Bacteria Isolated From Mines Effluent of National Iron Ore Mininig Project, Itakpe-Okene and Bioremediation Potential of Selected Species

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Abstract: The microbiology of mine effluent of Itakpe iron ore deposit was investigated in this study. Sample of mine effluent were collected from the iron ore deposit of the National iron project, Itakpe-Okene, Nigeria and were analyzed microbiologically. The effluent was bioremediated using *Bacillus circulans, Pseudomonas* sp and *Bacillus coagulans* along with 0.1 %w/v Ca $(OH)_2$. Extent of remediation was monitored with the physicochemical parameters of the effluent. Twenty seven different bacteria isolates were identified from the mine effluent. They are species of *Staphylococcus, Pseudomonas, Thiobacillus, Bacillus, and Micrococcus.* Initial physicochemical characteristics of the raw effluent were pH6.8; COD, 714.05mg/l; BOD, 23.17mg/1; TDS, 254.13mg/l; Total alkalinity, 240.0mg/1; Total hardness 230mg/1. Physicochemical results obtained after a seven day period showed that COD of effluent seeded with *Bacillus circulans* reduced to 598.55mg/1; while for effluent seeded with *Bacillus circulans* and 0.1%w/v $Ca(OH)_2$ recorded 49.78mg/1. The COD of effluent seeded with *Bacillus coagulans* was 369.90 mg/l and 668.80 mg/l for that with *Bacillus coagulans* 0.1%w/v $Ca(OH)_2$. *Pseudomonas* sp singly recorded 281.60mg/1 while a value of 563.20 mg/l was obtained when use in combination with the $Ca(OH)_2$2. The BOD followed a similar pattern with *Bacillus circulans* recording 12.67mg/1 and 14.33mg/1 with the addition of 0.1%w/v $Ca(OH)_2$. *Bacillus coagulans* reduced to 6.0mg/1 and in combination with 0.1%w/v $Ca(OH)_2$ reduced to 18.87mg/1 The TDS for *Bacillus circulans* was 160.6mg/1 and 158.8mg/1 for that with *Bacillus circulans* and 0.1%w/v $Ca(OH)_2$. *Pseudomonas* sp recorded 141.0mg/1 and 177.9mg/1 of w/v with addition of $Ca(OH)_2$. Evaluated parameter indicated that effluent seeded with *Pseudomonas* sp of gave the best remediation.

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1. Introduction

Iron, is the fourth most element in the earth crust (Greenwood and Shaw, 1984) and is the most abundant of the metal which naturally undergo redox changes (Armin and Friedrich, 1994). Its average concentration in the crust has been estimated to be 5.0% (Rankama and Sahama, 1950). It is found in a number of minerals in rocks, soil and sediments. However, when it is concentrated in appreciable quantity assay (>10% Fe_20_3) content. It forms iron deposits either as Banded Iron Formation, (BIF). Ferruginous quartzites, oolitic or pisolitic sedimentary ores.

Iron being a very reactive element. It easily oxidizes in its various oxidation states of 0, +2, and +3. Under anoxic conditions, moist environment, exposure to air or aerated at circumneutral to high pH, its ferrous form easily oxidizes to the ferric form of +3. In oxic environment, only the ferric state is energetically stable. Common forms of iron are for example, the poorly crystalline ferrihydrite (usually written as $Fe(0H)_3$) and the crystalline goethite (α – Fe00H) and haemetite (α – Fe_20_3) (Greenwood, and Show, 1984). In anoxic habitats, reduction to the ferrous state occurs either chemically, for example by sulphide or certain organic compounds (Ghiorse, 1988) or biologically in direct contact with dissimilar iron reducing bacteria.

From biogeochemical viewpoint, large scale microbial oxidation is important because of its resultant effect in extensive iron precipitation and microbial reduction resulting in final extensive iron solubilization. Microbial iron reduction plays an important role in the mineralization of organic carbon in some anaerobic environments. Example is in the formation of the Banded Iron Formation which consist of cherty magnetite ($Fe0_4$) and haemetite (Fe_20_3). Konhauser, (1997) reported the participation of microbes in the formation of many of these iron deposits.

Geomicrobiological studies deal with the role microbes have played and are still playing in geological processes. Examples of such processes are the weakening of rocks; soil, sediment formation and transformation; the formation and degradation of minerals alongside genesis and degradation of fossil fuels. These studies also apply to microbial processes currently taking place in modern sediments, of various bodies of water, in ground waters circulating through sedimentary and igneous rocks (Kuznestsol, 1963).

