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Bio-beneficiation of kaolin and feldspar and its effect on fired characteristics of triaxial porcelain

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Abstract. Presence of iron compounds as impurities in kaolin and feldspar, impart reddish colour to ceramic products manufactured using these minerals. The quality of kaolin and feldspar was enriched mainly through iron removal by biological methods. Bacteria isolated from kaolin of Indian origin were used for bioleaching. Biotreatment of kaolin and feldspar using indigenous bacteria not only lowered the iron content of the minerals but also improved their whiteness. The porcelain prepared with these biobeneficiated minerals was compared to that prepared with non-beneficiated one. Physico-mechanical properties of porcelain were distinctly improved by using biobeneficiated kaolin and feldspar, without affecting the individual mineralogical compositions of kaolin and feldspar.

Keywords. Triaxial porcelain; kaolin; feldspar.

1. Introduction

Last few decades have shown a remarkable development in vitrified porcelain tiles for application in building industry. Production of good quality coloured ceramic tiles demands for white-based porcelain to obtain maximum colour effect with minimum use of costly stains. A triaxial porcelain composition consists of 50% kaolin and 25% each of quartz and feldspar (Norton 1970). Purity of these raw materials plays a pivotal role in the ultimate colour and quality of the product. Iron oxides, which are often present as impurities in these raw materials, lead to coloration of the fired body (Kingery 1976; Bozdogan 1999; Kumar *et al* 2001; Styriaková *et al* 2003). The biological methods of iron removal have yielded encouraging results for various minerals (Drever and Stillings 1997) mending their characteristics in a way that the beneficiated minerals provided improved end products (Ambikadevi and Lalithambika 2000; Goudev 2001).

Microorganisms need iron as an essential element for growth and survival. They use iron as a component of cytochromes, iron–sulphur proteins and many enzymes (Arnold *et al* 1998; Bennett *et al* 2001). Some metal respiring anaerobes like *Geobacter sulfurreducens* AM-1 and *Shewanella putrefaciens* MR-1 derive energy from Fe(III)–Fe(II) redox couple and use Fe(III) as the terminal electron acceptor (Lovley and Phillips 1986, 1988; Gorby and Lovley 1991; Magnuson *et al* 2000). Although iron is abundant in nature, it remains mostly in the insoluble state as ferric oxides, hydroxides and other complexes. Under oxidizing conditions and at pH 7, Fe(III) is the stable oxidation state and sparingly soluble in water. But under reducing conditions at the same pH, Fe(II) is the stable oxidation state, which is highly soluble in water and available readily for biological consumption. Bioavailability of insoluble Fe(III) is increased by the presence of chemical chelators like nitrilotriacetic acid (NTA) (Arnold *et al* 1998; Lovley and Woodward 1996; Luu *et al* 2003) and biological Fe(III)-chelators like siderophores, citric acid, oxalic acid, ascorbates, phenols and extracellular polysaccharides (Arnold *et al* 1998; Barker and Banfield 1998).

Biobeneficiation is the method of removing undesirable elements like iron present as impurities in minerals by microbial action via oxidative or reductive dissolution of insoluble complexes of the same (Colmer *et al* 1980; Christinia 1992). This occurs either by direct (requires physical contact of viable microbial cells with the material to be leached) or indirect (leaching occurs due to chemicals produced by the microbes) mechanisms. While some bacteria can use Fe(III) as the terminal electron acceptor converting it directly to highly soluble Fe(II), others solubilize Fe(III) indirectly by secreting chemical agents like oxalic acid to retrieve this element from nature required for their growth (Lovley and Phillips 1986; Lovley and Woodward 1996). Thus, exploitation of the microorganisms towards developing bioleaching and

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related processes like biobeneficiation is nothing but harnessing of a natural phenomenon for commercial purposes.

