IRON REMOVAL FROM KAOLINS BY BACTERIAL LEACHING

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Microorganisms may play an important role in the dissolution of silicate structure in the rock weathering process and in the genesis of clay minerals. Bacteria of Bacillus species are considered to be relatively active in this process. The samples from Horná Prievrana kaolin pit are characteristic by a high number of bacteria, especially of Bacillus species. The dissolution activity of two selected Bacillus cereus strains from these samples was investigated on three kaolin samples contaminated with iron oxyhydroxides and mica. The experimental results showed that these bacteria are able to remove 43 % of free Fe occurring in kaolin in amorphous form of oxyhydroxides (KS1 sample) and approximately 15 % of Fe bound in mica (KS2 sample) after 1 month of bioleaching. The amorphous form of Fe is extractable by bacterial leaching easier than Fe bound in mica. The prolonged bioleaching time showed the removal of 53 % bound Fe from kaolin (KS3 sample), which was taken from Vyšný Petrovec. The biodegradation of iron oxyhydroxides and a partial destruction of mica structure were confirmed also by X-ray analysis. Bacteria subsequently destroyed some of mica surfaces, observed by SEM before bacterial leaching, when Fe ions were released from mica structure what resulting in illite development. The enrichment by fine-grained fraction as a result of bacterial leaching was ascertained by granulometric analysis especially in KS1 sample. The knowledge obtained could be important for the improvement of qualitative properties of kaolins and quartz used in ceramic and glass industry. An economical advantage of bioleaching is also suggested because the bacterial treatment is economically as well as ecologically more suitable than classical technologies such as magnetic separation and flotation, although not so rapid.

INTRODUCTION

Weathering of rocks generated large volumes of kaolin clays, quartz sands and micas, as well as other minerals, especially iron and titanium oxides. Kaolin is an economically important raw material often used in wide variety of ceramic applications, from high quality tableware and sanitaryware to electrical porcelain, tiles and glasses. There are some less common uses including glass fibre, white cement and refractory insulation bricks. However, oxyhydroxides of Fe are often deposited along with the kaolinite, contaminating and making much of the kaolin unusable for commercial applications due to insufficient whiteness. That is why the mined kaolins need to be beneficiated by removal of undesirable minerals - mica constituents and other iron-bearing minerals before they could be considered as a suitable raw material for some of the above-mentioned purposes.

According to the mineralogical, chemical and granulometric composition and corresponding technological properties, three kaolin types are distinguished: The 1st class kaolin of the best quality is created by kaolinite and hydromica; kaolin of the 2nd class differs from the best one mainly by increased content of colouring oxides with the lower whiteness in the rough state; 3rd class kaolin has increased content of Fe_2O_3 and worst technological properties [1].

Although physico-chemical procedures, such as magnetic separation and flotation, could be used for beneficiation of kaolin, they are expensive. On the other hand, biological methods could be cheaper and less adverse impact.

Field observations and laboratory experiments demonstrate that microbes can accelerate alumino-silicate mineral weathering reactions in direct contact with mineral surfaces, by producing organic and inorganic acids, creating metal-complexing ligands, changing redox conditions, or mediating formation of secondary mineral phases. Several strains of bacteria released by this way the cations from biotite (Si, Fe, Al), plagioclase, and feldspar (Si, Al) much more than abiotic procedures [2].

A process for biological removal of iron from quartz sands, kaolins and clays was developed as leaching at 90 ^{o}C with lixiviant produced as a result of the cultivation of acid-producing heterotrophic microorganisms, mainly strains of Aspergillus niger [3]. The iron content of different kaolins was lowered from 0.65 – 1.49 wt.% to 0.44 – 0.75 wt.% Fe_2O_3 and their whiteness was increased from 55 – 87 % to 86 – 92 % [3].

Calcium and iron removal from a bauxite ore by Bacillus polymyxa was demonstrated. Within a period of 7 days, the above organism could remove all the calcium and about 45 % of iron from the ore in the presence of 2 wt.% sucrose in a Bromfield medium [4].

