

# Bioleaching as Possible Method of Sorbent Regeneration

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**Abstract** The possibility to use the heterotrophic bacteria of *Bacillus* sp. for regeneration of natural montmorillonite and montmorillonite/iron oxide composite after the Cu(II) adsorption was studied and compared with the classical methods of chemical regeneration. After 10 days of bioleaching the bacteria released approximately 80% of loaded Cu(II), leaving the sorbents stable. Longer treatment and higher concentration of dissolved metal cations in the solution caused the reverse sorption of Cu(II) by bacteria. The treating of composite with adsorbed Cu(II) with organic acids (oxalic, citric) led to partial Cu(II) dissolution. The hydrochloric acid caused its structural destruction, liberated the Fe ions from the oxides precipitated on the bentonite surface. The bioregenerated sorbent was successfully repeatedly used for the Cu(II) adsorption.

**Keywords** Montmorillonite, Composite, Chemical Regeneration, Bacteria, Bioregeneration

## 1. Introduction

Due to the rapid increase in global industrial activities, heavy metal pollution became serious. Hazardous heavy metal pollution of wastewater is one of the most important environmental problems in the world. It is well known that adsorption by low-cost sorbents and biosorbents is an effective and economic method for wastewater treatment [1,2].

In spite of cumulation of sorbents after their use relatively small number of studies was performed on their regeneration. If the bound metals could be recovered from the sorbent and the sorbent regenerated and reused, it would further increase its cost effectiveness. The adsorbed ions can be removed by conventional methods, what are usually non economical processes, or processes cause the sorbents destruction. A few articles were devoted to the clinoptilolite regeneration [3-6]. The chitosan-clay composite was regenerated by rinsing with NaCl and NaOH to release the adsorbed sulphate and selenium [7]. Zhu et al. described in their review the regeneration of spent organoclays [8]. Stirk and van Staden

studied in their work different conditions of the cadmium desorption from biosorbents, as well as made a short review of works deal with the desorption of metal ions [9]. In most methods, either chemical agents (e.g., extraction agents, oxidation agents) or auxiliary (e.g., light, heat) have to be added, which make the regeneration process effective and/or rapid, but the costs are relatively high.

Biohydrometallurgical processes are often promoted as low cost and eco-friendly processes for the treatment of low-grade ores and wastes. They are essentially hydrometallurgical processes using microorganisms (i.e. bacteria, archaea and fungi) to enhance the dissolution of metals from ores, concentrates and wastes. In these processes, the exploitation of microorganisms is based on their inherent characteristics to oxidise/ utilise inorganic and organic substrates so as to generate lixiviant for dissolution of metals.

When the bioregeneration method is applied for the sorbent regeneration process, the microbes can grow without the addition of expensive chemical agents or auxiliary, which can significantly reduce the regeneration costs. In addition, the bioregeneration method can be performed in situ. However, the processing time is always longer.

Almost all knowledge of biohydrometallurgy accumulated up to now deals with chemolithoautotrophic microorganisms of the genus *Acidithiobacillus* and some other acidophilic bacteria while leaching mechanisms of heterotrophic microorganisms has been less studied. Heterotrophic microorganisms with leaching activity are mostly filamentous fungi and bacteria. Among heterotrophic bacteria, *Zooglea Pseudomonas* and *Bacillus* sp. with leaching activity have been isolated. Metal leaching by heterotrophic microorganisms generally involves an indirect process with microbial production of organic acids, amino acids and other metabolites. These metabolites dissolve metals from minerals by displacement of metal ion from the ore matrix by hydrogen ions or by the formation of soluble metal complexes and chelates [10].

The bioleaching of copper from a low grade manufacturing scrap TV circuit boards (STVB) using a mixed culture of mesophilic bacteria was studied by Bas et al. [11]. The results showed that the bioleaching process is

based essentially on the ability of bacteria to oxidize ferrous iron i.e. to generate ferric iron within the leaching environment. The simple laboratory bioleaching experiment for iron removal with heterotrophic bacteria on natural raw materials was conducted to explore a simple cyclic operation for potential use at the industrial scale. Heterotrophic bacteria growing in the presence of feldspar raw materials were able to dissolve iron. The bioleaching process involved acid attack, resulting in the solubilization of Si from the silicate phases and release of impregnated particles of iron minerals from silicate matrix [12]. The dissolution of iron from quartz sands by basin bioleaching under the static in-situ condition pointed at the possibility to improve the quality of quartz sand used for the glass industry. The bacterial activity was dependent and varied with the environmental conditions, addition of chelators and organic feedstock in the form of glucose [13]. The bacteria related to the *B. cereus* species and indigenous heterotrophic bacteria were studied in the bioleaching of fine-grained fraction obtained from industrial washing of quartz sands. Their activity was evaluated by the changes in chemical composition of the fine fractions before and after the reaction process. The bacteria assisted in releasing of the poorly ordered clay minerals from the surface of quartz particles via dissolution of amorphous Fe oxyhydroxides [14]. The bacterial leaching of Zn (II), Ni(II) and Fe(III) from the contaminated sediments using heterotrophic bacterial strains was also studied by Jablonovská et al. [15].