The aim of this study was to enrich the quality of kaolin and feldspar mainly through iron removal by biological methods. Bacteria isolated from kaolin of Indian origin were used for bioleaching. The beneficiated minerals obtained through biological treatment were incorporated in triaxial porcelain compositions. Physico-mechanical properties of the fired specimens including whiteness were evaluated and the results discussed.

2. Materials and methods

2.1 *Minerals and chemicals*

Feldspar was obtained from mines around Hyderabad, Andhra Pradesh, India. Colour of the mineral was reddish-white indicating the presence of iron oxides and hydroxides. Kaolin clay from Rajmahal, Bihar, India and quartz from Jharkhand, India were used to prepare triaxial porcelain composition. Chemical analysis of kaolin, feldspar and quartz (table 1) revealed that they were of the grade usually used to manufacture vitrified ceramic tile.

Chemicals of analytical grade were procured from SRL, Qualigens, Merck-India and Hi-Media. NTA and chemicals for molecular biology were obtained from Sigma and Bangalore Genei.

2.2 *Removal of magnetic iron*

Magnetic iron in feldspar was removed using a 1″ magnetic flea (Tarsons) sealed in a piece of parafilm (Kukier *et al* 2003). Feldspar (5 g) was taken in a 250 ml beaker and 50 ml of deionized water was added. The sealed magnetic flea was immersed in the beaker and the whole set was stirred on a magnetic base for 1 min. The beaker was then removed from the magnetic base and the magnetic flea was taken out. Magnetic iron particles attached to the parafilm was washed with deionized water in a beaker. The process was repeated to remove all magnetic iron. Collected particles were dried and weighed.

Table 1. Chemical analysis of raw material.

Oxides (mass%)	Kaolin	Feldspar	Quartz
SiO_2	45·13	66·78	98·65
Al_2O_3	34·71	18·22	0·39
Fe_2O_3	1·86	0·37	0·07
TiO_2	0·86	0·1	0·01
CaO	1·01	0·92	0·1
MgO	0·74	0·19	0·02
K_2O	0·45	10·87	0·11
Na_2O	0·88	1·62	0·08
LOI	14·72	0·57	0·36

2.3 *Bacterial leaching*

2.3a *Isolation and cultivation of bacteria*: Bacteria were isolated from kaolin (Mukdumnagar, Birbhum district, West Bengal). Kaolin was agitated with sterile 0·4% NaCl solution for 30 min on a rotary shaker at 30°C, and allowed to settle. The supernatant thus obtained was serially diluted with sterile water and spread on nutrient agar (I.P.) plates, which were incubated at 30°C. Representatives of the various types of bacterial colonies developed were selected and purified.

Bacterial strains were cultured and maintained in a medium having the following composition (g/l): glucose, 1·8; $(NH_4)_2SO_4$, 0·214; KCl, 0·007; $MgSO_4{\cdot}7H_2O$, 0·05; Na_2SO_4, 0·4; and yeast extract, 0·16; pH of the medium was adjusted to 6·5–6·7. Cultures were grown on a rotary shaker (100 rpm) for 14–16 h at 30°C. Bacterial cells were counted in a Petroff–Hausser counting chamber under microscope after appropriate dilutions.

2.3b *Bioleaching of feldspar and kaolin*: Preliminary experiments to screen the iron leaching capacity of all the isolate types were carried out using late log phase culture (~ 50 ml) of the isolates in 50 ml Schott Duran glass bottles containing 2·5 g methanol-sterilized minerals in presence of 2 mM NTA; in scale-up experiments, 12·5 g sterilized minerals and ~ 250 ml culture were taken in 250 ml bottles. All the bottles were filled up to the capacity to ensure little air entrapment required for microaerobic condition during leaching. Abiotic controls were maintained in each case. Contents of the bottles were mixed well and kept under stationary condition at 30°C. The bottles were inverted every alternate day for 1 min to mix the contents and left to settle for 30 min before collecting the supernatant for iron estimation.

