I J M R
International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975–9174, Vol. 3, Issue 2, 2011, pp-85-89
Available online at http://www.bioinfo.in/contents.php?id=27

CHARACTERIZATION OF *BACILLUS POLYMYXA* FROM JAMNAGAR MINE WATER AND BIOBENEFICIATION OF BAUXITE ORE FOR IRON THROUGH SURFACE MODIFICATION

VIJAYA B.*, JAYALAKSHMI N.R. AND MANJUNATH K.
Department of Microbiology and Biotechnology, Jnana Bharathi campus, Bangalore University, Bangalore, India - 560056
*Corresponding Author: Email- vsd_bhuvana@rediffmail.com

Received: August 01, 2011; Accepted: August 22, 2011

Abstract- Preliminary screening of the mine water sample from bauxite ore deposits in Jamnagar, India showed the presence of heterotrophic bacteria *B.polymyxa*. Growth conditions for the bacteria to bring about maximum beneficiation were standardized by using the enriched Bromfield medium. *B.polymyxa* brought significant changes in the surface modifications of the mineral magnetite. The interaction resulted in surface chemical changes both on the cell and on the mineral surface by studying their electrophoretic mobility using Zeta meter 3.0. Dissolution studies in the presence of microorganisms establish the foundation in which these processes could be used for the utility of beneficiation in the efficient separation of the impurities from the ore, thus confirmed that *B.polymyxa* has greater affinity towards magnetite and could be efficiently used to remove iron from magnetite. Experiments with respect to the bauxite ore was initiated after confirming the above result. Iron removal from bauxite ore by *B. polymyxa* has been demonstrated under 2% sucrose concentrations brought about 12.5% removals in four days and under similar conditions the control in absence of *B. polymyxa* only 6% iron removal was seen. Thus, *B. polymyxa* plays a significant role in biobenficiation of bauxite mineral. These observations clearly indicate that a direct mechanism through bacterial attachment to the ore and an indirect mechanism through leaching with metabolites are involved in the biobeneficiation process.
Keywords- Chemo-organotroph, Physico-chemical changes, zeta-potential, bauxite ore, dissolution, electrophoretic mobility, biobeneficiation, bioleaching

INTRODUCTION

Bauxite is an economically important mineral used in the extraction of aluminium and in the manufacture of refractory. The mined bauxite ore needs to be beneficiated (calcium and iron being major impurities) so as to remove undesirable mineral constituents before it could be considered as a suitable raw material for the commercial use [1]. Although physico-chemical processes such as froth floatation, gravity separation, reduction roasting and magnetic separation could be used to beneficiate bauxite, all of them are energy and cost intensive, less flexible and pose environmental problems. A biotechnological route on the other hand could prove to be cheaper, environmentally benign and less complex then physico-chemical process [2-4].

Microbial mining is a process of bioleaching which recovers metals from ores that are not suitable for direct smelting due to their low metal content. The use of microorganisms in ore leaching to extract metals such as copper, uranium, gold, silver and iron has been commercialized since 1960's [5, 6 and 7]. As different from bioleaching, biobeneficiation refers to the removal of undesirable mineral components from an ore by microbes, which bring about their selective dissolution by enriching the desired mineral constituents in the solid ore matrix [1]. When microorganisms interact with minerals, many consequences of mineral processing results like adhesion of microorganisms to mineral surfaces, oxidation- reduction reactions, adsorption or chemical interactions onto mineral surfaces etc., are resulting in biosurface modification [8, 9].

In the present study, biobeneficiation was studied for the major mineral impurity magnetite of bauxite ore using *B.polymyxa*, a gram positive facultative anaerobe, and a chemo-organotroph widely distributed in soil. The bacteria adhere to the mineral through surface proteins on cell wall, or by extra cellular polysaccharides and induce its subsequent uptake from ore matrix [10, 11]. Since significant amounts of polysaccharides, residual carbohydrates and organic acids are present in liquid phase, solubilisation of mineral occurs through chelation, reductive dissolution and acidolysis [12]. Thus, it can be expected that *B. polymyxa* can simultaneously remove magnetite from the ore.

The *B.polymyxa* isolated was characterized by microbiological and biochemical methods [13]. The electrophoretic mobility with pH as a functional aspect was determined for the mineral and bacterial cell, before and after interaction at different time intervals. The

influence of bacterial metabolite in changing the surface chemistry (zeta potential) through surface modification of the mineral magnetite was studied. The dissolution experiments [14, 15] were also conducted for confirmation of *B.polymyxa* affinity towards the mineral.