Berthelin et al. [5] have described the important role of bacteria in iron reduction when an enzymatic mechanism similar to dissimulative nitrate reduction should be involved in this reaction. Fe^{3+} is mobile only at very low *pH* values (*pH* < 3). Reduction enables the formation of Fe^{2+}, which is mobile in the normal range of soil *pH*. Consequently, if microorganisms and plants are able to reduce Fe^{3+}, they can have advantage in competition for available iron. In the case of silicates, an increase in Fe solubility generally occurs with acid and complex secretions, and feldspars and micas can be destroyed [6].

The present study aims at elucidating the possibilities to improve the quality of kaolins containing iron by means of bioleaching by Bacillus cereus strains. These bacteria are ubiquitous throughout the natural environment and most of strains are not pathogenic. For comparison, the genus Aspergillus is known to produce several toxins as well as to cause allergic reactions in humans and animals.

EXPERIMENTAL PART

Kaolin samples

Three samples of kaolin raw material from Lučenecká kotlina were investigated; two samples were taken from Horná Prievrana and one sample from Vyšný Petrovec deposits. Individual samples were different by form of Fe binding and by way of for-treatment. Their mineralogical and chemical characteristics are shown in table 1.

Bacteria and media

Many bacterial strains of Bacillus species were isolated from the kaolin deposit Horná Prievrana in Slovakia after heating them at 80 °C for 15 min to kill the non-sporoforming species. Two strains were chosen and purified by colonies reisolation on Nutrient agar No.2 (Imuna, Šarišské Michaľany) plates to obtain pure strain cultures. Both strains were identified by means of the Becton-Dickinson system (USA). For the species identification, the strains were cultivated on Columbia agar plates according to recommendation of the system producer. For experiment, these bacterial strains were grown in Nutrient broth No.2 (Imuna) at 28 °C for 18 hours. Bacterial cells were subsequently centrifuged at 4000 rpm for 15 min, subsequently washed twice with saline solution (0.9 wt.% NaCl) and added in a concentration of 10^{10} cells per ml to modified Bromfield liquid medium [7] with individual mineral samples as described below.

Bioleaching of mineral samples

Bioleaching of the 10 g kaolin samples was carried out in 300 ml Erlenmeyer flasks containing 100 ml of modified Bromfield medium (KH_2PO_4 - 0.5 g l^{-1}, $MgSO_4.7H_2O$ - 0.5 g l^{-1}, $(NH_4)_2$ SO_4 - 1.0g l^{-1}, NaCl - 0.2 g l^{-1}, glucose - 20 g l^{-1}) inoculated with a mixture of both Bacillus cereus strains. The microbial cultures were added to these flasks in the active logarithmic phase of growth under aseptic conditions. The flasks were incubated statically under anaerobic conditions. Two kaolin samples from Horná Prievrana were leached for 1 month and a sample of laboratory elutriated kaolin from Vyšný Petrovec deposit was leached for 3 months at 28 °C. However, Bromfield medium was changed three times during bioleaching (in kaolin samples from Horná Prievrana at 7-day intervals and in a sample from Vyšný Petrovec at 1 month intervals) under aseptic conditions. The abiotic controls were cultivated under the same conditions. After incubation, the culture solutions were separated from the biomass by means of membrane filtration.

The presence of vegetative bacterial cells in Erlenmeyer flasks and their morphology were regularly examined by light microscopy after Gram staining.

Chemical analyses

Quantitative changes of samples (solid and liquid phases) investigated from view of element composition stability were evaluated by standard analytical method – atomic absorption spectrometry on a VARIAN spectrometer AA - 30 apparatus (Varian, Australia) after dissolution of the samples by standard procedure.

