Flocculation behaviour of hematite–kaolinite suspensions in presence of extracellular bacterial proteins and polysaccharides

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Cells of *Bacillus subtilis* exhibited higher affinity towards hematite than to kaolinite. Bacterial cells were grown and adapted in the presence of hematite and kaolinite. Higher amounts of mineral-specific proteinaceous compounds were secreted in the presence of kaolinite while hematite-grown cells produced higher amounts of exopolysaccharides. Extracellular proteins (EP) exhibited higher adsorption density on kaolinite which was rendered more hydrophobic. Hematite surfaces were rendered more hydrophilic due to increased adsorption of extracellular polysaccharides (ECP). Significant surface chemical changes were produced due to interaction between minerals and extracellular proteins and polysaccharides. Iron oxides such as hematite could be effectively removed from kaolinite clays using selective bioflocculation of hematite after interaction with EP and ECP extracted from mineral-grown cells.

1. Introduction

The interaction of clays and iron oxide minerals with biopolymers such as proteins and polysaccharides is of interest to soil chemists and geologists to understand their biostability, mineral cycling, weathering and aggregate formation. Many types of soil microorganisms inhabiting clay and iron oxide mineralized soils and rocks adhere to mineral substrates and form biofilms resulting in secretion of several biopolymers constituting of proteins and polysaccharides, influencing their mineralogy and weathering [1]. Iron cycle in relation to clay mineralogy has been understood with respect to participation of different aerobic and anaerobic microorganisms [2]. Kaolinite formation is reported to be a consequence of bioinduced processes [3]. Iron oxide inclusion in kaolinite matrices is also bioinduced and hence a biological process may be appropriate to remove iron oxides from kaolin clays. Presence of iron in kaolin imparts a brownish colour which limits its industrial applications in ceramic, porcelain, cosmetics and paint manufacturing. Iron removal from clays is thus an important initial step for the production of commercially suitable kaolin containing kaolinite. Physicochemical methods including washing, acid dissolution, froth flotation and electrostatic separation commonly used to bring about iron removal from clays or clay removal from iron ores are often inefficient and not environment-friendly. An alternative approach of utilizing microorganisms as surface modifiers to bring about mineral beneficiation has great practical relevance due to its cost effectiveness and environmental acceptability [4].

Under the circumstances, an understanding of consequences interaction of bacterial cells and their extracellular polymeric metabolic products with clay minerals and associated iron oxide will be of great practical significance. Microbially-induced flocculation or dispersion of clay minerals such as kaolinite and iron oxide such as hematite would be useful in their mutual separation and also to achieve supernatant clarity from their suspensions. Since, flocculation–dispersion of mineral fines in an aqueous medium is an interfacial process, it is essential to understand the adsorption and electrokinetic behaviour of bacterial cells and the extracellular polymeric substances in the presence of the above minerals.

Use of *Bacillus spp.*, and fungi such as *Aspergillus niger* to remove iron from low grade kaolin have been reported earlier [5–8]. Iron reducing-microorganisms viz., *Bacillus cereus, Bacillus sphaericus, Bacillus pumilus, Mycoides* and *Pseudomonas mendocina* have also been used for the microbial refinement of kaolins [9,10]. However, all such microbial methods so far reported for iron removal from clays are based on reductive dissolution of iron oxides in organic and other acids produced through bacterial or fungal metabolism. The present work assumes great practical significance since it discusses a novel iron oxide-kaolinite separation process based on selective flocculation-dispersion induced by bacterial extracellular proteins and polysaccharides.

Bacillus subtilis, a Gram-positive soil microbe was chosen in this work to establish the adsorption behaviour of extracellular

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polymeric substances such as proteins and polysaccharides on a typical clay mineral, such as kaolinite and iron oxide, such as hematite. Use of *Bacillus subtilis* in the beneficiation of hematite to remove silica, alumina and calcite has already been reported in Ref. [11]. Bacterial interaction with clays and iron oxides was studied to understand differential bacterial surface affinities towards these minerals which occur closely associated together in nature. Such a study would facilitate development of a bioprocess to beneficiate both iron ores and clay minerals. Biologically-induced selective flocculation of hematite from its mixture with kaolinite was established in this work and its applications in the beneficiation of kaolin clays with respect to iron removal is emphasized. The results of this work are also significant with respect to removal of kaolin from hematitic iron ores.

2. Materials and methods

2.1. Minerals

Hand-picked pure mineral samples of hematite and kaolinite were obtained from Alminrock Indscer Fabriks and Indian Bureau of Mines, Bangalore, India, respectively. The minerals were subjected to dry grinding in a porcelain ball mill and sieved to obtain different size fractions. Fractions less than 10 μm sizes were obtained through sedimentation. Particle size was determined by Malvern Zetasizer (Nano ZS90) and an average particle size of ~8–10 μm was used for adsorption, flocculation and zeta potential studies. The purity of minerals determined through X-ray diffraction and mineralogical analysis was 99.8% hematite and 98.6% kaolinite.

