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Characterization of Bacillus *Polymyxa* from Jamnagar Mine Water and Biobeneficiation of Bauxite Ore for Calcite Through Surface Modification

[1]*B. Vijaya,* [1]*K. Manjunath,* [1]*N.R. Jayalakshmi and* [2]*G.S. Nagananda*

[1]Department of Microbiology and Biotechnology,
Jnana Bharathi Campus, Bangalore University, Bangalore-56, India
[2]Department of Biotechnology, CPGS, Jain University, Bangalore, India

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 calcite. The interaction resulted in surface chemical changes both on the cell and on the mineral surface by studying their electrophoretic mobilities using Zeta meter 3.0. Flocculation and settling 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 calcite and could be efficiently used to remove calcium from calcite. Experiments with respect to the bauxite ore was initiated after confirming the above result. Calcium removal from bauxite ore by *B.polymyxa* has been demonstrated under 2% sucrose concentration brought about 40% removals in four days and under similar conditions the control in absence of *B. polymyxa* only 20% calcium removal was seen. Thus, *B. polymyxa* plays a significant role in biobenficiation of bauxite mineral. These observations clearly indicated that a direct mechanism through bacterial attachment to the ore and an indirect mechanism through leaching with metabolites are involved in the biobeneficiation process.

Key words: Physico-chemical changes · Flocculation · Electrophoretic mobility · Bioleaching

INTRODUCTION

Bauxite is an economically important mineral used in the extraction of aluminium and in the manufacture of refractories. 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 [5, 6]. The use of microorganisms in ore leaching to extract metals such as copper, uranium, gold, iron, silver has been commercialized since 1960's [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., result in biosurface modification [8, 9].

In the present study, biobeneficiation was studied for the major impurity, calcium of bauxite ore using *B.polymyxa*, a gram positive facultative anaerobe and a chemo-organotroph widely distributed in soil. The bacteria attaches to the minerals through surface proteins on cell wall, or by extra polysaccharides: good chelating

Corresponding Author: B. Vijaya, Research Scholar, Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University, Bangalore-56, India, Email: vsd_bhuvana@rediffmail.com

agents for various metals including calcium [10-12]. When oxygen is present in limiting amounts, sugars are partially oxidized via fermentative pathway leading to production of organic acids such as acetic acid and lactic acid inducing reduction reactions by facilitating dissolution [13]. Thus, it can be expected that *B. polymyxa* can simultaneously remove calcium from the ore [14].

The *B.polymyxa* isolated was characterized by microbiological and biochemical methods [15, 16]. 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 calcite was studied. The flocculation and settling experiments [17, 18] 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 calcite 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 [6] and incubated at 30°C on a rotary shaker at 240rpm. The *Bacillus 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 surface behavior of bacteria and calcium mineral before and after interaction were done. 10% inoculum was inoculated to bromfield medium and at the expiry of 8 hours, corresponding to mid-logarthmic 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 calcite.

The influence of settling rates of the calcite mineral was studied with cells of *Bacillus polymyxa*. 5g of each sample were dispersed in 100ml of deionized double distilled water. The weight settled as a function of time and pH was monitored both in presence and absence of bacteria.

The adsorption of bacterial cells on calcite was estimated at different pH levels and concentration where, KNO_3 (0.001M) solution used as a base electrolyte. The number of cells after bacterial interaction in the supernatant was estimated using Petroff-Hausner counter.

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°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 calcium volumetrically by EDTA titration [21].

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

RESULTS AND DISCUSSION

The differential characteristics of *Bacillus 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 *Bacillus polymyxa* (Fig. 1), indicates, after an initial lag phase of about 2h, the cell number increases from about 6×10^7 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 [16].