Table 1. Mineralogical and chemical composition of a kaolin samples (KS1, KS2) from Horná Prievrana and of elutriated kaolin sample (KS3) from Vyšný Petrovec.

mineralogical composition (wt.%)				chemical composition (wt.%)			
	KS1	KS2	KS3		KS1	KS2	KS3
quartz	70 - 80	70 - 80	10 - 15	SiO_2	74.88	62.27	52.04
mica	3 - 7	6 - 14	30 - 40	Al_2O_3	15.64	14.53	29.09
kaolinite	7 - 15	10 - 20	45 - 50	Fe_2O_3	0.92	1.43	0.69
hydroxides	0.5 - 1.5	0.1 - 0.5	0.1 - 0.5	K_2O	0.83	1.05	2.27

Granulometric analysis

The particle size distribution of kaolin sample was measured by the laser radiation scattering on a Laser - Particle - Sizer Analysette 22 (Fritsch, Idar -Oberstein, Germany). The mean particle diameter d_m (0.9 -175 μm) was calculated from granulometric data.

Statistical evaluation of the results was done by one-way analysis of variance (ANOVA). Significance of differences between means was calculated by Tukey's test.

X-ray diffraction analysis

The structural destruction of bacterially leached samples was determined by X-ray diffraction analysis on a diffractometer DRON 3.0 equipped with goniometer GUR - 5 (Tekhsnabexport, Moscow, Russia) by using the following regime: radiation FeK_{α}, 30 kV, 20 mA, time constant 1 s, limit of measurement 10 impulses s^{-1}, rate of detector 2° min^{-1}, paper drive 2.4 m h^{-1}.

Scanning electron microscopy

The morphological changes in the surfaces of individual minerals were investigated by SEM (scanning electron microscopy) and the changes of chemical composition by energy-dispersion micro-analysis (EDS). All mineral samples were coated with carbon and subsequently examined in a scanning electron microscope Tesla BS 340.

RESULTS AND DISCUSSION

The release of Fe from mica or from oxyhydroxides was used as indicator of mineral dissolution and beneficiation of kaolin quality in this study.

KS1 sample of kaolin raw material (table 1) composed of 0.92 wt.% iron, which was found especially in the form of Fe oxyhydroxides (free Fe). The presence of Fe oxyhydroxides was identified by visible brown-red colour of this material.

KS2 sample (table 1) differed from KS1 in higher content of iron. KS2 sample contained 1.43 wt.% of Fe_2O_3, however, this material was of white colour with high mica dissemination. White colour suggests that a substantial part of iron in the sample is bound in the aluminosilicate lattice.

KS3 sample (table 1) contained 0.69 wt.% Fe_2O_3. Similarly to KS2, white sample colour suggests low content of Fe oxides in KS3. Iron is bound also here in aluminosilicate lattice. However, the presence of bound iron and aluminium was confirmed in KS3 sample by the method of sample dissolution in HCl according to Číčel et. al. [8]. The authors confirm that free hydrated oxides - Al_2O_3 and Fe_2O_3 are usually dissolved in HCl much more rapidly than from silicates.

According to Číčel et al. [8], the portion of bound and free iron and aluminium in a sample can be determined from the dependence 1 - α = f(t), where α is the amount of the readily soluble oxide. We can show this calculation on our KS3 sample.

The dissolution curves of Fe_2O_3 and Al_2O_3 in HCl (c = 4 mol l^{-1}) from KS3 sample are shown in figure 1. From the dependence 1 - α = f(t) for Fe_2O_3, we can subtract after extrapolation for t = 0 the value of 0.998, which represents the amount of Fe_2O_3 bound in octahedra. The difference (1 - 0.998 = 0.002) is approximately naught content of free Fe_2O_3 in the form of hydrates, which are easily soluble in HCl. Therefore total amount of 0.69 wt.% Fe_2O_3 is bound in octahedra. It means that the visual identification of the presence of bound iron was confirmed also by the method of the dissolution in HCl. The dependence 1 - α = f(t) for Al_2O_3 after extrapolation t = 0 displays the value of 0.983, which represents the amount of Al_2O_3 bound in octahedra and tetrahedra. The difference (1 – 0.983 = = 0.017) is free Al_2O_3 present in the form of hydrates. That is why from the total amount of 29.09 wt.% Al_2O_3 is bound 28.6 wt.%, and 0.49 wt.% of Al_2O_3 is found as free Al_2O_3. Extraction of Al is more rapid in comparison with Fe extraction because of decomposition of kaolinite with disorder structure by HCl. This fact is described also by Číčel et. al. [8].

