerance to varying environmental conditions such as acidic and alkaline PH, low and higher temperature. The impact of bacterial growth can be detrimental to both the operational process performance requirement of the system product.

## V. Conclusion

This research confirms the association of metal bio corrosion with microbiological entities – especially bacteria. Two species of bacteria namely *Bacillus subtilis* and *Bacillus cereus* associated with corroded metals have been isolated and indentified, and the result shows that there is a connection between the composition of bacteria and water quality. It is therefore recommended that modern machines are highly needed in the treatment plant for easier and faster identification of microbiologically influence corrosion (MIC).

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