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 surfaces. The bacterial cells were observed to adhere tenaciously onto the above mineral surface of calcite. There was a pronounced shift in isoelectric point (iep) to lower pH value of 3.0 over pH range of 2 to 12 in calcite (Fig. 3) and iep shifted to pH value of 8.9over a pH

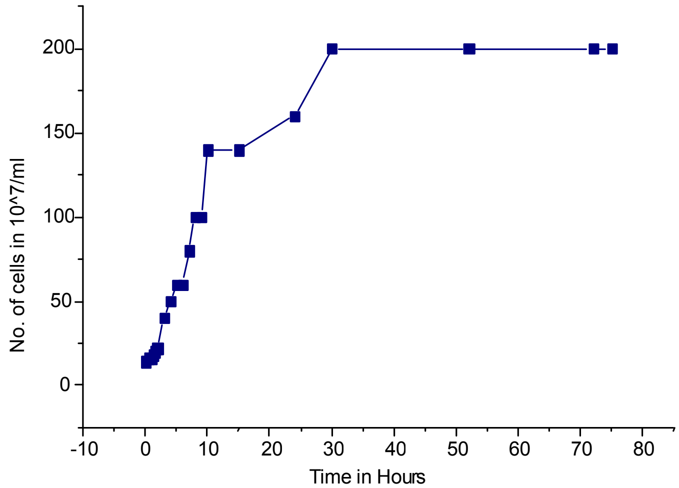

Fig. 1: Growth curve of *Bacillus polymyxa* in Bromfield medium

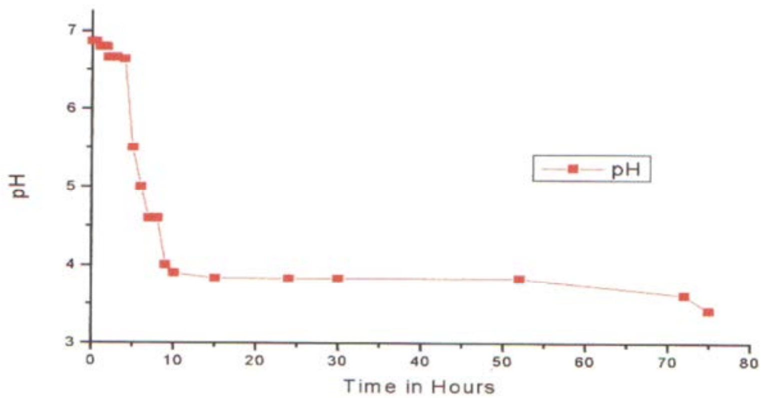

Fig. 2: Change of pH of growth curve of *Bacillus polymyxa*

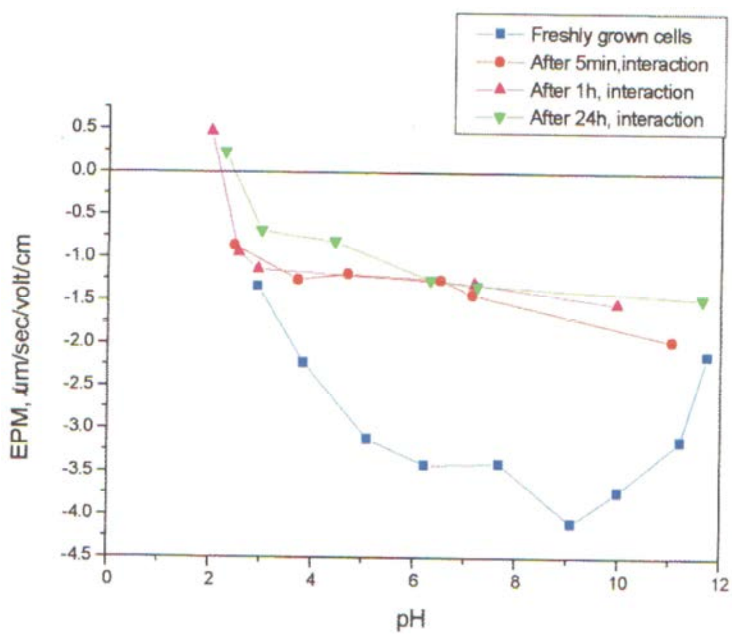

Fig. 3: EPM as function of pH for bacterial cells before and after interaction with calcite

