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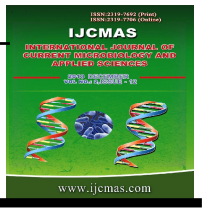

Original Research Article

Reduction of ferric iron in synthetic medium amended with acetate as a sole carbon source

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Keywords

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A B S T R A C T

Microbial iron reduction plays an significant role in the cycling of iron and organic matter in natural environment. Soluble and insoluble ions as well as the metallic forms frequently exhibit toxicity. Suitable remediation is required for the heavy metal contamination in soil especially ferric form of iron since it is highly toxic. In the present study, bioremediation process was applied because of its low cost, efficient and ecofriendly in nature. Iron resistant bacterial strains were isolated from soil samples obtained from various areas located in Salem district of Tamil Nadu. Among the iron resistant bacterial strains SK-PS13 was selected as potential Fe (III) reducer. It was selected by screening of amorphous Fe (III) reduction test in synthetic medium amended with acetate as a sole carbon source. Subsequently Fe (III) reduction was carried out in the medium enriched with humic acid and AQS. This was helped to lead 54 and 56 fold increased level of Fe(III) biosolubilization. The present study would be a lime light for further exploration of eco-friendly bioremediation of iron contaminated soil in Salem district in turn enhances the benefits for the human welfare.

Introduction

Fe (III) appears in the environment in a multiplicity of states such as oxides, hydroxides and oxyhydroxides; all these different forms are chiefly referred to as 'Fe (III) oxides'. Fe oxides are the most metallic oxides in soils, it constitute a great interest in soil chemistry and importance of plant nutrition but their low solubility is the main cause of Fe deficiency. Iron oxide like amorphous Fe (III) oxide ($Fe(OH)_3$, goethite (α-FeOOH), akaganetite (β-FeOOH), maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) those had been reported by previous studies (Wilkins *et al.*, 2006; Li *et al.*, 2012; Bae and Lee, 2013). The reactivity of a Fe oxide depends on mineralogy, elemental composition, crystal size and surface area. Two main parameters must be considered in order to

predict iron availability in soil: solubility and dissolution rate. Reduction of iron (III) oxides has an important influence on the geochemistry of anaerobic soils and sediments by direct microbial mediated process. Amorphous iron (III) oxide is considered to be the predominant form of Fe(III) reduced in sediment environments, because very little of the iron in crystalline iron(III) oxides (e.g., goethite, hematite) has appeared available for reduction by Fe(III)-reducing bacteria (Roden, 1996).

The metal resistant populations principally involved in the alteration of solubility of metals through their reduction, accumulation and in situ immobilization by extracellular precipitation (Roane, 1999). In general microorganisms like bacteria, fungi and algae, yeast are known to resist and also accumulate heavy metals (Ahmad *et al*., 2005). These adaptations largely recognized due to a diversity of chromosomal, transposon and plasmid mediated resistance systems in bacteria. Malik (2000) reported that the prevalence of plasmid-bearing strains is more in metal containing sites.

Through microbial established remediation technique, the biotransformation process is precise interest on interpretation of their eco friendly approach by two different metabolic pathways like assimilatory and dissimilatory metabolism (Akinci and Guven, 2011). In assimilatory metabolism elements are integrated into cellular structures (anabolism) while in dissimilatory metabolism elements are oxidized or reduced, and the organism derive the energy released in the process (catabolism). Frequently all microorganism need Fe for their diversity of metabolic functions and Fe is a central structural component of several diverse enzymes. Dissimilatory iron reduction is the progression by which organisms reduce Fe (III) to Fe (II), concerning the energy released in the process to drive their cellular reactions requiring energy input. Transformation and solubilization are energetic mechanisms in dissimilatory iron reduction by means of a model organism like *Shewanella* sp. (Lovley, 1987; Babechuk *et al*., 2009; He *et al*., 2010). In specific, the metal oxides serve as terminal electron acceptors for the oxidation of organic matter by iron reducing bacteria, adding to this an important oxidation pathway of organic matter degradation and producing soluble ferrous iron. Maximum of the work in that field has submitted that Fe (III) reduction may have been one of the first microbial respiratory processes on Earth. Based on this concept, a large number of studies have been passed out in Fe (III) dissimilatory reduction because of its high removal efficiency and low cost (He and Qu, 2008; Li *et al*., 2011). At this point of view microbial Fe(III) reduction can have a superior impact on soil geochemistry, affecting the minerals in the subsurface, the cycling of organic compounds and also the dissolution of a wide variety of insoluble iron oxide.

Numerous works pointed out that microbial dissimilatory iron reduction processes subjected to electron transfers to the cell surface underwrite to the oxidation of organic carbon sources like glucose, acetate, lactate in a variability of anaerobic habitats (Zhang *et al.,* 2007; Ayyasamy *et al.,* 2009). The significance of these respiratory processes reflect that Fe(III) as a redox-active metal via the electron-shuttling mechanism, in this process Fe(II) species capable of accepting electrons from biotic oxidation of organic matter and donating these electrons to the target contaminants (Bishop *et al.,* 2011; Gorski

et al., 2010). The Fe (III) was in an amorphous form, a wide range of fermentation products like acetate, lactate and H_2 were metabolized with concomitant Fe (III) reduction (Lovely *et al.,* 1995).

