JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 677-680

Investigation on Iron Oxides Precipitated from $FeSO_4$ by *Klebsiella sp* SU- 14

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(Received: 02 May 2013; accepted: 17 June 2013)

Results of observation and preliminary analysis on mineralogical composition, chemical and structural characteristics of iron oxides precipitated from $FeSO_4$ by *Klebsiella sp* SU-14 are presented here. In this study, *Klebsiella sp* SU-14 was allowed to grow in $FeSO_4$ rich medium, the organism was able to convert $FeSO_4$ into iron oxides extracellularly through biologically induced mineralization (BIM). These extracellular precipitated biogenic iron oxide mineral particles were analyzed by following physicochemical methods: Fourier transformed infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) combined with energy dispersive X-rays (EDX) analysis. From these analyses the phase composition and physicochemical properties of iron oxides were obtained. Observations of these results revealed that the biogenic iron oxides particles were confirmed as magnetite (Fe_3O_4) which has different morphological characteristics with micrometer size distributions.

Key words: Iron oxides, *Klebsiella sp* SU-14, FT-IR,
Thermal field emission scanning electron microscope –EDX.

In recent years, research on iron oxides (a group name for oxyhydroxides and oxides) has been considerably focused due to their potential applications in sensors, pigments, magnetic targeting etc[1]. Goethite (α-FeOOH), lepidocrocite (γ-FeOOH), feroxyhyte (δ-FeOOH), hematite (α-Fe_2O_3) and Fe_3O_4 (magnetite) are normally precipitated from aqueous $FeSO_4$ solutions. Mostly these iron oxides are produced by chemical methods and which also occurs naturally by biologically controlled mineralization (BCM). Microbial iron reduction has been studied extensively for the cycling of the iron metal in natural ecosystem or in contaminated ecosystem[2-4] and often produce iron oxides such as magnetite[5,6]. There are many approaches for the synthesis of magnetic nanoparticles such as size reduction through ball milling, chemical precipitation and microbial synthesis[5].

MATERIALS AND METHODS

Isolation of the bacterial strain

The bacterial strain was isolated from the garden soil of Sathyabama University, Chennai, TamilNadu-600 119, India. Soil sample collected was inoculated into the medium containing $FeSO_4.7H_2O$ (5 g/l), $Na_2H PO_4$ (12.8 g/l), KH_2PO_4 (3 g/l), NaCl (0.5 g/l), NH_4Cl (1 g/l), $MgSO_4.7H_2O$ (2.4 g/l), glucose (10 g/l) and left undisturbed for two weeks at room temperature. After two weeks 1ml of sample was used for the serial dilution and pour plated on above mentioned selective iron rich medium with agar (20 g/l) and incubated at 37 ^{0}C for 3 to 4 days. The organism which grown on the iron rich medium

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were able to sustain the high concentration of $FeSO_4$ were selected and inoculated into 50ml of same iron rich liquid medium. These procedures were repeated for three times in order to stimulate an enrichment culture. Then the culture was subjected for iron oxide precipitation.

Identification of microorganism

Identification of microorganism was done by performing routine biochemical tests and 16S rRNA sequencing.

Activity of ferrous salt on *Klebsiella sp* SU-14

The bacterial isolate (*Klebsiella sp* SU-14) was inoculated in a iron rich liquid medium containing $FeSO_4.7H_2O$ (5 g/l), Na_2HPO_4 (12.8 g/l), KH_2PO_4 (3 g/l), NaCl (00.5 g/l), NH_4Cl (1 g/l), $MgSO_4.7H_2O$ (2.4 g/l), glucose (10 g/l) and incubated at 37 ^{0}C for 144 h under aerobic condition and control was also maintained (without bacterial inoculation).

Separation of extracellular iron oxides

After 144 h incubation the culture medium was removed out of the conical flask and the remained precipitate in the conical flask was collected washed twice with de-ionized water and used for further analysis.

Characterization of iron oxides

The extracellular synthesized biogenic iron oxide particles, mineralogical composition, chemical and structural characteristics were analyzed by physicochemical methods of Fourier transform infrared spectroscopy (FTIR- Perkin Elmer Spectrum1 instrument, operating by transmittance between 450- 4000 cm^{-1} using KBr method), and thermal field emission scanning electron microscope –EDX (FE-SEM- model JSM-7000F).

RESULTS AND DISCUSSION

The bacterium was isolated and identified by its biochemical characteristics and 16SrRNA sequence as *Klebsiella sp* SU-14. The 16SrRNA sequence was submitted in GENBANK (accession number: JQ756126). When the *Klebsiella sp* SU-14 was grown in $FeSO_4$ rich medium at 37^{0}C under aerobic condition, with controlled pH, there was a gradual change in colour of the medium was observed. After 144 h incubation it was converted as black coloured iron oxide particles at acidic pH, but it was not found in control where the medium was not inoculated with the organism. These results are because of the oxidation process of ferrous (Fe^{2+}) ions is converted to ferric (Fe^{3+}) ions may be takes place in culture medium.

FT-IR analysis revealed that the iron oxide formed by *Klebsiella sp* SU-14 is magnetite as it showed typical Fe–O–H bending bands of magnetite, whereas the shoulder at 569 and the band at 543cm^{-1} can be can be Fe-0 bond vibration mode of magnetite (Figure. 1). Ellid *et al*.,[7] found the FT-IR spectrum of substoichiometric magnetite $Fe_{2^{.}91}O_4$ showed two IR bands at 586 and 404cm^{-1}.