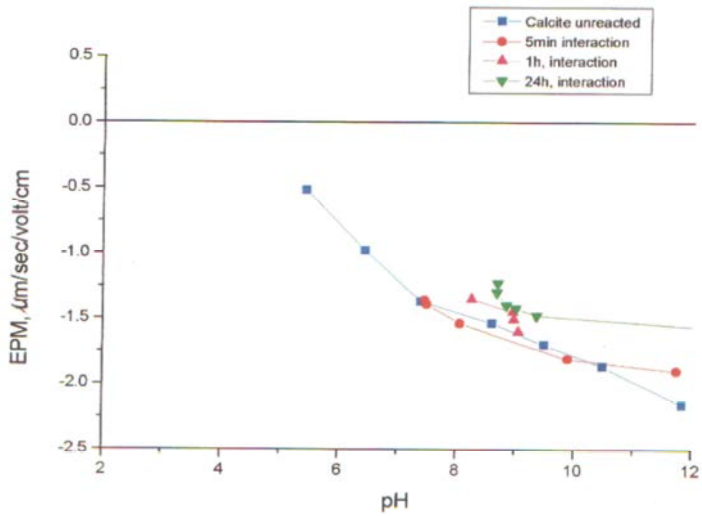

Fig. 4: EPM as function of pH for calcite before and after interaction with cells of *Bacillus polymyxa*

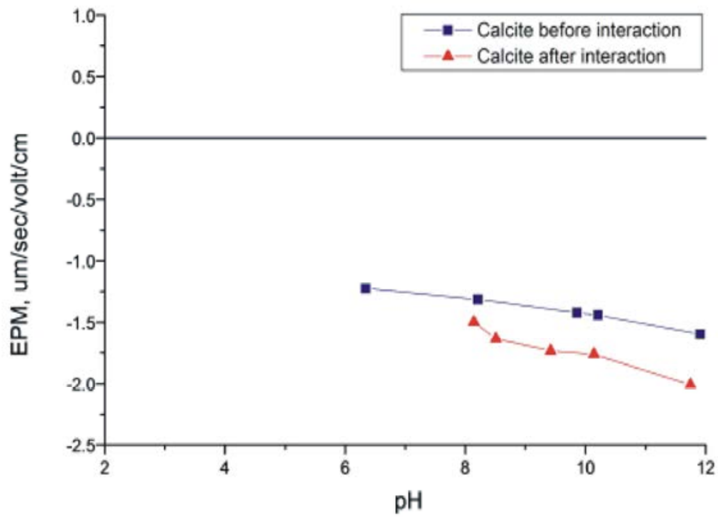

Fig. 5: Influence of bacterial metabolite on electro kinetic behaviour of calcite

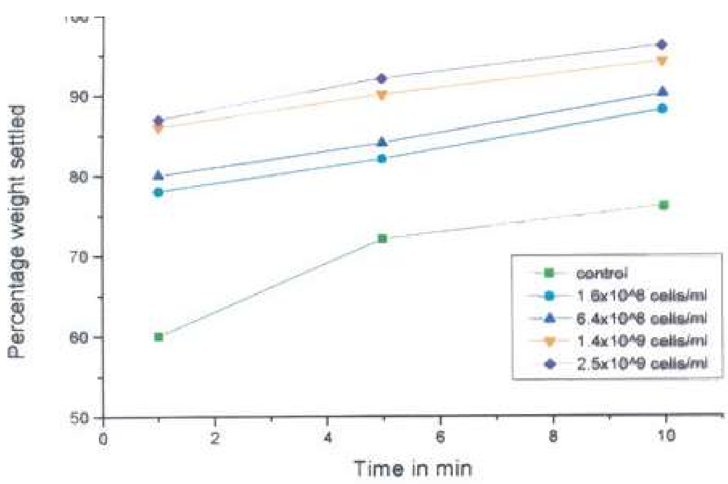

Fig. 6: Settling behavior of calcite in presence and absence of *Bacillus polymyxa*