2.3c *Identification of bacteria*: a. Biochemical characterization was done by API 20E (Bio Mírieux San, France) identification system for Enterobacteriaceae family and b. molecular biological characterization was carried out.

Isolation of genomic DNA: Genomic DNA was extracted by the method of Stefan (1999) and purified by phenol chloroform. The purified DNA was spooled with a clean glass rod and dissolved in TE buffer. The DNA solution was treated with RNase and extracted again with phenol chloroform.

The genomic DNA was dissolved and diluted with 0·1% SSC to an absorbance of 0·4–0·5 at 260 nm. Denaturation of DNA was carried out and monitored in a temperature controlled GBC Cintra 10e spectrophotometer (Australia) in the temperature range 40–75°C with a rise in temperature of 1°C/min. The G + C mol% was calculated by the following equation as described by Schildkraut and Lifson (1965)

$$G + C \text{ mol\%} = 2{\cdot}44(T_m - 81{\cdot}5 - 16{\cdot}6\log M),$$

where M is the molarity of sodium ion in the buffer. For 0·1% SSC, the value of 16·6log$M$ is –28·3584.

To obtain the 16S rRNA gene, the genomic DNA isolated from bacterial strains was amplified by using universal 16S rRNA gene primer in a Gradient PCR (MJ Research). Amplified product was electrophoresced on 1% agarose gel to separate the ~1·5 kb gene and subsequently purified from the gel by electro-elution following standard procedure. The purified DNA was then sequenced with an automated ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using ABI dye terminator sequencing reagents. The reaction was conducted in 10 μl containing 0·3 U Taq polymerase (Banglore Genei), 2 pmoles of each primer (forward primer: AGT TTG ATC CTG GCT TCA, reverse primer: ACG GCT ACC TTG TTA CGA CTT) and PCR buffer containing 0·2 mM dNTPs.

2.4 *Preparation of triaxial porcelain (TP) specimens*

Composition (mass %) of TP was: Kaolin 50, feldspar 25 and quartz 25. TP-R stands for triaxial porcelain containing raw (untreated) kaolin and feldspar. The bio-treated kaolin and feldspar were used for the TP-B composition. Quartz remained the same in both the compositions. Specimens were prepared following standard processes of mixing, compaction, drying and firing. Bars (average size: 6 × 0·8 × 0·9 cm) were prepared utilizing hydraulically operated pressing machine at 35 MPa pressure. Firing was conducted in an electrically heated furnace with on/off control system in the temperature range of 1250 and 1300°C. Physico-mechanical properties of the porcelain specimen, such as bulk density, water absorption, apparent porosity, mechanical strength and shrinkage after firing were measured following standard methods (Bhattacharya *et al* 2005). The colour measurements of the fired porcelain bodies were done using Hunterlab colorimeter and results are reported as $L^*a^*b^*$ values.

2.5 *Scanning electron microscopy (SEM)*

Morphological changes of the kaolin and feldspar grains before and after bioleaching were investigated by field emission scanning electron microscope (ZEISS SUPRA 35VP) with ultra-high performance variable pressure FESEM. The powder samples were dispersed in water by sonication. A drop of dispersion on glass slide was dried to obtain a thin film of the sample. The dried films were coated with gold by sputtering (Edwards, Scancoat) to make the surface conducting for viewing through SEM.

2.6 *Iron estimation*

Iron content in solutions was measured using ferrozine by a modified method originally described by May and Fish (2002). The reagents used are as described below:

Reagent A: The 'iron-releasing' reagent A was prepared in a hood immediately before use by mixing equal volumes of 1·2 M HCl and 0·285 M (4·5%, w/v) potassium permanganate solution.

Reagent B: 100 ml of reagent B (reducing iron-chelating reagent) contained 38·8 g ammonium acetate, 35·2 g ascorbic acid, 320 mg ferrozine and 320 mg neocuproine, which were dissolved sequentially; respective final concentration of the chemicals was 5 M, 2 M, 6·5 mM and 13·1 mM.