Iron is a source of energy for bacteria, however because of the easy tendency of iron to oxidize in aerated solution at pH values above 5, enzyme catalysed iron oxidation in circumneutral air-saturated solution is difficult though this is possible at pH below 5 under air saturated solution. However, the concept of iron bacteria includes bacteria that can precipitate iron through active (enzymatic) oxidation or by passive accumulation of ferric oxides or hydroxides about their cells.

The participation of bacteria in the formation of iron deposits is suspected to have arisen from the following: (i) Presence in some deposits of fossilized microbes with implied iron-oxidizing or accumulating potential. (ii) The presence of Fe(III) reducing bacteria in recent deposits of magnetite. (Fe_30_4) (iii) Iron – accumulating bacteria in currently forming deposits of ferric oxide (Fe_20_3 or Fe00H) and (iv) The presence of sulphate – reducing bacteria in currently forming iron sulfide deposits (Konhauser, 1997).

The iron ore deposits in Nigeria are located at the middle belt geopolitical zone. They are extensive enough to be economically exploitable as iron ore. They are characterized by alternating layers rich in chart, a form of silica (S102) and layers rich in iron minerals such as haematite (Fe203), magnetic (Fe304), the iron silicate chamosite and even siderite. Geochemical studies of Itakpe iron ore reveals that except for Fe and Mn, the concentratons of heavy metals in the water are well below the maximum permissible levels recommended by United States Environmental Protection Agency (USEPA), 1996 and WHO, 1993. The elevated concentration of Fe in water is to be expected as the study area is uncertain by banded iron formation. The metal concentrations in the water were found in the following ranges (ppb) Cr (3.8-7), Pb (0.1-8.1), Zn (4.4-24.4), Cu (1.0-26.6), Fe (10.0-1636.0), As (0.5-1.9), Mn (0.39-1709.74), C0 (0.04-4.68) and Ni (0.2-5.2). (Okunlola and Soetan, 2006). They also reported that there was a down stream in the total metal levels in the water. The profiles revealed that the highest concentration of each of the heavy metal was at a distance of between 3.5 km - 4.5 km from the beginning of the river within the study area. This may be attributed to the fact that the mine is located at a distance of about 4.2 km from the beginning of the river and the effluent from the mining site drains into the river resulting in elevated values of the heavy metals. However, Pb, Fe, Mn, Co and Ni showed a mark decrease in concentration about 2.5 km from the river. The aims and objectives of this research is to

i. Determination of physiochemical parameters of the effluent sample
ii. Isolation, and identification of bacteria from water samples at different locations
iii. Study of bioremediation potential of selected bacteria strains

2. MATERIALS AND METHODS

Sample colledtion

Mine water was collected in twenty litres and one litres jerry cans from the National Iron Ore Mining Company, Itakpe mine site, Itakpe-Okene. Nigeria.

Microbiology analysis

1ml of selected dilution (10^{-3}, 10^{-6}) were plated in duplicate using Nutrient agar, Pseudomonas agar, MacConkey agar, using pour plates techniques while wire loops were used for further isolation

Pure cultures of bacteria isolates were subjected to various biochemical tests to determine the probable identify of the bacteria isolates. The result of each test was recorded and the probable identity to the isolates was deduced with reference to Bergey's manual of Determinative Bacteriology (Bucchanam and Gibbons, 1974).

Physico-chemical analyses of the Mine waters

The physico-chemical parameters of mine water samples carried out were temperature, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total alkalinity, total hardness and conductivity. All were done according to standard methods of APHA, (1992)

Bioremediation Studies

Three bacterial isolates were used for study. They were grown on nutrient broth singly. These were then incubated for 48 hours at 37^0C. The growth culture was read on a spectrophotometer at 610 nm and standardized at 0.5 optical densities. 5 mls each of the isolated was introduced into 1L of the mine effluent in the following manner

a. Uninoculated mine effluent (*b*). Mine effluent +*Bacillus circulans* (*c*) Mine effluent +*Pseudomonas* (*d*) Mine effluent +*Bacillus coagulans* (*e*) Mine effluent + 0.1% (w/v) calcium hydroxide + *Bacillus circulans* (*f)* Mine effluent +0.1%(w/v) calcium hydroxide +*Pseudomonas* (*g*) Mine effluents+0.1% (w/v) calcium hydroxide +*Bacillus coagulans*. The total set up was seven and they were incubated at 28^0C for seven days.