MATERIALS AND METHODS

To study the physico-chemical characteristics, the mine water sample was obtained from Orient Abrasives limited; Jamnagar water mines Gujarat, India.

Pure mineral sample of magnetite was obtained from Alminrock Indscer Fabriks, Bangalore, India. The sample was dry grounded, fractioned (minus 400 mesh fractions) and then screened for adsorption and electrokinetic studies.

The **physico-chemical studies** were done by inoculating the mine water sample into Bromfield medium [16] and incubated at 30^{o}C on a rotary shaker at 240 rpm. The *B. polymyxa* isolated was characterized by Gram staining and other biochemical tests including indole, methyl red, Voges-Proskauer, citrate utilization, gelatin liquefaction, starch hydrolysis and catalase tests.

Electrokinetic measurements for observing electrophoretic mobility was determined by using Zeta meter model 3.0 to observe the zeta potential exhibited by minerals and bacterial cells. Surface behavior of magnetite mineral, bacterial metabolite and bacterial cells before and after interaction was done. 10% inoculum was inoculated to Bromfield medium and at the expiry of 8 hours, corresponding to mid-logarithmic phase of growth; the cells were harvested and centrifuged to separate cells from the metabolite. Experiments were performed by the interaction of the mineral with either the cells or the metabolites separately. After interaction the mineral samples were separated from the metabolite or the cells, as the case may be, and the surface charge characteristics of the interacted mineral as well as the cells were ascertained using zeta potential measurements. The electrophoretic mobility of cells of *B. polymyxa* as a function of pH was performed before and after 5min, 15min, 1h, 24h, of interaction with magnetite.

Dissolution of magnetite was conducted in erlenmeyer flasks at pulp density of 5% magnetite and cell density/ population of 109 cells/ml of *B. Polymyxa.* After each test at given interval of time, pulp was centrifuged and supernatant was analyzed for pH, cell count and for dissolved iron.

Biobeneficiation of bauxite ore using *B.polymyxa* was carried out in Bromfield medium. 10% of active inoculum was inoculated into sterile medium containing 5% bauxite ore and the flask was incubated at 30^{o}C on a rotary shaker at 240rpm to allow the growth of the cells and leaching of the ore. At regular intervals the solid residue of the beneficiated bauxite remaining in the flasks was analyzed for iron by ammonium chloride method [16].

All experiments were carried out in triplicate and the reported values are on the average value.

RESULTS AND DISCUSSION

The differential characteristics of *B. polymyxa* was positive for catalase production, gelatin liquefaction, starch hydrolyses, and Voges Proskauer test where as negative for indole test, methyl test and citrate utilization test.

The cell density measurement of mine water after inoculating into Bromfield medium showed an increase in cell density with time ["Fig. (1)"]. The change in pH of mine water dropped from pH 7.5 to pH 4 showing the capacity of the organism to produce acids ["Fig. (2)"]. The growth curve of *B. polymyxa* ["Fig. (1)"] indicates, after an initial lag phase of about 2h, the cell number increases from about 6 x 10^7 to 10^9 cells/ml in 16h, and attains stationary phase thereafter. Middle logarithmic phase was observed at around the eighth hour. It is also observed that the pH of the medium drops from about 7 initially to about 3 in 20h, due to the production of organic acids such as acetic acid, lactic acid and formic acid [15].

The experiments with surface chemical behavior of bacteria and minerals before and after interaction showed a significant chemical changes, both on the cell and on the mineral surface. The bacterial cells were observed to adhere tenaciously onto the above mineral surface of magnetite. "Fig. (3), (4) and (5)" shows the electrophoretic mobility of magnetite, bacterial metabolite and bacterial cells as a function of pH, both before and after 5 min, 1h and 24 h interaction with cells. It is apparent that the iep of magnetite is located around 5.8. After interaction the iep is shifted to around pH 5. It is interesting to note that the zeta potential becomes less positive or more negative after interaction and iep is marginally shifted to pH 4.5 for magnetite after interaction with cells. Further increase in period of interaction for 24 h, there is a pronounced shift in iep to lower pH values approximately around 3.4. Thus, the bacterial cell has higher affinity towards magnetite, depicting the metabolic product is mainly responsible for surface chemical changes. There by considering the role of the organism in bringing surface modification [17]. Similar studies are done in *Bacillus circulans* and *Bacillus mucilaginosus* (biobeneficiation of bauxite ore for silica) **[**14**].** *Aspergillus niger* (for removal of iron from clay) [18]*, Hypomicrobium Sp.* (biobeneficiation of manganese ore from silica) [19].