In a 2 ml centrifuge tube, a small volume (≤ 0·5 ml) of properly diluted sample solution containing < 3 μg iron was taken and it was diluted to 1 ml with water and 0·5 ml 0·02 (N) HCl. Freshly prepared reagent A (0·5 ml) was then added and the mixture was digested for 2 h at 60°C. After cooling to room temperature, 0·1 ml reagent B was added; the reaction was allowed for 30 min at room temperature to produce a magenta coloured complex which was measured against water within 20 h in a Shimadzu Pharmaspec UV-1700 spectrophotometer at 562 nm. In the blank set, 1 ml of 0·01 N HCl was taken instead of sample. Amount of iron in the samples was estimated from a standard curve drawn freshly after making necessary corrections in experimental values.

3. Results and discussion

3.1 *Magnetic separation*

Beneficiation of feldspar through iron removal was initially carried out by magnetic separation. About 0·05% of the feldspar was removed as magnetic iron. With the same method, no magnetic iron could be separated from kaolin. The minerals were washed, dried and were then treated chemically or biologically for further beneficiation.

3.2 *Microbiological experiments*

3.2a *Screening of microbial isolates*: Among the bacterial colonies developed on nutrient agar, 19 different types were found and selected. Bioleaching experiments were conducted with all the isolates and only two of them exhibited some leaching activity from both the minerals. The strains were designated as KA-10 and KA-25.

3.2b *Bacterial leaching*: NTA plays a vital role in bioleaching of iron (Lovley and Woodward 1996). Leaching experiments without NTA showed nil to very little (< 0·01%) iron dissolution from feldspar. Very slow dissolution of iron was observed in case of kaolin (8% in 30 days, data not shown). We observed linear leaching rate up to 2% NTA, and used this concentration. It was observed that the mixed culture or co-culture of the bacteria

leached more iron from the minerals than individual strains (figures 1 and 2), therefore, in subsequent experiments the bacterial co-culture was used for iron leaching. A maximum of 14% and ~30% of non-magnetic iron was leached from the feldspar and kaolin, respectively in 30 days in presence of 2 mM NTA and no significant leaching was detected thereafter. NTA alone could not leach any iron from the minerals in absence of bacteria. After biological treatment, the minerals were washed thoroughly with deionized water and dried in an air-oven at 60°C. The feldspar and kaolin thus obtained were much whiter in colour and used to study the firing characteristics of the triaxial porcelain bodies made from them.

Microbiological and biochemical characteristics of bacterial isolates: Both the isolates are mesophilic, flagellated rods. Isolate KA-10 was gram variable spore forming bacillus, whereas isolate KA-25 was gram-negative small rod. The strains could utilize citrate, lactate and glucose as carbon and energy source. They showed nitrate reductase activity and could liquefy gelatin. KA-10 is both oxidase and catalase positive, whereas, KA-25 is oxidase negative and weakly catalase positive.

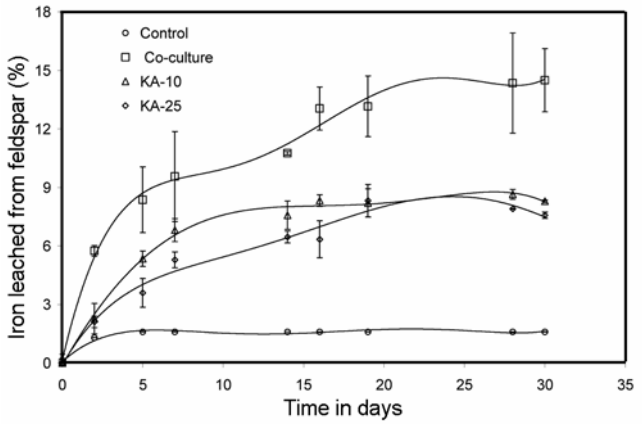

Figure 1. Bioleaching of feldspar using bacterial isolates and their co-culture.