Thus, the ability of HCl (c = 4 mol l^{-1}) to dissolve Fe was used as an indicator of the presence of iron bound in mica structure. In the case of acid leaching, the iron removal was only about 5 % and the aluminium removal about 12 % after 6 hours of leaching. This low acidic solubility of iron was reached because Fe is bound in mica structure.

Iron is present in mica, oxides and oxyhydroxides, and in a third form, that is of low-order crystallinity (amorphous form) where it is more available for bacterial leaching [6]. The different mineral species containing Fe in kaolin raw material cause the differences in the percentages of iron removed by Bacillus cereus strains from kaolin samples KS1, KS2 and KS3.

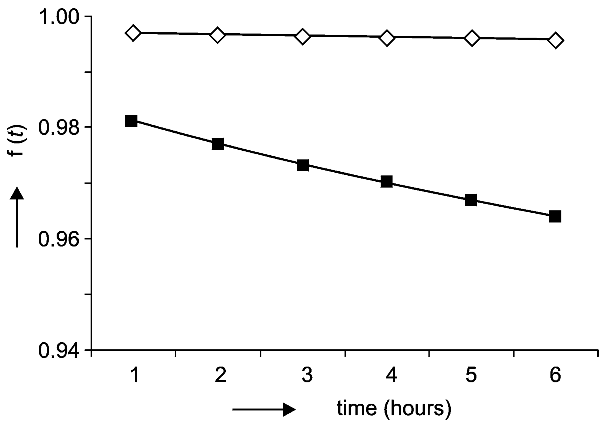

Figure 1. The dissolution curves of Al_2O_3 and Fe_2O_3 in HCl (c = 4 mol l^{-1}) from KS3 sample (Vyšný Petrovec).
◇ - Fe, ■ - Al

Samples from Horná Prievrana
deposit - KS1 and KS2

As can be seen from the results presented in table 2, the Bacillus cereus strains used in this study removed about 43 % of free iron under anaerobic conditions from KS1 within 28 days. The removal of structural Fe ions from KS2 under similar conditions was not so efficient because only about 15 % of Fe was removed within 28 days. This fact can also suggest more difficult availability of iron for bacterial removal because of probable binding of iron in mica. The lower intensities of diffraction lines of mica d = 0.997 nm and 0.333 nm (figures 2) refer to a partial bacterial destruction of mica included in KS2 sample. The portion of resistant quartz in the sample was increased after bacterial leaching to the detriment of a decrease of kaolinite portion.

In the presence of the kaolin during bacterial growth, the *pH* of the Bromfield medium was decreased from 7.0 to about 4.0 within 7 days. During this time, most of the bacteria were adsorbed on the mineral surfaces, and the bulk solution was exchanged with fresh glucose containing Bromfield medium three times during 28 days. The cultivation of Bacillus cereus strains under anaerobic conditions provided to be better for facilitation of Fe dissolution with prevention of subsequent back-oxidation of dissolved Fe. Although acids produced by Bacillus cereus strains were not measured in these experiments, in our previous assays were detected several organic acids, such as acetic, butyric, pyruvic, lactic, and formic acids after bioleaching of aluminosilicate samples by Bacillus spp. strains [9]. It is well known that many organic compounds produced by microorganisms, such as acetate, citrate and oxalate [10] can increase mineral dissolution rates in laboratory experiments [11, 12]. Carboxylic acid groups, which were shown to promote dissolution of silicates [13] are also common in extra cellular organic material.