3. RESULTS

Twenty-seven bacteria isolates were obtained from the Itakpe mine site water samples and were identified. The bacterial isolates were made of seventeen gram positive, *Bacillus* spp, six gram negative (Thiobacillus and *Pseudomonas*), one gram positive coccibacilli (Micrococcus) and one cocci (*Staphylococus saprophyticus*), based on Biochemical characterization of the bacterial isolates and with reference to Bergey's manual of Determinative Bacteriology (Bucchanam and Gibbons, 1974).

The conductivity of the mine water sample treated with the three bacteria showed gradual increase from an initial level of $106\mu^{S}$ to 558, 499 and 432 for the Bacillus circulan , *Pseudomonas* sp, and ***Bacillus coagulans*** respectively as shown in Table 1. When the mine sample were treated with the bacteria in combination with 0.1% (w/v) Ca $(0H)_2$ the conductivity value did not change for the first 96 hours. Table 2 showed the total hardness of the mine effluent treated with the bacterial isolates. There was early increase in the value of the total hardness from 240 mg/l in the first three days of bioremediation in all treatment. The trend however changed in Day 4 and 5 where there were visible decrease before a little rise in Day 6 and 7. The total alkalinity result is shown in Table 3. It could be deduced that the result did not follow a consistent trend, however, there were slightly increase in the values in the first day from the initial value of 220mg/ml. Sample D and F mine water which was treated with *Bacillus* sp and Pseudomonas sp recorded a decreased in value on the last day of the treatment.

Table 1. Conductivity (μ^{S}) of Acid Mine water sample after treatment with different bacteria and calcium hydroxide

Samples	Day1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7
C	520	601	641	623	792	678	558
D	471	529	493	430	581	577	499
F	417	540	456	458	470	496	432
HC	1	1	1	-1	945	1	319
HD	1	1	1	-1	696	1412	552
HF	1	1	1	-1	722	1721	386
UT	372	387	406	406	425	448	390

Initial Conductivity = $106\mu^{S}$

C= mine water + Bacillus sp; D= mine water + Pseudomonas sp; F= mine water +Bacillus coagulans; HC= mine water + Bacillus sp + 0.1%(w/v) Ca $(0H)_{2;}$

HD= mine water + Pseudomonas sp + 0.1%(w/v) Ca $(0H)_2$; HF= mine water + Bacillus coagulans + 0.1% (w/v) Ca (0H)2; UT=Uninoculated mine water

Table 2. Total Hardness (mg/l) of Acid Mine water sample after treatment with different bacteria and calcium hydroxide

Samples	Day1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7
C	300	350	500	400	250	750	400
D	500	400	1000	300	250	400	500
F	400	350	5000	200	250	500	400
HC	1800	1800	2400	1280	1000	600	700
HD	2400	2200	1800	800	800	1200	800
HF	1800	1500	2100	1000	1000	800	800
UT	250	200	350	400	200	400	500

Initial total hardness: 240 mg/ml

Table 3. Total alkalinity (mg/l) of Acid Mine water sample after treatment with different bacteria and calcium hydroxide

Samples	Day1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7
C	400	380	300	380	500	400	410
D	400	320	320	410	490	200	150
F	400	400	350	340	400	250	100
HC	2400	2200	2200	2000	1800	1500	1600
HD	2200	2200	2000	2000	1600	1300	800
HF	2400	2000	2000	2000	1700	1400	1000
UT	400	500	310	440	390	410	410

Initial total alkalinity: 220mg/ml

Table: 4 Physicochemical parameters of Acid Mine water sample after treatment with different bacteria and calcium hydroxide

Sample code	BOD mg/l	COD mg/l	TDS mg/l
C	12.67 $\pm$ 2.88	598.55 $\pm$ 50.0	160.6
D	5.67 $\pm$ 1.96	281.60 $\pm$ 49.78	141.0
F	6.00 $\pm$ 1.89	369.90 $\pm$ 24.98	115.3
HC	19.33 $\pm$ 1.09	528.00 $\pm$ 49.78	158.8
HD	14.33 $\pm$ 1.96	563.20 $\pm$ 49.78	177.9
HF	18.67 $\pm$ 2.18	668.80 $\pm$ 49.78	166.
Control	10.00 $\pm$ 1.89	299.20 $\pm$ 24	109.5