Generally acetate is considered to be one of the key organic intermediates driving the dissimilatory iron reduction processes (Francis *et al.,* 2000). Although many microorganisms have been shown to have the capacity to anaerobically reduce Fe(III) (Lovley *et al.,* 1997), only a limited number of organisms are known to couple acetate oxidation to Fe(III) reduction the only other mesophilic organisms known to possess this acetate-oxidizing, Fe(III)-reducing capacity are *Geothrix fermentens* (Lonergan *et al.,* 1996), *Geovibrio ferrireducens* (Caccavo *et al.,*1996), *Ferribacterium limneticum* (Cummings *et al.,* 1999) all of which are obligate anaerobes. The best-characterized group of facultatively anaerobic Fe(III) reducers are within the genus *Shewanella,* which is capable of using a wide variety of electron acceptors, including oxygen, but their ability to utilize electron donors is somewhat limited, in that they are unable to use acetate anaerobically. Recently, facultative organisms within the genus *Aeromonas* (Knight and Blakemore, 1998), as well as the species *Sulfurospirillum* (formerly *Geospirillum*) *barnesii* (Laverman *et al.,* 1995 and Stolz *et al.,* 1999) and *Ferrimonas balaerica* (Rosello-Mora *et al.,* 1999), have also been shown to utilize Fe (III) as an anaerobic electron acceptor, but, like *Shewanella* species, they are unable to use acetate as an electron donor. In the reductive dissolution study, investigation of reduced Fe (II) using the colorimetric 1,10-phenanthroline method is a general method essentially proceeds from the formation of color due to a reaction between level of Fe(II) with phenanthroline reagent (Herrera *et al*., 1989; Wang *et al*., 2013).

Frequently the reductive dissolution process carried out in the presence of electron donors (Coates *et al*., 1998; Marsh *et al*., 2001). Humic substances and semiquinone moieties alleviates the need for direct microbe-mineral surface interactions, increasing the rates of Fe(III) reduction, these are naturally occurring and also anthropogenic derivatives may influence the biogeochemistry through their ability to act as "electron shuttles" for extracellular redox processes such as Fe(III) reduction (Williamson *et al.,* 2013).

Since, physical and chemical process of soil remediation is expensive and produces concentrated waste brines requiring further treatments. Therefore, in the present study, potential iron resistant organisms were isolated from metal contaminated soil and screened to select efficient organisms for the remediation of metal contamination in the natural system. Further, in the combined system was attempted, using acetate as the carbon source incorporated with humic acid and ADS for the efficient Fe (III) transformation.

Materials and Methods

Source of organisms

A total of three soil samples were collected in the depth of 15 cm) from Kanjamalai (Latitude: 11°36'58.57"**,** Longitude: 78°3'23.41"**)** forest reserve located in Salem district, Tamil Nadu, India. The collected soil samples were air-dried, ground and passed through a stainless steel mesh (0.6 mm).

Sample analysis

Significant physico-chemical characteristics of the soil samples were analyzed after proper digestions. The concentration of the heavy metals was also measured using Atomic Absorption Spectrophotometry (Electronic Corporation of India- AAS4129M) according to the methods reported by Abida Begum *et al*., (2009). Measurement of pH was done by preparation of aqueous soil extracts (1:2.5, w/v) using a pH-meter at 20°C (Piotrowska-Seget *et al*., 2005). For bacterial isolation, serially dilution was made using nutrient agar at 37°C incubation. Independently growing colonies were preferred on the basis of morphology and color. All the strains were purified by streaking on nutrient agar. The isolated bacterial cultures were named as SK-SS1 to SK-SS20, SK-JS1 to SK-JS40 and SK-PS1 to SK-PS21 and maintained on nutrient agar at 4°C for further studies.

Primary screening of Fe (III) resistant bacteria

Among the 90 bacterial strains isolated, Fe(III) resistant were screened by using analytical grades of filter-sterilized different concentration (100 to 1000 ppm) of ferric chloride, amended in 50mM Tris-buffered nutrient agar media (Suzana *et al.*, 1997). The screened Fe (III) resistant were identified based on the biochemical characteristics as given in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Secondary screening of Fe (III) reducing bacteria in the presence of acetate

Presumptively screened 14 iron resistant isolates were cultivated individually in serum bottles capped with black butyl rubber stoppers and aluminum crimp sealed under N_2 atmosphere. A bicarbonate-buffered anaerobic medium (Francis *et al.,* 2000) supplemented with 10 mM acetate and 40 mM of solid $Fe(OH)_3$ was used for enrichment of acetate oxidizing iron reducing bacterial isolates. The efficient isolate was selected based on the Fe (II) production measured by spectrophotometrically using the 1,10 - phenanthroline method (Li *et al.,* 2008).

Biosolubilisation of ferric iron

Preparation of synthetic amorphous ferric oxide

Synthetic amorphous Fe(III) oxide was prepared by neutralizing 0.4 M solution of $FeCl_3.6H_2O$ with 1 M NaOH and then washing with distilled water which was used as an iron (Fe^{3+}) source for this complete study (Roden *et al.,* 1996).