Microorganisms produce enzymes (e.g., chitinase and cellulase, often bound to the cell wall) specifically to degrade these substrates. It is possible that extracellular polysaccharides of some microorganisms in lichens (as well as other in environments such as soils) contain enzymes that function in ways analogous to chitinase and cellulases, i.e. they specifically break down mineral structures and extract elements required for metabolism or structural purposes (e.g., “mineralases“) [14]. This may be especially important for ions such as Fe^{3+} and Al^{3+}, which are expected to be rather insoluble [15, 16].

KS1 sample was heated at 600 °C for 3 hours and the amorphous forms of Fe oxyhydroxides were changed to hematite. The ratio of hematite in the KS1 elementary sample (figure 3*a*) and bioleached sample (figure 3*b*) was increased by its standard addition up to level of the X-ray detection limit and therefore it was possible to detect the decrease of iron-bearing minerals after bioleaching. The lower intensities of diffraction lines of hematite (d = 0.269 nm) after bacterial leaching also confirm the partial destruction of this mineral found in the KS1 sample. The enrichment by fine-grained fraction as a result of bacterial leaching

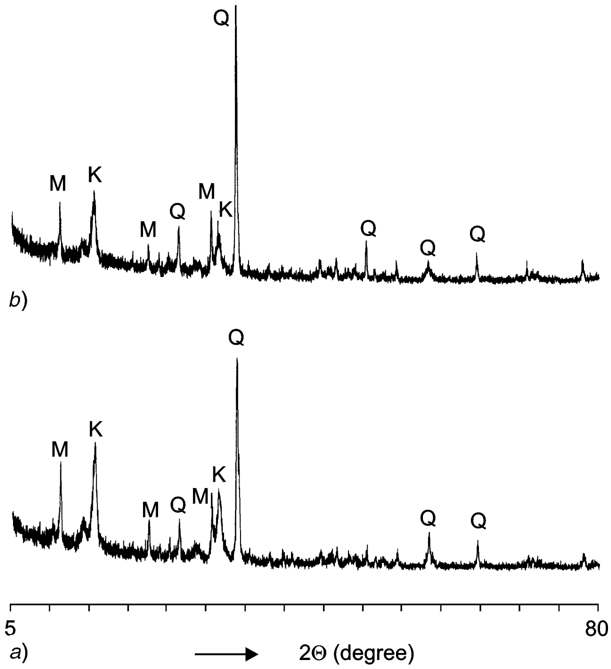

Figure 2. The mineral composition of KS2 sample according to X-ray diffraction spectrum.
a) - before bioleaching, *b*) - the diffraction lines of mica structure were decreased (K- kaolinite, Q - quartz, M - mica) after bioleaching

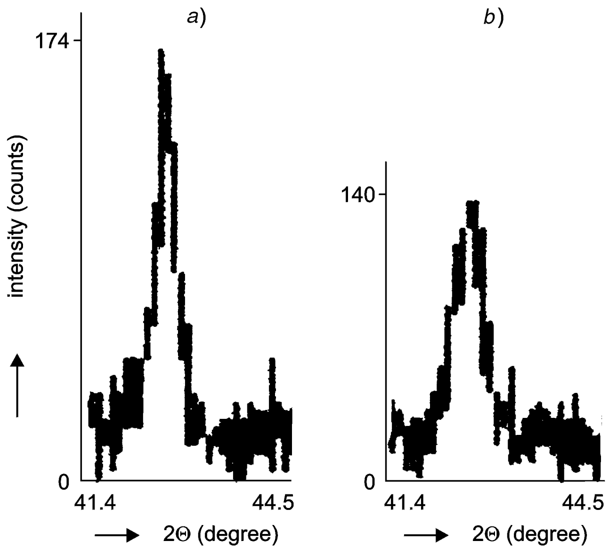

Figure 3. The diffraction lines (d = 0.269 nm) of hematite (600 °C) in elementary sample (KS1).
a) before bioleaching, *b*) the lower intensities of diffraction line of hematite after bioleaching

(figure 4) is important also for the quality improvement of kaolins. The fraction from 12.50 to 61 μm was in KS1 sample significantly (significance levels are between $P < 0.5$ and $P < 0.001$ in dependence on individual particle sizes comparison) decreased, and on the other hand, the distribution of fine-grained fraction with particle size between 3.10 and 10.50 μm ($P < 0.01$ or $P < 0.001$) and also finest-grained fraction with particle size from 0.9 to 1.8 μm ($P < 0.001$), were significantly increased.