The above tests were further confirmed by dissolution experiments on magnetite, where maximum dissolution of magnetite was to around 250 ppm of iron with cells alone and with metabolite it is comparatively lesser and is around 120 ppm. The bacteria and cells exhibited higher affinity and chemical interaction with magnetite, where the presence of ions and polysaccharides in the metabolite, and the enzymes secreted by bacteria favored the dissolution of iron ["Fig. (6)"]. Similar experiments were made by [20] and had shown that when cells growing in the medium i.e., in the growing mode the cells brought about greater dissolution of iron from magnetite. However, detailed experiments are

warranted, varying the parameter such as cell density, time of interaction etc.

Finally, experiments with respect to removal of iron from the ore were initiated by *B. polymyxa* under 2% sucrose concentrations. There was 12.5% iron removal in 4 days when compared to the control (in absence of *B. polymyxa*) with 6% removal ["Fig. (7)"]. These observations clearly indicate that both a direct mechanism through bacterial attachment to the ore and an indirect mechanism through leaching with metabolites are involved in the biobeneficiation process there by correlating the work done by [14].

CONCLUSION

In this regard the reported results have opened up a practically significant and commercially viable biotechnological approach to mineral beneficiation.

ACKNOWLEDGEMENT

I immensly thank the Jamnagar water mines officials for providing the water sample from which we isolated *Bacillus polymyxa.*. I also thank the Alminrock Indscer Fabriks Bangalore, for providing the pure minerals to have an experimental data to work with the bauxite ore. I am grateful to Prof K.A. Natarajan, Emeritus Professor and Dr. Subramanian, for giving an opportunity to work in the Dept of Metallurgy, Indian Institute of Science, Bangalore.

REFERENCES

[1] Brandle H. (1998) *German,* 143: 157-164.

[2] Ehrlich H.L. (1999) *Process Metallurgy,* 9: 3-12.

[3] Groundev S.N., Genchev F.N., Groundeva F.N., Petrov E.C. and Mochev O.J. (1983) *Progress Biohydrometallurgical Cagliari,* 442-450.

[4] Groundev S.N., Genchev F.N., Mochev O.J. and Petrov E.C. (1985) *Proceedings of XY International Mineral Process Congress,* 378-387.

[5] Agate A.D. (1996) *World Journal of Microbiology and Biotechnology,* 12: 487-495.

[6] Deo N. and Natarajan K.A. (1997) *Miner Process Metallurgy,* 14: 32-39.

[7] Bromfield S.M. (1954) *Journal of General Micrbiology,* 11: 1-6.

[8] Mishra D., Kim D.J., Ahn J.G. and Rhee Y.H. (1986) *Metals and Materials International*, 11: 249-256.

[9] Ehrlrich H.L. (1992) *Academic Press. London,* 140-234.

[10] Deo N., Vasan S.S., Modak M.J. and Natarajan K.A. (1999) *Process Metallurgy,* 9: 463-472.

[11] Mankad T. and Nauman E.B. (1992) *Biotechnology Bioengineering,* 40: 413-426.

[12] Deo N. and Natarajan K.A. (1998) *Mineral Engineering,* 11: 717-738.

[13] Buchanan R.E. and Gibbons M.E. (1974) *The Williams and Wilkins Corporation, Baltimore, 324-278.*

[14] Natarajan K.A., Modak J.M. and Anand P. (1997) *Mineral Metallurgical Process,* 14: 47-53.

[15] Phalguni A.M., Jayant M. and Natarajan K.A. (1996) *International Journal of Mineral Process,* 48: (1-2, 51-60).

[16] Vogel A.I. (1978). *ELBS, London,* 45-51.

[17] Karavaiko G.I., Avakyan Z.A., Ogurtsova L.V. and Safonova O.F. (1989) *CANMET, Ottawa,* 93-102.

[18] Devasia P., Natarajan K.A., Sathyanarayana D.N. and Rao G. (1993) *Applied Environental Microbiology,* 59: 4051-4055.

[19] Mishra A.K., Roy Mahapatra, S.S., Roy, P.,Mohanty, B.K., Majumdar,S., and Ray, P.K., 1986. Bioleaching of silica from manganese ore. Ind. J. of Tech., 24: 770-772.