Before Treatment: **COD = 714.05 mg/l, BOD = 23.17 mg/l, TDS = 54.12 mg/l**

All the seven samples recorded a decrease in value in the BOD at the end of the bioremediation period. The initial BOD before treatment was 23.4mg/l. Sample C, being mine water inoculated with *Bacillus* sp recorded a BOD of 12.67 mg/l. Sample D, containing mine water treated with *Pseudomonas* sp gave a BOD of 5.67 mg/l while F being sample containing mine water with *Bacillus coagulanss* recorded 6.00 mg/l. The COD for all the samples decreased at the end of the bioremediation period from initial COD of 714..25 mg/l. Sample C, mine water inoculated with *Bacillus* sp had a COD of 598.55 mg/l. Sample D, of mine water and *Pseudomonas* sp had a value of 281.60 mg/l.Sample F, mine water with *Bacillus coagulans* was 369.90 mg/l. Sample HC, mine water with the addition of 0.1%(w/v)$Ca(0H)_2$ and *Bacillus* sp had a value of 528.00 mg/l Sample D, consisting of mine water, 0.1%(w/v)$Ca(0H)_2$ and *Pseudomonas* recorded 563.20 mg/l. Sample HF of mine water, 0.1%(w/v)Ca $(0H)_2$ and *Bacillus coagulans* had a value of 668.80 mg/l. The control sample had the least COD value of 299.20 mg/l. The total dissolved solids for all the samples increased at the end of the bioremediation period. The initial TDS was 54.1 mg/l. For the sample C the TDS increased to 160.6 mg/l while sample D increased from 54.12 mg/l to 141.0 mg/l.

4. DISCUSSION

The microbiological analysis of the mine water from the various locations indicated low diversity in the microbial flora within the mine site. The environment supported limited microbial diversity, a community that face physically and chemically restriction poses threat to the microbial inhabitant. Bacteria isolated were *Bacillus* sp, *Thiobacillus* sp, *Micrococcus sp* and *Staphylococcus sp.* Gram positive had the highest occurrence in the samples. The non predominance of gram negative in the environment is in accordance with Edwards (1999), who had earlier reported less than 50% of gram negative bacteria of the total viable microbial population in an acidic mine environment. The isolation of gram negative purple bacterial in mine waters could be supported Johnson (1995).

The effective remediation of the iron ore effluent with the three different bacteria isolates monitored with some water quality parameters i.e. conductivity, total hardness and total alkalinity showed that the mine effluent treated with only bacterial isolates had better remediation effect compared to those inoculated with the bacterial isolates and calcium hydroxide. Increase in conductivity which tends to decrease towards the end of the treatment could be attributed to enzymes or metabolites which might have acted as surface active agents helping in dissolution of both organic and inorganic elements present in the effluent. Levin and Gealt (1993) observed microbes produced enzymes or metabolites that acts as surface active agents. The higher values in conductivity, alkalinity and total hardness in samples inoculated with calcium hydroxide could be due to the increase in the pH by the addition of the chemical. The changes observed in the physicochemical parameter of the mine waste effluent during the period of bioremediation could have resulted from the influence that the bacteria might have exerted on the metals present in the water. This is in agreement with the work of Ehrlich, (1999) that reported that microbes may act as producers or consumers of certain geo-chemcially active substances and thereby influence the rate of geo-chemical reaction in which such substances are reactants.

However, all the samples irrespective of treatment had a decrease in BOD and COD, which are indicative that the organic matter in all the samples, were mineralized by the microorganisms. It also showed that indigenous microorganisms could promote targeted changes in Acid Mine Drainage water chemistry and thereby facilitates bioremediation of these polluting wastewaters. The uninoculated sample under anaerobic condition however gave the least BOD value of 4.00 $\pm$ 0.00 mg/l, this low BOD and COD observed in the effluents sample that was kept under anaerobic condition is in agreement with report of Bridge and Johnson (1998) who reported that one of the advantages of mineral bi-oxidation operation is that they are not usually subject to contamination by unwanted microorganisms especially in continuous flow tank, the continual wash out of mineral together with their attached microbes as well as the organisms in suspension provides strong selection for improved organisms (Rawlings 2005). The increase in the TDS could be due to soluble metabolites produced by the microorganisms in comparison to the chemical oxidation of ferrous which form insoluble iron at neutral pH (Prescot *et al*., 2005)

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