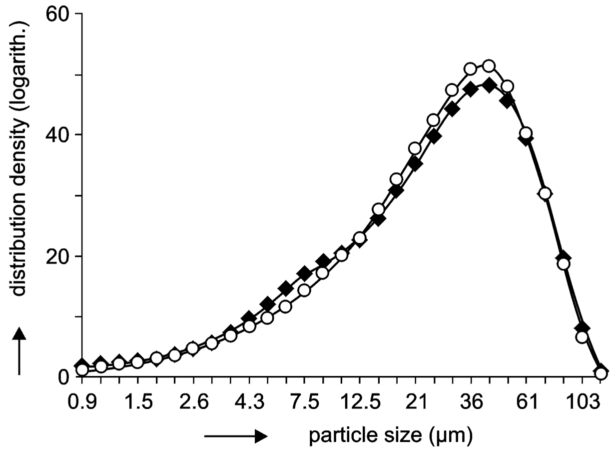

Figure 4. The enrichment by fine-grained fraction in KS1 sample as a result of bacterial leaching.
○ – kaolin sample before bioleaching, ◆ – kaolin sample after bioleaching

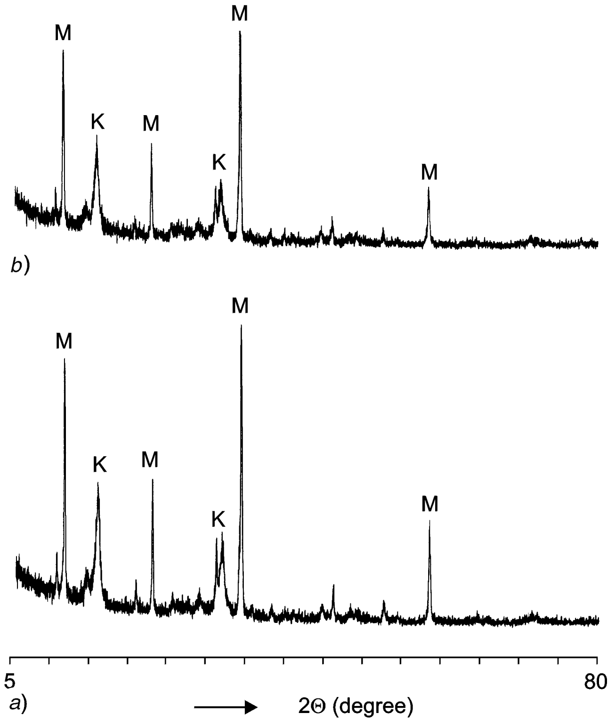

Figure 5. The mineral composition of KS3 sample according to X-ray diffraction pattern (K- kaolinite, M - mica).
a) - before bioleaching, *b*) - the lower intensities of the diffraction line of mica after bioleaching

Samples from Vyšný Petrovec deposit - KS3

Probable mechanisms involved in the biological removal of iron from elutriated kaolin were examined on sample KS3 from Vyšný Petrovec.

The prolongation of bioleaching time from 1 month to 3 months and thus longer production of organic acids and metal-complexing substances by Bacillus cereus strains caused the increase of iron removal from mica. There was observed after 3 months the 52 % extraction of Fe atoms from octahedral position in mica when Al removal was only about 2 %.