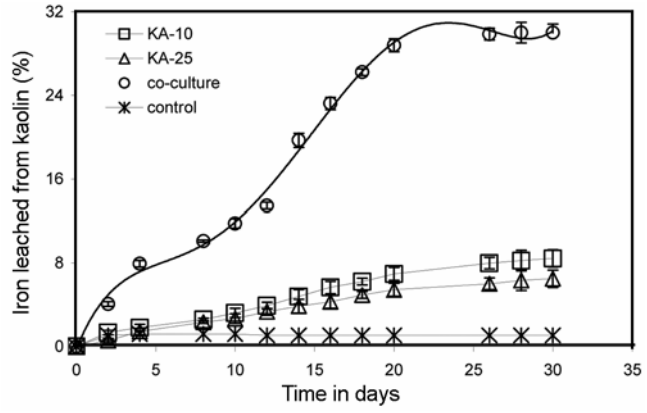

Figure 2. Bioleaching of kaolin using bacterial isolates and their co-culture.

3.2c *Molecular biological identification of bacterial strains*: DNA melting temperature and $G + C$ content: The DNA melting temperatures or denaturation temperature (T_m) was obtained as 66·8 and 69·8°C for KA-10 and KA-25, respectively. The $G + C$ content was calculated as 41 and 46% for KA-10 and KA-25, respectively.

16S rDNA sequencing of isolate KA-10 16S rRNA gene showed the organism is probably a strain of *Bacillus cereus*. The sequence is designated GenBank accession number DQ855661.

Isolate KA-25 is an unknown species as predicted by the 16S rRNA gene sequence. The sequence is designated GenBank accession number DQ855662.

3.3 *Changes in fired properties of triaxial porcelain*

Since kaolin and feldspar are major industrial raw materials for the production of triaxial porcelain, the effect of bioleaching on these raw materials on the final product 'porcelain' was studied with respect to few important physical properties. Table 2 shows the various fired properties of the experimental specimens at different temperatures.

Densification phenomenon always led to shrinkage of the ceramic body during heating and firing shrinkage (FS) is a measure of densification. It can be seen that both PR-R and PR-B have the same FS value (8·92%) at 1300°C. At 1250°C, however, PR-B shows higher FS, that indicates higher densification compared to PR-R. Elimination of pores occurs during sintering of porcelain body and densification can be looked upon as a process on removal of pore phase. Open pores are available for permeation of a fluid from outside of the body which is measured by water absorption (WA) value. WA showed a decrease from 0·76%–0·34% for biobeneficiated mineral containing porcelain at 1300°C. This indicates that biobeneficiation helped in attainment of higher vitrification in PR-B samples. Higher vitrification in PR-B also resulted in increase of flexural strength, from 38·1 MPa for PR-R to 50·2 MPa at 1300°C (table 2).

Table 2. Fired properties of porcelain.

	P–R		P–B	
Fired characteristics	1250°C	1300°C	1250°C	1300°C
FS (%)	8·51	8·92	8·76	8·92
WA (%)	1·89	0·76	1·88	0·34
MOR (MPa)	33·2	38·1	37·1	50·2
Y (GPa)	5·8	7·0	6·8	8·4
Green strength (MOR, MPa)	1·4 At room temperature		1·58 At room temperature	

An increase in the Young's modulus and green strength of the porcelain body was also found using biobeneficiated minerals. Young's modulus increased to 1 GPa at 1250°C and 1300°C, respectively and the increase in green strength was 0·18 MPa (table 2).

Due to removal of iron, which imparts colours to the fired body, whiteness of the fired samples containing biobeneficiated feldspar and kaolin (PR-B) increased, and the 'a^*' value indicating redness decreased (table 3). At 1300°C, L^* values of PR-R and PR-B are 68 and 82. This increase in 'lightness' is very significant since porcelain tile manufacturers usually incorporate expensive zircon opacifier to increase L^* value. Thus the increase in whiteness of porcelain made from biobeneficiated minerals is of significant commercial interest.