Inoculum preparation

The isolated metal reducing bacteria were grown in 500 ml of triptic soy broth containing (g l^{-1}) 17 g of casein, 5 g of NaCl, 3 g of soybean meal, 2.5 g of K_2HPO_4, 2.5 g of glucose and also 1000 ppm concentration of ferric chloride incorporation leads to maintenance of metal tolerance, which was incubated on a rotary shaker at 30 °C at 120 rpm. The 1% inocula (10^7 CFU ml^{-1}) of 12 hours culture were used as the inoculum throughout the study (Ayyasamy *et al*., 2012).

Effect of acetate as a carbon sources on Fe (III) reduction in Mineral salt medium (MSM)

MSM supplemented with 1% of acetate was prepared and sterilized at 121°C for15

min at 15 lbs (Wu *et al.*, 2010). To this 0.5% of synthetic amorphous ferric oxide, 1 mg/l synthetic electron mediators such as humic acid and 9,10-anthraquinone-2-sulfonate (AQS) were added respectively. Further, about 1% (v/v) of screened efficient acetate oxidizing Fe (III)-reducing isolate SK-PS13 (10^7 CFU/ml of the cells) were aseptically inoculated. Strict anaerobic conditions were maintained by flushing N_2 (99.99%) gas for 15 min, sealed with butyl-rubber stoppers and crimped with aluminum caps and keeping the test materials in a shaker (120 rpm) at 30°C for 30 days. Samples were withdrawn aseptically at the time intervals of 24 hours and analyzed ferrous ion by 1,10- phenanthroline method.

Statistical analysis

The biosolubilization experiments were conducted in triplicates and the rate of dissolution and the percentage of iron removal from soil was calculated with standard error bars. Both mean and standard deviation were performed wherever appropriate, using the statistical package within Microsoft® Excel (Version 2007).

Results and Discussion

Characteristic features of soil samples

The pH of the collected soil samples were slightly acidic to neutral ranges, they were 6.6, 6.9 and 7.1 in different sites. The micro element concentration analyzed by using Atomic Adsorption Spectrometry (AAS), among the selected metals in the given soil, the concentration of Fe was maximum as 58.8 ppm in site 1 sampling area in Kanjamalai forest soil (Table 1). The heterotrophic bacterial counts range were ranged from 3.5, 2.9 and 2.2 x 10^3 CFU/g respectively in each sites. The P value was 0.0, F-ratio was 103.40, since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean bacterial populations (CFU/g) from one level of sampling location to another at the 95.0% confidence level (Figure 1). Ayyasamy *et al.*, (2009) reported that the soil samples from a mountain slope was found to be neutral and rich in heavy metals predominantly, iron concentration was higher as 73,996 mg/kg. Mohamed *et al.*, (2012) noted that the soil samples from different places at roadsides had high prevalence of heavy metal resistant microbes its potential as bioremediation agents.

Primary screening of Fe (III) resistant bacteria by spot assay

Among 90 bacterial isolates, 14 strains resistant up to 1000ppm ferric iron. So, the isolated heterotrophic microbial load decreased with the increase in concentration of heavy metals indicating toxic effect of the heavy metals on the growth of microorganisms (Figure 2). Similar results reported that high concentrations of heavy metals not only cause serious health hazards but also disturb the ecological status of biota (Malik *et al.*, 2000). Nearly15.55% of the isolates could resist up to 1000 ppm concentration of Fe (III) and also 10% of the isolates were very sensitive to Fe (III). The difference in the metal tolerances of the bacterial isolates could be explained by the nature of habitats and the physiological characteristics of each bacterial isolate (Hassan *et al.*, 2008).