[20] Deo N. and Natarajan K.A. (1996) *Int. J. Mineral Processing* 14(2): 47-53.

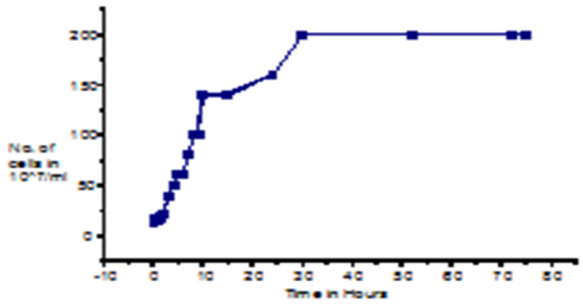

Fig. 1- Growth curve of *Bacillus polymyxa* in Bromfield medium

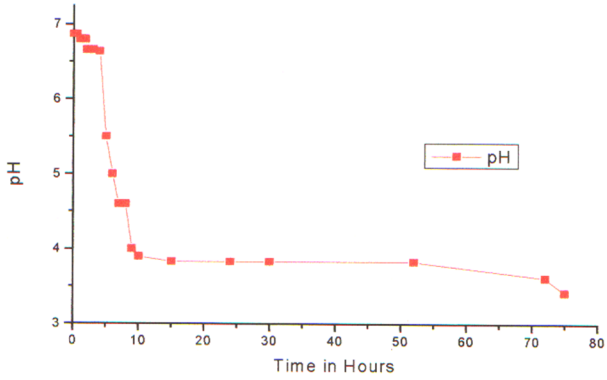

Fig. 2-Change of pH of growth curve of *Bacillus polymyxa*

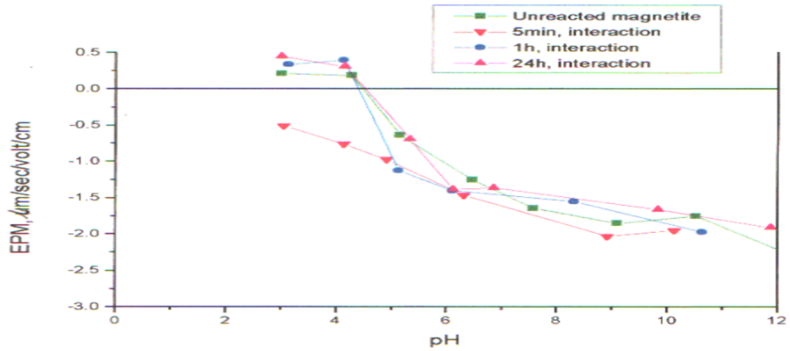

Fig. 3-EPM as function of pH for **magnetite** after interaction with *B. polymyxa.*

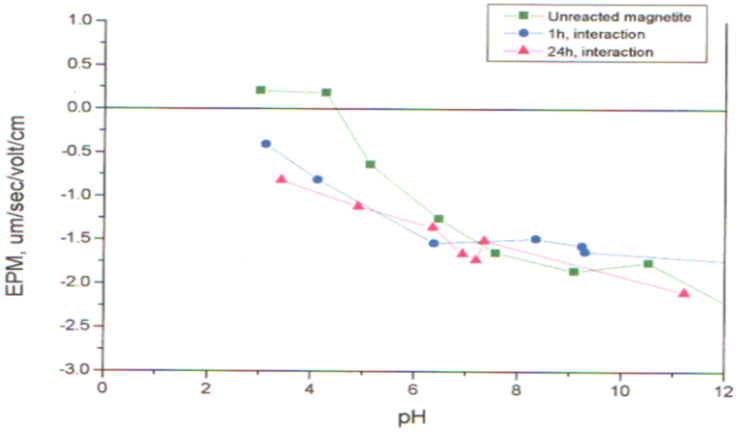

Fig. 4-EPM as function of pH for **bacterial metabolite** after interaction with *B. polymyxa.*