Table 3. Colour indices of the triaxial porcelain bodies.

Sample	Temperature	L	a	b
P–R	1250°C	69	2	13
	1300°C	68	1	8
P–B	1250°C	75	2	19
	1300°C	82	0	8

3.4 *Scanning electron microscopy*

Figure 3a shows the untreated feldspar particles, the individual grains are sharp edged and no etching is observed on the surface. Figure 3b shows biologically treated mineral, in which notable proportion of smaller, irregular and extensively etched particles are observed. Similarly, in case of kaolin, the usual hexagonal shape of the particles (figure 4a) was lost and irregular shaped grains were observed after bioleaching (figure 4b). The difference in mineral morphology can be explained in terms of dissimilatory reduction of Fe(III) to Fe(II) by microorganisms. This reduction of Fe(III) to Fe(II) by microorganisms occurs widely in nature whereby insoluble ferric oxides/hydroxides are solubilized. We consider dissimilatory

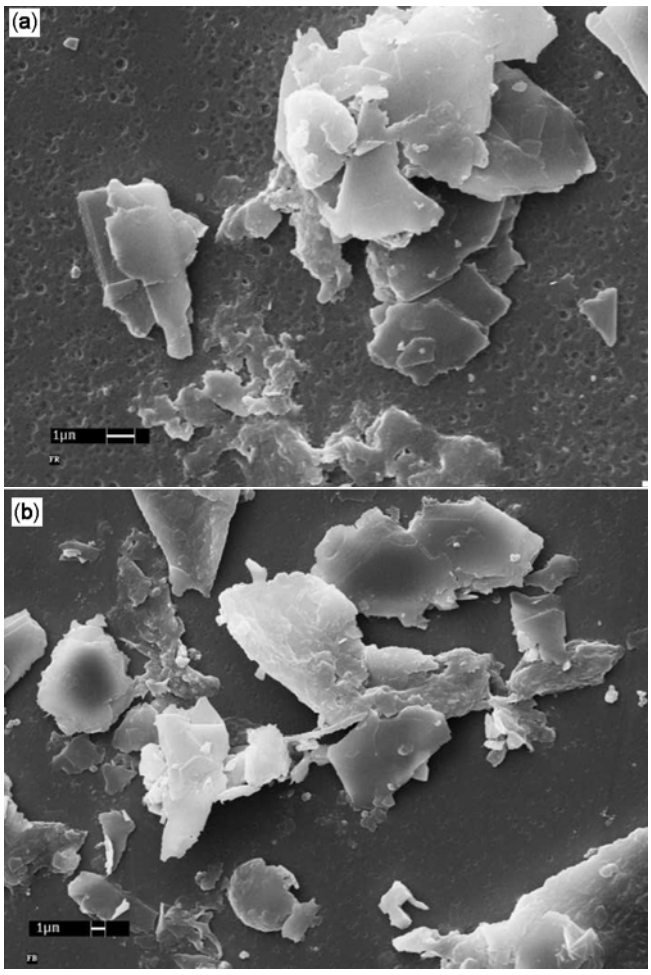

Figure 3. SEM of untreated (**a**) and biologically treated (**b**) feldspar.