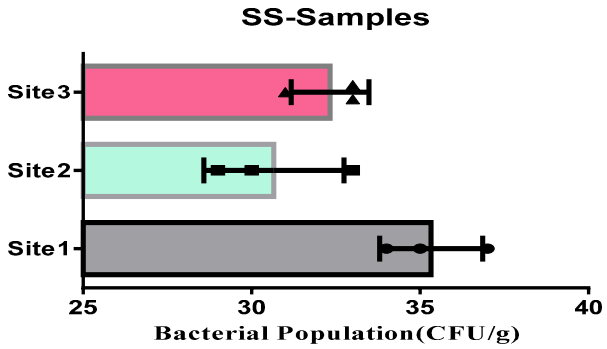
Metal Tolerant Bacteria

In the 1000ppm concentration of ferric iron amended in 50 mM Tris-buffered

Table.1 The physicochemical parameters of collected soil samples

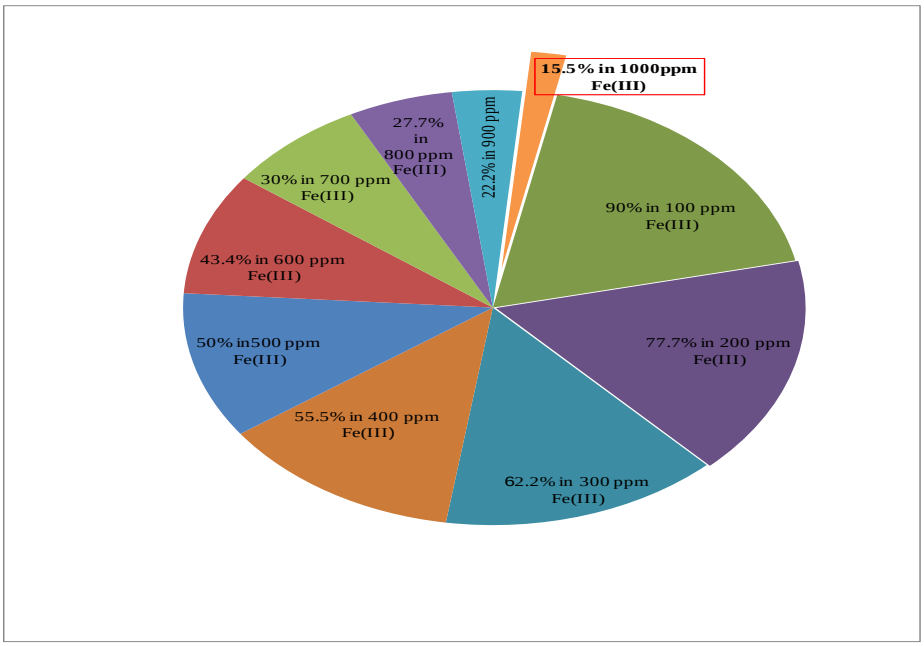
S. No	Parameters	Site-1	Site-2	Site-3
1	pH	7.1	6.9	6.6
2	Fe (ppm)	58.8	30.8	17.4
3	Mn (ppm)	18.4	12.4	11.2
4	Zn (ppm)	0.4	0.2	0.8
5	Cu (ppm)	0.6	0.2	0.8

Figure.1 Microbial analyses from collected soil samples



P value is < 0.0001

Figure.2 Percentage of Fe (III) resistant bacteria



Nutrient Agar (NAT) media, showed only 15.55% of Fe(III) resistant bacterial isolates and they were selected and identified upto generic level. Based on gram staining and biochemical tests the genera were identified as *Bacillus, Micrococcus, Acinetobacter, Pseudomonas, Alkaligenes, Arthrobacter, Flavobacterium, Vibrio, Enterobacter, Aeromonas* and *Corynebacterium*. The bacterial isolates identified in this study were mostly represented by gram-negative, and also few gram-positive bacteria are believed to be tolerant to a Fe(III) stress environment. Despite the apparent diversity of Fe(III)-reducing bacteria, microbial community analysis of subsurface environments where Fe(III) reduction occurs has suggested that in many cases, so the efficient Fe(III) reducing bacterial isolation from place rich in Fe(III) and its reduction was focused. Scala *et al.* (2006) reported that the bacterial species mediate Fe(III) reduction are phylogenetically diverse, include both facultative and obligate anaerobes, these can be categorized as fermentative, sulfur-oxidizing, hydrogen oxidizing or organic-acid-oxidizing organisms. Dissimilatory Fe(III)-reducing microorganisms can be categorized into two major groups: those that support growth by conserve energy during the electron transfer to Fe(III) and those that do not in that fermentative bacteria belongs to this latter group (Balboa *et al.,* 2010).

Acetate oxidizing Fe (III) reducing isolate

Isolate PS13 from SK site-1, was efficient acetate oxidizing and Fe(III) reducing isolate selected based on the Fe (II) production was measured spectrophotometrically using the 1,10 - phenanthroline method (Figure 3). These results suggest that this group of organisms is important in iron reduction coupled to acetate oxidation and hence to dissimilatory iron reduction in general because acetate is believed to be a major electron donor for this process. Most of the iron reducers were not the main contributors to acetate oxidation (Vandieken *et al.,* 2013). Fermentative and terminal electron-accepting processes interact very closely so that the products of fermentation do not accumulate but are used immediately as substrates for respiration, with typical acetate concentrations in the micromolar range and turnover rates of 10-1200 nmol cm^{-3} day^{-1} for marine sediments (Finke *et al.,* 2007) and also he reported that acetate is an important substrate for anaerobic respiratory organisms like iron and sulfate reducers. Iron reduction was active and also stimulated by the addition of acetate (Achtnich *et al.,* 1995). Lovely (1992) reported that Fe(III) reduction could be a significant process for organic matter mineralization, in addition to that iron reduction which occurs during fermentation, the products of fermentation particularly acetate could also be linked with Fe(III) reduction.

Effect of biosolubulisation of Fe(III) in the presence of electron mediators

The biosolubulisation of Fe(III) by using acetate oxidizing bacterial isolate SK-PS13 isolate was examined over a period of 30 days using MSM with 1% acetate with synthetic electron mediators like humic acid and AQS separately. Every 24 h, the reduced Fe(II) in each set of sample were analyzed. In this study, the given isolate reduced the maximum level of Fe (II) during 2^{nd} to 3^{rd} week in MSM containing 1% acetate and 1 mg/l concentration of redox elements like

Figure.3 Biosolubulisation rate of screened acetate oxidizing Fe(III) reducing isolate