The mineral composition of this sample is shown on X-ray diffraction pattern (figure 5*a*). This pattern indicates that kaolinite and mica are major constituents of the KS3 sample. The X-ray diffraction pattern of KS3 sample after bioleaching suggests a partial destruction of mica structure because the diffraction lines (d = 0.997 and 0.333 nm) were decreased (Figure 5*b*).

The chemical composition of the mica surfaces with bound iron ions was investigated by EDS analysis (figure 6). Some mica surfaces, observed by SEM before bacterial leaching (figure 7*a*), were subsequently destroyed by bacteria (figure 7*b*) when Fe ions were released from mica structure what resulted to illite development. The chemical composition of illites is typical by the absence of iron, which is important for metabolical processes of bacterial cells, however, it also depreciates kaolin.

Kaolin mined at Vyšný Petrovec deposit is economically interesting raw material but its properties could be improved by removal of unsuitable minerals (mica, oxyhydroxides). After such an improvement, it could be a suitable raw material for more extensive use in ceramic industry.

Table 2. The removal of Fe by bacteria of Bacillus cereus after 1 month of bioleaching.

concentration of Fe (wt.%)	kaolin sample KS1	kaolin sample KS2
elementary sample	0.92	1.43
bioleached sample	0.53	1.29
iron removed within 1 month from kaolin	43 %	15 %

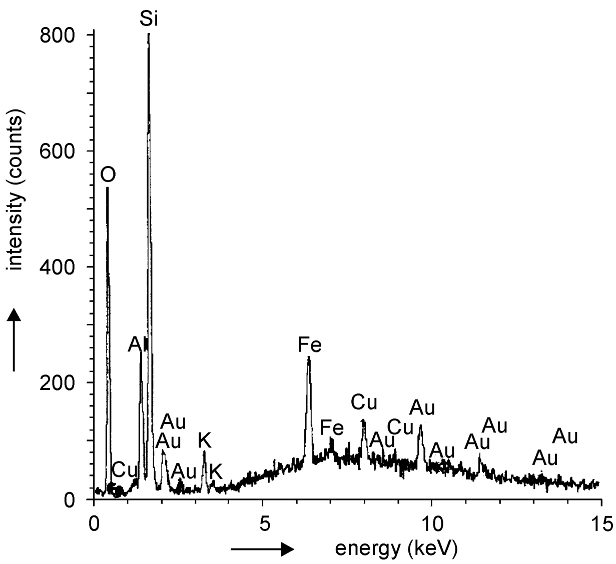

Figure 6. The chemical composition of the mica surfaces with iron composition investigated by EDS analysis.

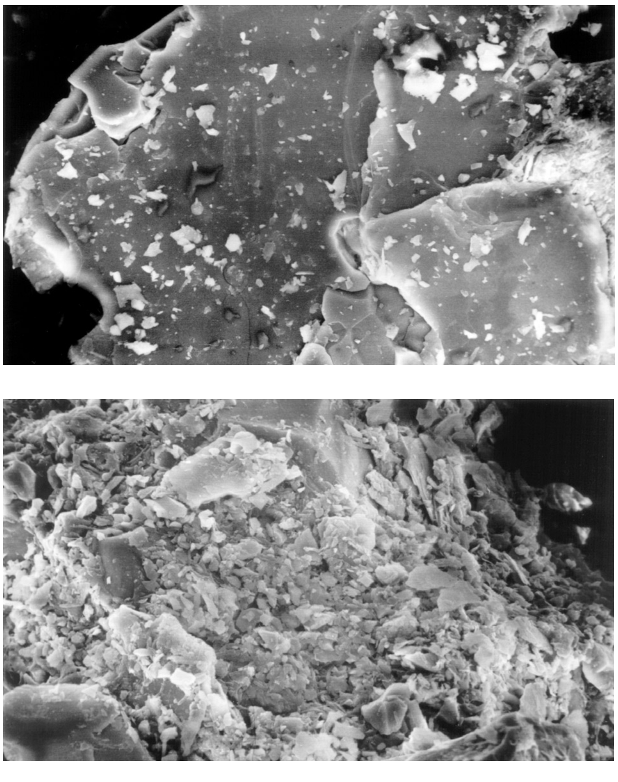

Figure 7. The mica surface observed by SEM.
a) before bacterial leaching, *b*) the formation of illite after destruction of mica

