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Full Length Article

Bioflocculation of (Iron oxide – Silica) system using *Bacillus cereus* bacteria isolated from Egyptian iron ore surface

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ABSTRACT

In mineral bio-beneficiation, it is very important to understand the microbial surface characteristics and its behaviour onto the mineral surface. *Bacillus cereus* bacterium has never been used before as a bio-reagent for separation of different mineral systems. In this work, complete characterization of such type of bacteria, isolated from Egyptian iron ore surface, including gram stain, growth curve, Biolog microbial identification, Zeta potential characterization, Fourier Transform Infrared Spectrometer, FTIR characterization as well as protein and polysaccharide analysis have been studied. The results confirmed that *Bacillus cereus* is a gram positive bacterium, rod shaped, smooth and circular with different types of by-products as polysaccharides, carboxylic acids and amino acids that gives an amphoteric behaviour on the cell surface. The results of zeta potential showed that the iso-electric points (IEP) of iron oxide (≈6.3) and silica (≈1.8) were significantly displaced to low values (≈2.2) and (≈1) respectively after treatment with the bacterial isolates. The results obtained showed a better affinity of *Bacillus cereus* to hematite mineral surface rather than silica surface and could be used in separation of such mineral from its associated gangue minerals. On applying ***B. cereus*** bacterial strain as a sole flocculating agent, to selectively separate hematite from its mixture with silica, succeeded in the removal of 80% of SiO_2 as a concentrate containing about 2% SiO_2 and 98% Fe_2O_3 with 82% flocculated by Wt. and a good recovery of 89.20%.

1. Introduction

The necessity to process and beneficiate ores with lower grades, which are more refractory and finely disseminated, has resulted in the conventional mineral processing and hydrometallurgical operations becoming inadequate and inefficient. Further, stringent environmental regulations have constrained the processing of ores. It thus becomes imperative to develop novel technologies for mineral processing and waste remediation. Recent developments in biotechnology hold promise to process such difficult-to-treat ores as well as to safeguard the environment. The growing importance of mineral bioprocessing can be gauged from the conferences exclusively dedicated to this topic [1–4]. An excellent overview of mineral bioprocessing has been compiled by Smith and Misra [5]. There are typical examples of the potential applications of microbes in mineral beneficiation [6,7], flotation collectors [8] and flotation modifiers [9,10]. These bacterial isolates act as bio-reagents and may induce hydrophobic properties once they can adhere selectively onto the mineral surface [11]. The micro-organism cell surface is conformed by functional groups like polymers, peptides, phospholipids, proteins and organic acids [12]. Those groups must adhere to the mineral surface directly and utilize cell surface associated or extracellular biopolymers to catalyse chemical reactions on the mineral surface [1,13] and [14]. Like traditional reagents, the bacterial isolate interacts with the mineral surface and gives amphoteric characteristics to it, [15]. Application of bio-reagents as collectors involves several fundamental aspects: surface charge, presence of specific hydrophobic groups and polymers compounds which deeply affect their adhesion to the mineral surface [13,16] and [17]. Bacillus cereus is frequently isolated from both the natural environment (soil and growing plants) and foods, including raw and pasteurized milk and milk products [18], dried products [19], pulses and cereals [20], spices [21], meat products, raw meat and meat product additives [22], fresh vegetables and ready-to-eat vegetable-based foods [23]. There is the first time in isolating *B. cereus* from mineral surface and studying its behaviour

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in mineral processing. In this paper, complete characterization of *B. cereus* bacterium isolated from iron oxide surface and its adsorption on the mineral surface has been studied.

2. Materials and methods

2.1. Materials

Sample of single minerals of hematite (Fe_2O_3) and silica (SiO_2) was delivered from 'Wards' Company, USA. The purity (99.9%) of the samples was confirmed using XRF. The –200 mesh fractions were used in adsorption studies. Analytical grade HCl and NaOH, from Aldrich, were used for pH regulations.

2.2. Methods

2.2.1. Bacterial growing and Isolation

A suspension containing 0.5 gm of mineral sample in 10 ml distilled water was prepared. After that, 1 ml of suspension was taken and sprayed onto a nutrient agar plate surface then incubated for 24–48 h at 30 °C. The developed colonies were picked up and streaked on nutrient agar plates and incubated at 30 °C for 24–48 h. The final step was repeated several times until pure colonies have been obtained. Separate colonies were picked up, streaked on nutrient agar slopes, stored at 4 °C and sub-cultured monthly [24].

2.2.2. Growth media

Two forms of nutrient media were used, solid form (nutrient agar) and liquid form (nutrient broth). Nutrient agar (NA) [25] including peptone, 5 gm; beef extract, 3 gm; sodium chloride, 8 gm; agar, 12 gm and distilled water, 1000 ml. The constituents were dissolved with heating, adjusted to pH 6.8–7.0 and sterilized at 120 °C for 20 min.