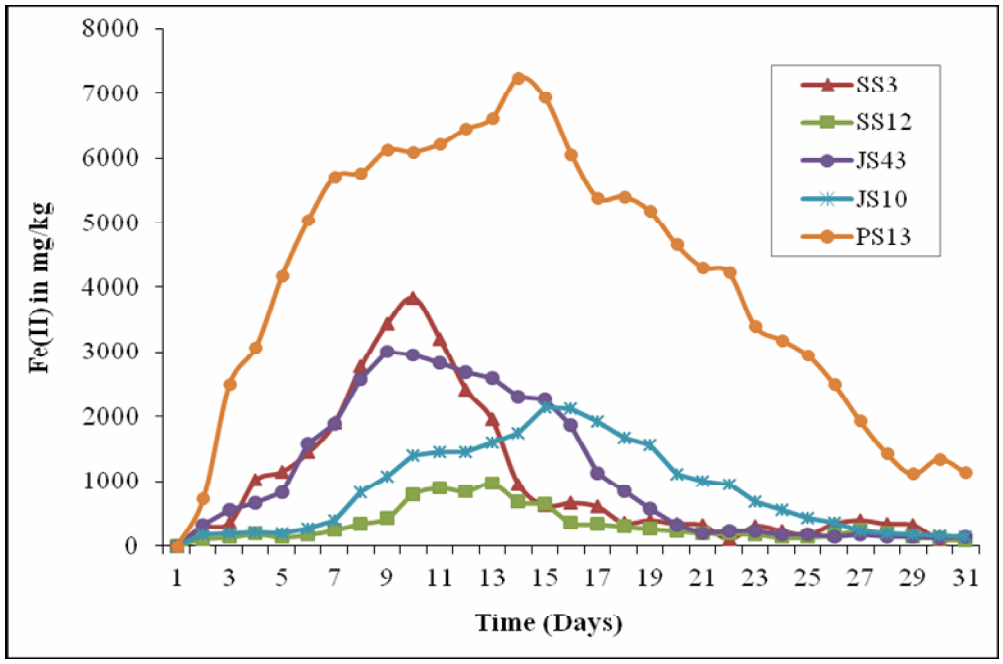
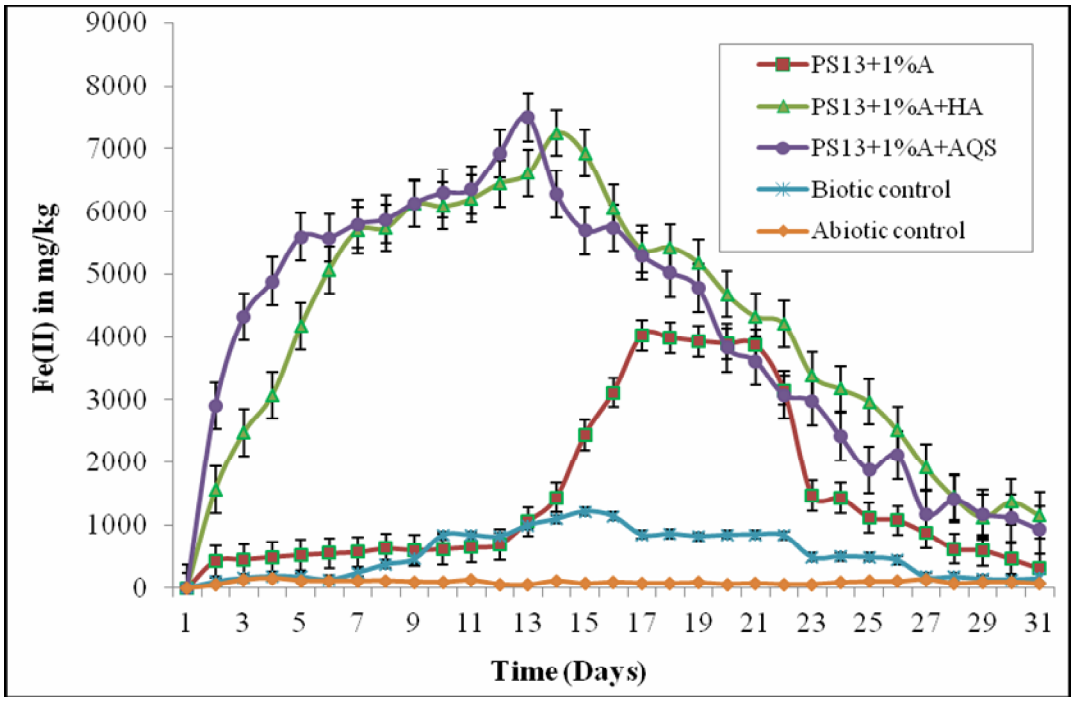


Figure.4 Effect of acetate in the presence of electron mediators for biosolubulisation study



humic acid and AQS (Figure 4). The higher reduction rate of Fe(II) measured as 7,494.77 mg/kg was recorded on the 12th day of the acetate with AQS added experiment. In the case of acetate incorporated with humic acid, the level of reduced Fe(II) was 7,245.54 mg/kg, in the absence of synthetic electron mediators acetate itself reduced 4,017.37 mg/kg of Fe(II) at on 14th and 16th day, respectively. In all test carried out with the acetate as a carbon source, the biosolubulisation of Fe(III) increased rapidly up to the 3rd week and then decreased thereafter. The reduction of synthetic amorphous Fe(III) oxide was negligible when the mineral salt medium did not contain any carbon source or bacterial inoculum.