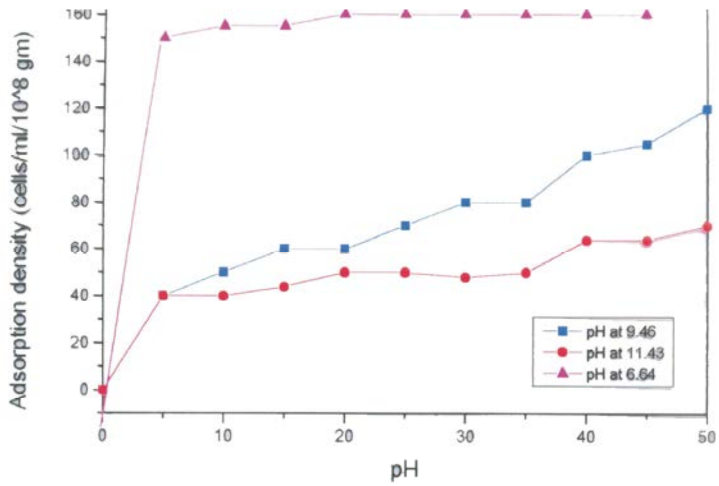

Fig. 7: Adsorption of cells on calcite as a functionof pH

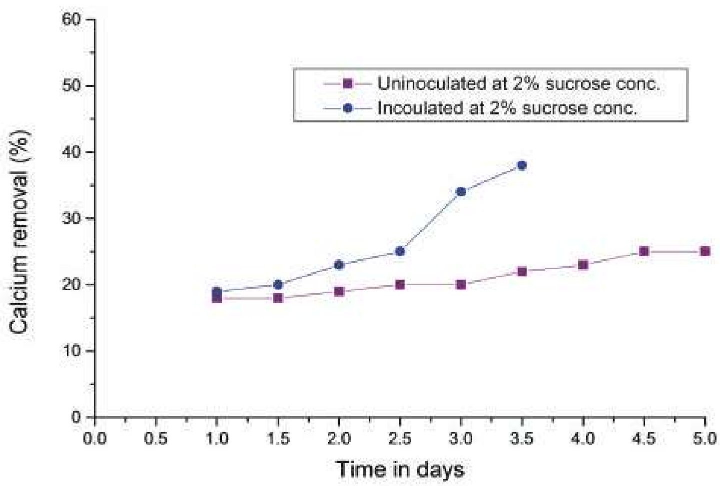

Fig. 8: Calcium removal by *Bacillus polymyxa* at 2% sucrose concentration

range of 2 to 12 (Fig. 4). There by considering the role of the organism in bringing surface modification of dissolved calcium from calcite [12].

The effect of bacterial metabolite with electro kinetic behavior on calcite was studied. There was a pronounced shift in iep of magnetite to lower pH values when compared with cells alone. The bacterial cell has higher affinity towards calcite, depicting the metabolic product is mainly responsible for surface chemical changes [19, 20] (Fig. 5).

The above tests were further confirmed by settling rate experiments on calcite, where the enzyme secreted by bacteria favored the dispersion of the bacteria (Fig. 6). Cell adsorption on to calcite with pH studied showed the bacterial cell have higher affinity towards calcite because adsorption increase with decrease in pH (Fig. 7). However, detailed experiments are warranted, varying the parameter such as cell density, time of interaction etc. The above partial experiments confirmed that *B. polymyxa* has greater affinity towards calcite and could be efficiently used to remove calcium from calcite. Hence, the above preliminary studies show *B. polymyxa* can be efficiently used for removal of impurities from the bauxite ore.

Experiments with respect to bauxite ore was initiated after confirming the above results. Calcium removal from the ore in presence of *B. polymyxa* under 2% sucrose concentrations, brought about 40% calcium removal in 4 days and under similar conditions the control (in absence of *B. polymyxa*), only 20% of calcium removal could be estimated. 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 [17] (Fig. 8).

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

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