2.2. Bacterial growth conditions

A strain of *Bacillus subtilis* (NCIM 2655) obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India, was used. Bacterial cells were subcultured in Luria Bertani (LB) medium by inoculating 10 ml of pure strain in 90 ml of LB medium in 250 ml Erlenmeyer flasks and incubated in an Orbitek rotary shaker at 30 °C and 200 rpm [11]. The cell population was estimated using a Petroff-Hausser counting chamber under a Leitz phase contrast microscope (LABORLUX K Wild MPS 12).

Bacillus subtilis cells were also grown in the presence of hematite and kaolinite at a pulp density of 10% and adaptation to minerals was considered achieved when the growth rate of adapted strain was identical to that observed in the absence of minerals. Mineral-grown adapted cells were preserved and periodically subcultured in the presence of respective minerals. The mineral samples were also sterilized before interacting with bacterial cultures in order to ensure prevention of contamination from exogenous microorganisms.

2.3. Extraction of extracellular protein (EP) and polysaccharide (ECP)

A volume of 1 l solution-grown or minerals-grown bacterial culture was taken and centrifuged at a speed of 8000 rpm for 5 min. The supernatant was filtered through 0.2 μm sterile membrane filter paper to obtain cell free extract (CFE). Analytically pure ammonium sulphate was slowly added to a saturation level of 65% with constant stirring at 4 °C. The precipitated protein was dissolved in a minimal volume of 0.1 M Tris–HCl buffer of pH 7, dialyzed and kept overnight at 4 °C [12].

For ECP extraction, 1 l of two days solution-grown or minerals-grown bacterial culture was centrifuged and the supernatant filtered through 0.2 μm sterile membrane using Millipore vacuum suction pump. The cell free extract was lyophilized in Modulyod 230 freeze dryer at a temperature of −50 °C and a vacuum of 100 millitorr. The solids obtained were dissolved in 10 ml of double distilled water and allowed to cool down to 10 °C, followed by addition of 20 ml of ice cold ethanol to selectively precipitate all the polysaccharide. The precipitate was centrifuged and redissolved in double distilled water followed by ethanol precipitation which was repeated thrice. The precipitated polysaccharide was then dissolved in a minimal volume of double distilled water, dialysed and kept overnight at 4 °C in a refrigerator [13].

2.4. Isolation and characterization of EP by SDS-PAGE

Proteins can be characterized in terms of the molecular size of the constituent polypeptides by SDS-PAGE. Electrophoresis was carried out in vertical position which provides longer separations and the supporting medium was impregnated with buffer solution. The buffer compartments were separated physically to avoid contamination with the products of the electrolysis formed in the electrode compartment. 12% SDS-PAGE gel was used for the purpose and procedures involved are detailed elsewhere [11,12].

2.5. Adsorption studies

For adsorption tests, one gram each of individual mineral sample was suspended in 100 ml of 1 mM KNO$_3$ solution at the desired pH in the presence of known concentration of bacterial extracellular proteins or polysaccharides in 250 ml Erlenmeyer flasks. The suspension was agitated for 15 min on an orbital shaking incubator at 30 °C and 250 rpm and equilibrated at different pH levels. The suspension was then centrifuged at 2000 rpm for 5 min to separate the mineral particles with adsorbed proteins and polysaccharides. The supernatant was further filtered through Whatman 42 filter paper and analyzed for residual protein and polysaccharide [14,15].

2.6. Zeta potential studies

The effect of bacterial interaction on the surface charge of the minerals was studied by Zeta-potential measurements using Malvern Zetasizer (Nano ZS90). For zeta potential studies, 1 g of desired mineral sample was taken in 10^{-3} M KNO$_3$ and interacted with known amount of EP and ECP at desired pH and period at room temperature. After interaction, mineral particles were separated by filtration and then washed twice or thrice to remove the loosely adsorbed reagents.

Zeta potentials of bacterial cells grown in the presence and absence of minerals were also similarly determined as a function of pH.

2.7. Flocculation tests

For flocculation tests, 1 g of the desired mineral sample was taken in 100 ml of known bacterial EP and ECP concentration in a measuring cylinder. The cylinder was tumbled 10 times and kept still for 5 min. The supernatant as well as settled products were carefully decanted, filtered and weighed.

Selective flocculation tests using 1:1 mineral mixtures were also similarly carried out.

2.8. SEM micrographic analysis

SEM studies were carried out using a FEI Sirion, high resolution electron microscope as per procedure described elsewhere [16].