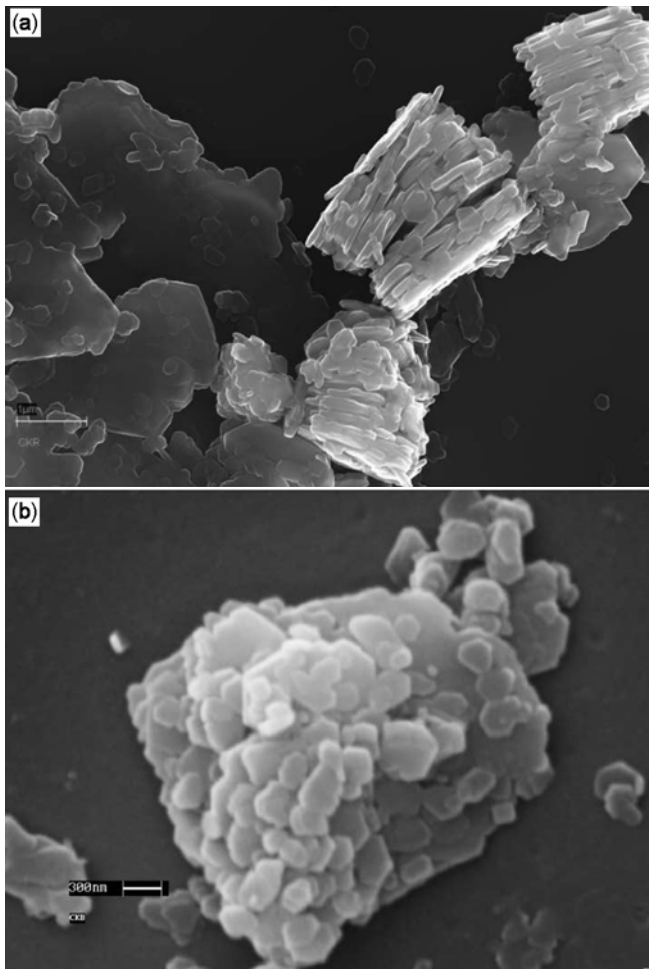

Figure 4. SEM of untreated (**a**) and biologically treated (**b**) kaolin.

Fe(III) reduction as the major pathway of Fe(III) dissolution from feldspar because of the fact that culture filtrates of the individual and mixed bacterial cultures could not leach iron from the minerals under identical experimental conditions nullifying participation of any excreted chemicals in the Fe(III) dissolution process. Moreover, NTA by itself could not leach recognizable amount of iron from the minerals, it tremendously augment iron leaching in presence of the bacterial culture indicating its role in the reductive dissolution of Fe(III) oxides (Lovley and Phillips 1988; Arnold *et al* 1998). Further support in favour of the dissimilatory Fe(III) reduction in these cases of iron leaching was the observation that nitrate inhibited the process (Christinia 1992). Since, both the bacterial strains can utilize nitrate, the nitrite produced from the same inhibited the Fe(III) reduction as suggested (Obuekwe *et al* 1981). Another probability of nitrate inhibition may be the participation of nitrate reductase that can also reduce Fe(III) under microaerobic conditions in absence of nitrate in some bacteria (Ottow 1970). When nitrate is not available, nitrate reductase uses Fe(III) as the terminal electron acceptor converting it to Fe(II).

The chemical environment produced by the bacteria in vicinity of the minerals have varying gradients which are highly effective in localized etching of the minerals (Weisberg *et al* 1991; Yu *et al* 2001). As iron and other macro nutrients are solubilized from the surface and interstitial spaces of the mineral, the grains are broken down into smaller irregular sized, etched particles.

The role of individual bacterial strain in the iron leaching process remains unanswered. In all probability, the co-culture of the bacterial strains acted by producing metabolites which not only allowed better enzyme function but also helped breakage of the mineral particles.

4. Conclusions

Two different types of bacteria isolated from kaolin were used for the leaching process that together leached significant amount of iron from both kaolin and feldspar in 30 days. They failed to leach any iron from the minerals under aerobic conditions or in presence of nitrate, which suggest that the process for iron reduction was dissimilatory in nature. Biotreatment of kaolin and feldspar using indigenous bacteria not only lowered the iron content of the minerals and improved their whiteness, the porcelain prepared with these biobeneficiated minerals was of better quality. Physico-mechanical properties of porcelain were improved by biobeneficiation, without affecting the individual mineralogical compositions of kaolin and feldspar.

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