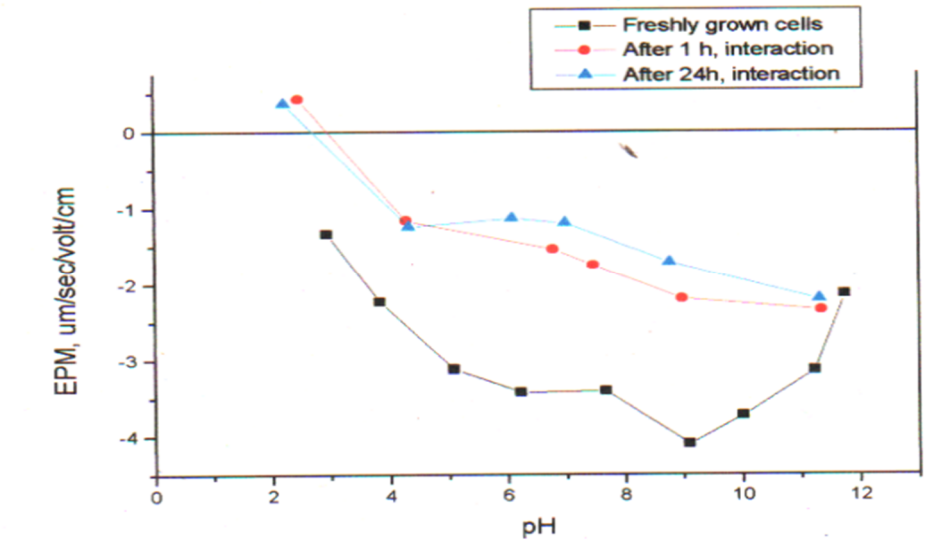

Fig. 5-EPM as function of pH for **bacterial cells** after interaction with *B. polymyxa.*

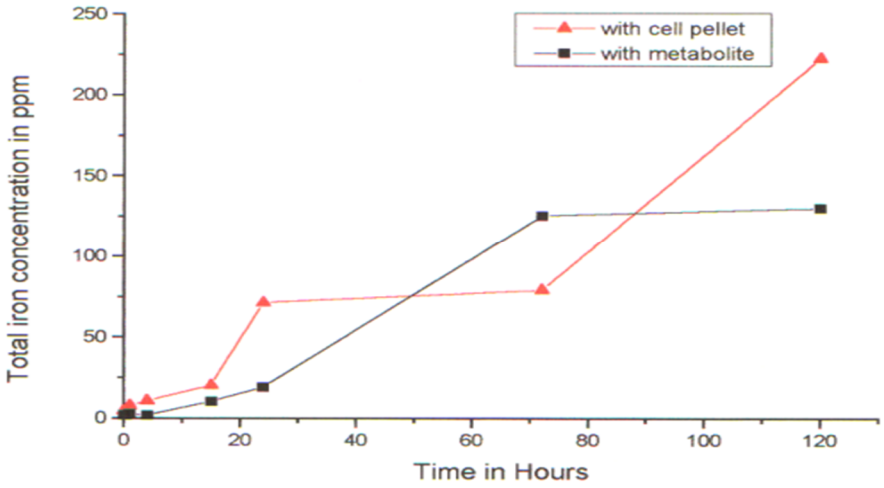

Fig. 6-**Dissolution** of magnetite as a function of time under different conditions.

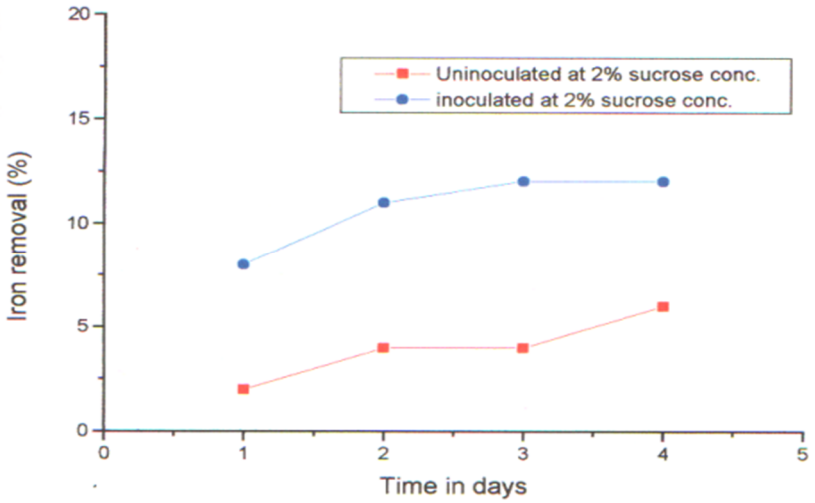

Fig. 7-**Iron** removal by *B. polymyxa* grown in Bromfield medium at 2% sucrose concentration.