Bacteria and fungi interact with clay minerals and synthesize an array of organic compounds that have been shown to affect the mobility of metal ions. Their biological activity may be high and dependent on production of organic acids. The samples from Horná Prievrana kaolin pit are characteristic by a high number of bacteria especially of Bacillus species. This fact suggests that they are the representatives of microbial community, which is active in this environment. They play an important role in the iron removal as well as in the destruction of silicate minerals.

CONCLUSION

Bacterial leaching is a suitable way for the improvement of qualitative properties of kaolins used in ceramic industry. Bacteria of Bacillus spp. can decrease the content of free Fe as well as of Fe bound in mica, which often contaminates kaolins. An enrichment of kaolin samples by fine-grained fraction is also an accompanying positive fact of bacterial leaching.

This process is time consuming from the technological view but very advantageous with the respect to ecology and economical costs.

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ODSTRÁNENIE ŽELEZA Z KAOLÍNOV BAKTERIÁLNYM LÚHOVANÍM

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Mikroorganizmy zohrávajú významnú úlohu v rozpúšťaní silikátových štruktúr v horninovom zvetrávacom procese a genéze ílovitých minerálov. Za relatívne aktívne v tomto procese sú považované baktérie rodu *Bacillus.* Vo vzorkách z kaolínového ložiska v Hornej Prievrane boli zistené vysoké počty baktérií, špeciálne druhov *Bacillus*.

Solubilizačná aktivita dvoch kmeňov *Bacillus cereus* izolovaných zo vzoriek kaolínu z Hornej Prievrany bola skúmaná na troch vzorkách kaolínov z Hornej Prievrany a Vyšného Petrovca znečistených oxyhydroxidmi železa a sľudou. Z výsledkov experimentov vyplynulo, že po mesačnom lúhovaní sú tieto baktérie schopné odstrániť až 43 % voľného železa obsiahnutého v amorfných formách oxyhydroxidov kaolínov a približne 15 % viazaného železa v sľude. Amorfné formy železa sú ľahšie extrahovateľné bakteriálnym lúhovaním ako viazané formy Fe v sľudách. U kaolínu z Vyšného Petrovca sa pri predĺžení doby lúhovania na 3 mesiace podarilo odstrániť až 53 % viazaného železa v sľude. Biodegradácia oxyhydroxidov železa a čiastočná deštrukcia štruktúry sľudy bola potvrdená aj röntgendifrakčnou analýzou. Rastrovacou elektrónovou mikroskopiou bolo potvrdené, že niektoré povrchy sľudy boli porušené baktériami, pričom ióny železa boli uvoľnené zo štruktúry sľudy, čo malo za následok vznik illitov.

Z hľadiska skvalitnenia kaolínov je významné aj nabohatenie jemnozrnnej frakcie po bakteriálnom lúhovaní, čo bolo zistené granulometrickou analýzou. Na úkor zníženia obsahu frakcie s veľkosťou zŕn od 12,50 do 61 μm sa zvýšila distribúcia jemnozrnnej frakcie s veľkosťou zŕn od 3,10 do 10,50 μm a čiastočne aj najjemnejšej frakcie s veľkosťou zŕn od 0,9 do 1,8 μm.

Získané poznatky by mohli byť významné pre zlepšovanie kvalitatívnych vlastností ako kaolínov tak aj kremenných pieskov, používaných v keramickom a sklárskom priemysle. Je reálny predpoklad, že využívanie bakteriálnej úpravy je v porovnaní s klasickými technológiami, magnetickou separáciou a flotáciou, ekonomicky a ekologicky výhodnejšie, najmä pri neustálom raste ceny energie.