2.2.3. Graphical Expression of bacterial growth

Bacterial growth was measured through inoculating the preserved bacterium into 100 ml nutrient broth and incubated overnight in 250 ml measuring flask after that optical density was measured to determine the start point. Then 1 ml of the previous culture was taken in 100 ml nutrient broth followed by measuring OD550 along time intervals of 30 min. OD550 was measured using "Perkin-Elmer" Spectrophotometer" model Lambda 3B. Growth curve was obtained by plotting the logarithm of OD550 versus time, [1,24].

2.2.4. Preparation of inoculum

A liquate of 350 ml was dispensed into 1 litre flask, then sterilized at 120 °C for 20 min. and after that inoculated with a loop full of the bacterial strain under test and incubated at 30 °C for 48 h according to growth curve of three strains of bacteria under test.

2.2.5. The gram stain technique

A loopful of tap water was placed on a slide; using a sterile cool loop transfer a small sample of the colony to the drop, and emulsify. The film was allowed to be air dried. The dried film was fixed by passing it briefly through the Bunsen flame two or three times without exposing the dried film directly to the flame. The slide should not be so hot as to be uncomfortable to the touch. The slide with crystal violet solution was flooded for up to one minute and was washed off briefly with tap water (not over 5 s) and drained. After that, the slide was flooded with Gram's Iodine solution, and be allowed to act (as a mordant) for about one minute followed by washing off with tap water and drainage. Excess water was removed from slide and blot, so that alcohol used for decolourization is not diluted. The slide was flooded with 95% alcohol for 10 s and washed off with tap water. (Smears that are excessively thick may require longer decolourization). This is the most sensitive and variable step of the procedure, and requires experience to know just how much to decolorize). The slide after that was drained, flooded with safranin solution and be allowed to counterstained for 30 s. Finally, the slide was washed off with tap water, drained and blotted dry with bibulous paper and didn't be rubbed. All slides of bacteria must be examined under the oil immersion lens.

2.2.6. BIOLOG microbial identification system

Bacteria identification was done using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on BIOLOG recommended agar media and incubated at 30 °C. Inoculums were prepared where the cell density was in the range of 90–98%T. precisely 100 μl of the cell suspension was transferred by multichannel pipette into the wells of BIOLOG micro-plate. The plates were incubated for 36 h at 30 °C into the Omni-Log incubator/reader. The BIOLOG micro-plate tests the ability of an organism to utilize or oxidize a pre-selected panel of 95 different carbon sources. The dye tetrazolium violet is used to indicate utilization of substrates. A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for each organism in which the manufacturers literature terms a "Metabolic Fingerprint". Finally; micro plate was read using BIOLOG's Microbial Identification Systems software through biology reader

2.2.7. Zeta potential measurements

A laser Zeta Meter 'Malvern Instruments Model Zeta Sizer 2000' was used for zeta potential measurements. 0.05 g of ground sample was placed in 50 ml double distilled water with definite concentration of the bacterial isolate at fixed ionic strength of 10^{-2} M NaCl. NaOH and HCl were used as pH modifiers. The suspension was conditioned for 60 min during which the pH was adjusted. After shaking, the equilibrium pH was recorded. It was then allowed to settle for 3 min, after which 10 ml of the supernatant was transferred into a standard cuvette for zeta potential measurement. Solution temperature was maintained at (25 °C ± 2). Five measurements were taken and the average was reported as a measure for zeta potential [1,13].

2.2.8. Measuring selectivity of bacteria to mineral surface

A laser particle size analyzer (FRITSCH Model Analyst 22) was employed for measuring size analysis of single minerals before and after treatment with bacteria. Fixed volume 10 ml of *Bacillus cereus* was conditioned with one gram of each mineral for 60 min before recording the change in size distribution [1,13].

2.2.9. FTIR measurements

Infrared absorption spectra were recorded for haematite and bacterial isolate before and after interactions using Fourier transform infrared spectrometer (Model FT/IR 6300). After interaction with bacteria, the mineral samples were thoroughly washed using double distilled water and vacuum dried. The KBr pellet technique was used to record the spectra [1,13].

2.2.10. HPLC analysis

Samples were filtered through a 0.45 μm membrane (Smith et al., 1986). Analysis of the carbohydrate in the filtrate was performed using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector, LC-16ADVP binary pump, DCou-14A degasser and Shodex PL Hi-Plex Pb column (Sc 1011 No. H706081), Guard column Sc-Lc Shodex, and heater set at 80 °C. The mobile phase water, and the flow rate 1 ml/min. Standard solutions of individual sugars with analytical

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