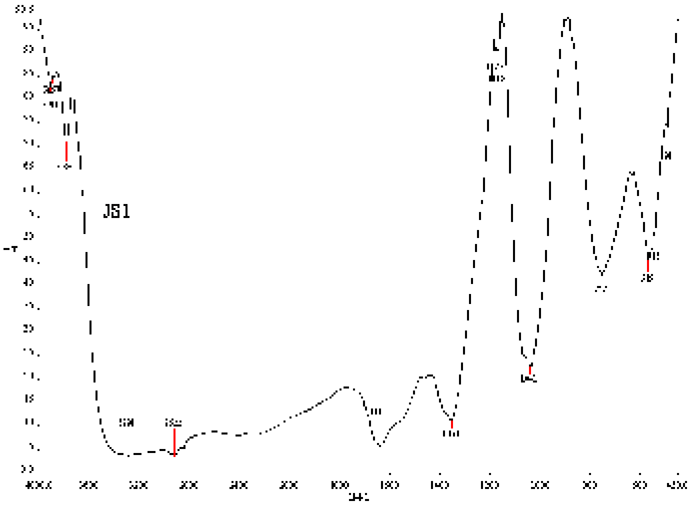

Fig. 1. FTIR spectra of the extracellular precipitated biogenic magnetite (Fe_3O_4) mineral of *Klebsiella sp* SU -14

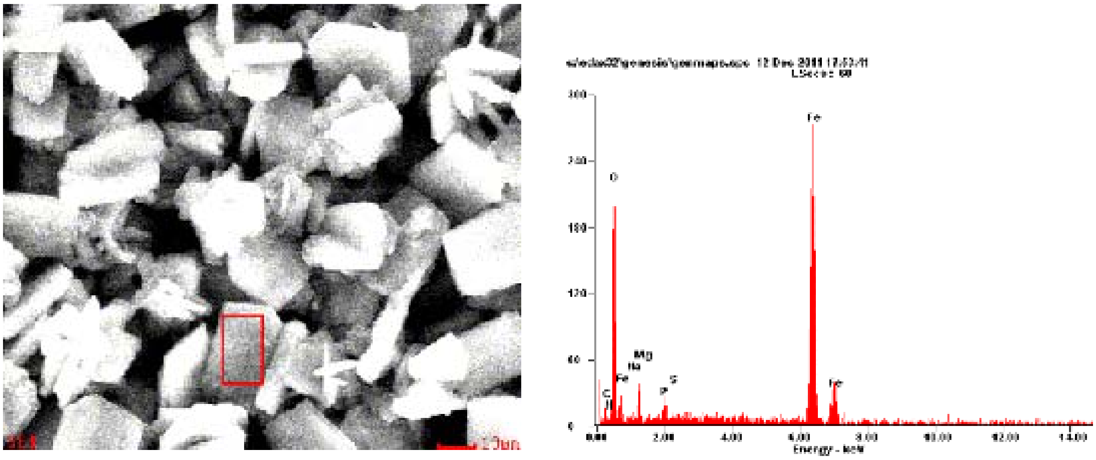

Fig. 2. FESEM micrograph and EDX spectrum of extracellular precipitated magnetite (Fe_3O_4) mineral of *Klebsiella sp* SU -14

Ishii *et al.*,[8] assigned the IR bands at 565 and 360cm^{-1} for Fe_3O_4. The absorption band at 1642 cm^{-1} is due to H_2O bending vibration. Other absorption bands at 1179, 1168 and 1041 cm^{-1} may be because of absorbed sulphate groups. The acidic pH created by *Klebsiella sp* SU-14 might be the reason for the absorption of these sulphate groups, even Gotic and Music[1] also found increased absorption of sulphate group on iron oxide at acidic pH. At low pH, oxidized Fe (III) are soluble and it favors active mineralization by organisms, which was also found mesophilic, autotrophic bacteria *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*)[9] and also in *Leptospirillum spp*. which oxidized Fe (II) to iron oxyhydroxide[10,11].

FE-SEM-EDX analysis (Figure.2) the morphology and chemical composition of iron oxides precipitated from $FeSO_4$ by Klebsiella sp SU- 14. The SEM observations indicated the presence of poorly crystalline iron oxides with µM-size distribution. Qualitative element analysis of energy dispersive X-ray spectra (EDX) of iron oxides (Fe_3O_4) particles identified their major elements as Fe and O with varying concentrations of minor elements such as C, N, Na, Mg, P and S. The iron and oxygen percentages in the EDX spectrum are also very similar to the previously reported magnetite (72.36% Fe; 24.64% O)[12]. The minor elements (C, N, Na, Mg, P and S) present in EDX spectrum are the residual salts from media. These results indicate that the precipitated biogenic iron oxides particles as magnetite (Fe_3O_4).

CONCLUSION

Klebsiella Sp SU-14 involved in the biologically induced mineralization (BIM) of $FeSO_4$ (Fe^{2+} to Fe^{3+}) into extracellular micrometer sized magnetite mineral at acidic pH, which have been confirmed by FT-IR and thermal field emission scanning electron microscope –EDX Spectroscopy.

ACKNOWLEDGEMENTS

The authors are thankful to Sathyabama University, Chennai, Tamil Nadu, India for providing infrastructure facilities for this study and also faculty members, Department of biotechnology for their help. Last, but not the least the authors are also grateful to Mr. G. Narendrakumar, Lecturer, Arunachalam An, and Gerald Manual Pravin Fernando, Sathyabama University, Chennai for their valuable help and suggestions.

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