The rate of microbial dissolution of iron oxide can vary significantly depends on different environmental conditions (Zhang *et al.,* 2007; Salas *et al.,* 2010; Ayyasamy *et al.,* 2012), the bacterial specificity/ capability of reduction process and nature of iron oxides (Li *et al.,* 2012), the presence of electron mediators (Ayyasamy and Lee, 2009), and the interaction between iron oxides and bacteria. The given purified SK-PS13 enrichment isolates, can derive energy for growth by coupling the reduction of Fe(III) to the oxidation of organic carbon source acetate, which is a key fermentation end products in such metabolism and also, given their abundance and ubiquity, acetate as formerly evident electron acceptor for iron reduction. Bae and Lee (2013) reported that the 59% as a highest concentration of 0.5 M HCl extractable Fe(II) was obtained in the presence of AQS (23.4 mM) compared with other ETMs like AQC (49%), riboflavin (33%), FMN (36%), and FAD (32%) by using *S. putrefaciens* CN32 able to reduced 40 mM lepidocrocite in deaerated deionized water, and also the continuous electron shuttling by the redox couples can significantly enhanced bioreduction of lepidocrocite in the presence of different ETMs. Wilkins *et al.,* (2006) explained that generally, the most obvious mechanism in some bacteria are able to access the iron oxides directly, by using outer membrane bound c-type cytochromes transfer of electrons from the cell onto the Fe(III) oxide surface in iron reduction process. In the given study, the SK-PS13 isolate was motile, in the absence of acetate and synthetic electron mediators the organism alone able to reduce in 1,211.06 mg/kg of reduced Fe(II) as a higher rate at the end of 2nd week in biotic control. Childers *et al.,* (2002) reported that the flagellated *G. metallireducens* enable to move towards Fe(III) minerals during reduction study. Electron-shuttling compounds found in the environment afford another mechanism by which bacteria can reduce Fe(III) oxides lacking the need for direct contact with the mineral phase. In this report, from 4th day onwards swiftly increase in reduction occurs particularly in the presence of humic acid and AQS. Lovley *et al.,* (1998, 1999) experiments using *G. metallireducens* and *S. alga* have demonstrated that respiration on humic acid as the sole electron acceptor can yield energy to support cell growth. The reduction of structural Fe(III) present in clays such as smectite was also stimulated by the addition of humic acids and the humic analogue 2,6-anthraquinone disulfonate (AQDS) (Lovley *et al.,* 1998) and also the dissolution and redox transformation of heavy metals in the soil from a mountain slope in the Bucheon area, South Korea by using *Shewanella* sp. (HN-41) influenced by the carbon source, humic acid and ADQS able to reduce 25.6% of iron reduction (Ayyasamy and Lee, 2009). Carbon source-mediated and

electron-shuttling compounds-mediated iron oxide reduction by indigenous bacterial isolate from iron ore containing or contaminated soil are the two most studied mechanisms involved in biotransformation study. A further study can be carried out based on different factors. Thus the present study serve as a good exploration of eco-friendly bioremediation of iron contaminated soil in turn enhances the benefits for the human welfare.

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References

Abida Begum, M., Harikrishna, R., Khan, I. and Veena, K. 2009. Analysis of Heavy Metals Concentration in Soil and Lichens from Various Localities of Hosur Road, Bangalore, India. E-J. Chem. 6(1): 13-22.

Achtnich, C., Bak, F. and Conrad, R. 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soils. Biol. Fertil. Soils. 19: 65–72.

Ahmad, I., S. Zafar and Ahmed, F. 2005. Heavy metal biosorption potential of *Aspergillus* and *Rhizopus* sp. isolated from waste water treated soil. J. Appl. Sci. Envol. 9: 123–126.

Akinci, G. and Guven, D. E. 2011. Bioleaching of heavy metals contaminated sediment by pure and mixed cultures of *Acidithiobacillus* sp. Desalination, 268: 221–226.

Ayyasamy, P.M. and Lee, S. 2009. Redox transformation and biogeochemical interaction of heavy metals in Korean soil using different treatment columns in the presence of *Shewanella* sp. Chemosphere. 77: 501–509.

Ayyasamy, P.M. and Lee, S. 2012. Biotransformation of Heavy Metals from Soil in Synthetic Medium Enriched with Glucose and *Shewanella* sp. HN-41 at various pH. Geomicrobiol. J. 29: 843-851.

Ayyasamy, P.M., Chun, S. and Lee, S. 2009. Desorption and dissolution of heavy metals from contaminated soil using *Shewanella* sp. HN-41 amended with various carbon sources and synthetic soil organic matters. J. Hazard Mater.161: 1095–1102.

Babechuk, M. G., Weisener, C.G., Fryer Paktunc, B.J. and Maunders C. 2009. Microbial reduction of ferrous arsenate: Biogeochemical implications for arsenic mobilization. Appl. Geochem. 24: 2332–2341.

Bae, S. and Lee, W. 2013. Biotransformation of lepidocrocite in the presence of quinines and flavins. Geochimica et Cosmochimica Acta, 114: 144–155.

Balboa, C. G., Bedoya, I.C., Gonzalez, F., Blazquez, M.L., Munoz, J.A. and Ballester, A. 2010. Bio-reduction of Fe(III) ores using three pure strains of *Aeromonas hydrophila*, *Serratia fonticola* and *Clostridium celerecrescens* and a natural consortium. Biores. Technol. 101: 7864–7871.

Bishop, M. E., Dong, H., Kukkadapu, R.K. and Edelmann, R. E. 2011. Microbial reduction of Fe(III) in multiple clay minerals by *Shewanella putrefaciens* and recativity of bioreduced clay minerals toward

Tc(VII) immobilization. Geochimica et Cosmochimica Acta, 75: 5229-5246.

Caccavo, F. J., Coates, J.D., Rosello-Mora, R.A., Ludwig, W., Schleifer, K.H., Lovley,D.R. and McInerney, M. J. 1996. *Geovibrio ferrireducens*, a phylogenetically distinct dissimilatory Fe(III)-reducing bacterium. Arch. Microbiol. 165: 370–376.

Childers, S. E., Ciufo, S. and Lovley, D.R. 2002. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. Nature, 416: Macmillan Magazines Ltd. pp. 767-769.

Coates, J. D., Councell, T., Ellis, D.J., and Lovley, D. R. 1998. Carbohydrate oxidation coupled to Fe(III) reduction, a novel form of anaerobic metabolism. Anaerobe. 4: 277–282.

Cummings, D.E., Caccavo, F.J., Spring, S. and Rosenzweig, R.F. 1999. *Ferribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. Arch. Microbiol. 171: 183–188.

Finke, N., Vandieken, V. and Jorgensen, B. B. 2007. Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. FEMS Microbiol. Ecol. 59: 10–22.

Francis, C. A., Obraztsova, Y.A. and Tebo, B. M. 2000. Dissimilatory Metal Reduction by the facultative anaerobe *Pantoea agglomerans* SP1. Appl. Environ. Microbiol. 66(2): 543-548.

Gorski, C. A., Nurmi, J.T., Tratnyek, P.G., Hofstetter, T.B. and Scherer, M.M. 2010. Redox behavior of magnetite: Implications for contaminant reduction. Environ. Sci. Technol. 44: 55-60.

Hassan, S.H.A., Abskharon, R.N.N., Gad El-Rab,S.M.F. and Shoreit, A.A.M. 2008. Isolation, characterization of heavy metal resistant strain of *Pseudomonas aeruginosa* isolated from polluted sites in Assiut city, Egypt. J. Microbiol. 48: 168-176.

He, J. Z. and Qu, D. 2008. Dissimilatory Fe(III) reduction characteristics of paddy soil extract cultures treated with glucose or fatty acids. J. Environ. Sci. 20: 1103–1108.

He, Y. T., Fitzmaurice, A.G., Bilgin, A., Choi, S. O'Day, P., Horst, J., Harrington, J., Reisinger, D. R. Burris and Hering, J. G. 2010. Geochemical processes controlling arsenic mobility in ground water: A case study of arsenic mobilization and natural attenuation. Appl.Geochem. 25: 69–80.

Herrera, L., Ruiz, P., Aguillon, J.C. and Fehrmann, A. 1989. A new spectrophotometric method for the determination of ferrous iron in the presence of ferric iron. J.Chem.Technol. Biotecnol. 44: 171–181.

Holt, J. G., Kreing, N. R., Sneath, P.H.A., Staley, J.T. and Williams, S. T. 1994. Bergey's manual of Deteminative Bacteriology. Lippincott Willams and Willkins, Baltimore. USA. ISBN-13:978.0683006032. 9., pp. 787.

Knight, V., and Blakemore, R. 1998. Reduction of diverse electron acceptors by *Aeromonas hydrophila*. Arch. Microbiol. 169: 239–248.

Laverman, A. M., Switzer Blum, J., Schaefer, J.K., Phillips, E.J., Lovley,R. and Oremland, R. S. 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. Appl. Environ. Microbiol. 61: 3556–3561.

Li, W., Liu, N., Cai, Jiang, J.L.and Chen, J. M. 2011. Reduction of Fe(III) chelated with citrate in an NOx scrubber solution by *Enterococcus* sp. FR-3. Bioresour.Technol. 102: 3049–

3054.

Li, X., Liu, T., Li, F., Zhang Shungui Zhou, W. and Yongtao Li. 2012. Reduction of structural Fe(III) in oxyhydroxides by Shewanella decolorationis S12 and characterization of the surface properties of iron minerals. J. Soils Sediments. 12:217–227.

Li, X.M., Zhou, S.G., Li, F.B., Wu, C.Y., Zhuang, L., Xu, W. and Liu, L. 2008. Fe(III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumonia* strain L17. J. Appl. Microbiol. 106: 130–139.

Lonergan, D.J., Jenter, Coates, J.D., Phillips, E.J.B., Schmidt, T.M., and Lovley, D. R. 1996. Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J. Bacteriol. 178: 2402–2408.

Lovley D. R. 1997. Microbial Fe(III) reduction in subsurface environments. FEMS Microbiol. Rev. 20: 305–313.

Lovley, D. R. 1987. Organic matter mineralization with the reduction of ferric iron: a review. Int. J. Geomicrobiol. 5: 375–399.

Lovley, D. R. 1995. Bioremediation of organic and metal contaminants with dissimilatory metal reduction. J. Ind. Microbiol. 14: 85–93.

Lovley, D. R., Caccavo, F. and Phillips, E. J. P. 1992. Acetate oxidation by dissimilatory Fe(III) reducers. Appl. Environ. Microbiol. 58: 3205–3206.

Lovley, D. R., Philips, E. J. P. and Caccavo J. Jr. 1992. Acetate oxidation by dissimilatory Fe(III) reducers. Appl. Environ. Microbiol. 58: 3205–3208.

Lovley, D.R., Fraga, J.L., Blunt-Harris, Hayes, L.A., Phillips, E.J.P. and Coates, J. D. 1998. Humic substances as a mediator for microbially catalyzed metal reduction. Acta. Hydrochim. Hydrobiol. 26: 152-157.

Lovley, D.R., Fraga, J.L., Coates, J.D. and Blunt-Harris, E.L. 1999. Humics as an electron donor for anaerobic respiration. Environ. Microbiol. 1: 89-98.

Malik, A. and Jaiswal, R. 2000. Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater. World J. Microbiol.Biotechnol.16: 177–182.

Marsh, T. L. and McInterney M. J. 2001. Relationship of hydrogen bioavailability to chromate reduction in aquifer sediments. Appl. Envol. Microbiol. 67: 1517–1521.

Mohamed, R. M. and Abo-Amer, A. E. 2012. Isolation and characterization of heavy-metal resistant microbes from roadside soil and phylloplane. J. Basic Microbiol. 52: 53–65.

Piotrowska-Seget, Z., M. Cycon and Kozdroj, J. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. J. Appl. Soil. Ecol. 28: 237–246.

Roane, T. M. and Kellog, S. T. 1996. Characterization of bacterial communities in heavy metal contaminated soils. Can. J. Microbiol. 42: 593–603.

Roden, E. E. and Zachara, J. M. 1996. Microbial Reduction of Crystalline Iron(III) Oxides: Influence of Oxide Surface Area and Potential for Cell Growth. Environ. Sci. Technol. 30: 1618-1628.

Rossello-Mora, R.A., Caccavo, F.J., Osterlehner, R.N., Springer, N., Spring, S., Schuler, D., Ludwig, W., Amann, R., Vannacanneyt, M. and Schleifer, K. H. 1999. Isolation and taxonomic characterization of a halotolerant, facultative anaerobic iron-reducing bacterium. Syst. Appl.

Microbiol. 17: 569–573.

Salas, E.C., Berelson, W. M., Hammond, D.E., Kampf, A.R. and Nealson, K. H. 2010. The Impact of Bacterial Strain on the Products of Dissimilatory Iron Reduction. Geochim Cosmochim Acta. 74: 574-583.

Scala, D. J., Hacherl, E.L., Cowan, R., Young and Kosson, D. S. 2006. Characterization of Fe(III)-reducing enrichment cultures and isolation. J. Res. in Microbiol. 157: 772–783.

Stolz, J. F., Ellis, D.J., Switzer Blum, J., Ahmann, D., Oremland, R.S. and Lovley, D. R. 1999. *Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilus* sp. nov., new members of the *Sulfurospirillum* clade of the Proteobacteria. Int. J. Syst. Bacteriol. 49: 1177–1180.

Suzana, T., Britz, L.M. and Bhave, M. 1997. Detection of heavy metal ion resistance genes in Gram-positive and Gram-negative bacteria isolated from a lead-contaminated site. Biodegradation. 8: 113–124.

Vandieken, V. and Thamdrup, B. 2013. Identification of acetate-oxidizing bacteria in a coastal marine surface sediment by RNA-stable isotope probing in anoxic slurries and intact cores. FEMS Microbiol. Ecol. 84: 373–386.

Wang, X., Zhou, Z. and Jing, G. 2013. Synthesis of Fe3O4 poly(styrene–glycidyl methacrylate) magnetic porous microspheres and application in the immobilization of *Klebsiella* sp. FD-3 to reduce Fe(III)EDTA in a NOx scrubbing solution. Biores. Technol. 130: 750-756

Wilkins, M.J., F. R. Livens, D. J. Vaughan and Lloyd, J. R. 2006. The impact of Fe(III)-reducing bacteria on uranium mobility. Biogeochemistry. 78: 125–150.

Williamson, A. J., Morris, K., Shaw, S., Byrne, J.M., Boothman, C. and Lloyd, J.R., 2013. Microbial Reduction of Fe(III) under Alkaline Conditions Relevant to Geological Disposal. Appl. Environ. Microbiol. 79(11): 3320–3326.

Wu, C.Y., Zhuang, L., Zhou, S.G., Li, F.B. and Li, Z.M. 2010. Fe(III)-enhanced anaerobic transformation of 2,4-dichlorophenoxyacetic acid by an iron-reducing bacterium *Comamonas koreensis CY01*. FEMS Microbiol. Ecol. 71: 106–113.

Zhang, S.H., Li, W., Cheng, J.W., Chen, H. and Yao, S. 2007. Reduction of Fe(II) EDTA-NO by a newly isolated *Pseudomonas sp.* strain DN-2 in NOx scrubber solution. Appl. Microbiol. Biotechnol. 76: 1181–1187.