The main aim of this study was to perform the introductory experiments of bioregeneration of the sorbents with adsorbed Cu(II) cations. The bacteria were isolated from the Cu(II) contaminated soils in East-Slovakia region. The conditions of release of Cu(II) adsorbed on the montmorillonite and composite by bacteria were studied and compared with the classical chemical methods of sorbents regeneration.

## 2. Materials and Methods

### 2.1. Sorbents and Adsorptive

Bentonite, containing montmorillonite with crystallochemical formula:  $[\text{Si}_{7.95}\text{Al}_{0.05}][\text{Al}_{3.03}\text{Fe}_{0.22}\text{Mg}_{0.75}]\text{O}_{20}(\text{OH})_4(\text{Ca}_{0.42}\text{Mg}_{0.04}\text{Na}_{0.01}\text{K}_{0.01})$ , originated from the Slovak deposit Jelšovský potok [16]. The composite material was prepared by the method of precipitation of the iron oxide on the bentonite surface in weight ratio 1:1 [17].

All chemicals used in this study were of analytical grade and solutions were prepared using the double distilled water. The stock solution of  $100\text{ mg Cu(II) L}^{-1}$  was prepared by dissolving the solid  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  in distilled water. The sorption experiments were made by a batch technique at the ambient temperature using the rotary shaker. The experiments were conducted with 10 grams of sorbents per liter of solution, the pH of the solution was 5. The suspensions were shaken for 24 hours and then consequently

filtered using the filter paper. The supernatant solutions were analyzed by the atomic absorption spectroscopy (AAS, Varian 240 RS/2400).

The structural changes of the composite loaded with Cu(II) before and after the bacterial treatment was studied by diffractometer Bruker D8 Advance (USA) with CuK $\alpha$  radiation (40 kV, 40 mA). The experimental data were processed by Diffra<sup>plus</sup> Basic software with PDF2 database.

### 2.2. Chemical Regeneration of Composite

The classical methods of metal dissolution enable the using of organic acids, as oxalic and citric, as well as the inorganic hydrochloric acid. The selected organic acids might be also present in bacterial metabolites [18]. Their efficiency in regeneration of composite sample was studied and compared with the bacterial regeneration. The experiments were realized in test tubes containing the same ratio (1:100) of powdered sample and the liquid medium as during the Cu(II) sorption. The samples of 0.1 grams were added to 10 ml solution of 0.1 M oxalic acid, 0.1 M citric acid and 0.5 M hydrochloric acid, including double distilled water and left under the static conditions at the ambient temperature for 2 days. To determine the concentration of dissolved Cu(II), the solutions were filtered through a  $0.2\ \mu\text{m}$  filter.

### 2.3. Bacteria and Bioregeneration

Bacteria were isolated from two localities in eastern part of Slovak republic. The first source was the contaminated soil near the metallurgical factory Kovohuty Krompachy (denoted as K bacteria), which effectively recovers the copper from the secondary scrap materials. The second source was the sediment from the river Hornád, locality near the Ružín dam, loaded with Cu(II) from the metallurgical industry (denoted as R bacteria). The strains were purified by heating at  $80\ ^\circ\text{C}$  for 15 min followed by streak plating on nutrient agar cultures. The isolates were identified with the BBL Crystal Identification System (Becton, Dickinson & Co., Franklin Lakes, NJ). For identification, the isolates were cultivated on Columbia agar plates according with the instructions of the manufacturer. The flasks were incubated under the static conditions at  $25\ ^\circ\text{C}$ . The presence of vegetative bacterial cells in the culture flasks and their morphology were regularly examined by light microscopy after the Gram staining. The strains of *Bacillus* sp. (*Bacillus cereus*, *B. subtilis*, *B. megaterium*, and *B. pumilus*) were identified among the isolates.

Prior to the experimental use, the bacterial strains were grown in Nutrient broth No.2 (Imuna, Slovak republic) at  $28\ ^\circ\text{C}$  for 18 hours. The bacterial cells were subsequently centrifuged at 4000 rpm for 15 min, washed twice with saline solution (0.9 % NaCl) and added to the liquid medium in a concentration of  $10^7$  cells per ml.

The composite sample after the Cu(II) sorption was first inoculated with mixture of heterotrophic bacteria of *Bacillus*

sp. (separately by K and R bacteria). The experiments were realized in test tubes containing the same ratio of powdered sample and the liquid medium as during the Cu(II) sorption. The medium consisted of  $0.05 \text{ g L}^{-1}$  of yeast extract,  $0.5 \text{ g L}^{-1}$   $\text{MgSO}_4$ ,  $1 \text{ g L}^{-1}$   $(\text{NH}_4)_2\text{SO}_4$ ,  $2 \text{ g L}^{-1}$   $\text{K}_2\text{HPO}_4$ ,  $0.1 \text{ g L}^{-1}$   $\text{CaCl}_2$ ,  $0.075 \text{ g L}^{-1}$   $\text{NaCl}$ ,  $0.4 \text{ g L}^{-1}$  of urea,  $0.085 \text{ g L}^{-1}$   $\text{NaNO}_3$  and chelating reagent  $\text{Na}_2\text{EDTA}$ . The tubes were incubated under the static conditions for 22 days. The liquid supernatant was sampled after 2, 5, 7, 9, 12, 16 and 22 days and content of desorbed metal was evaluated. Also, the Fe(III) dissolution by bacteria from the composite sample was monitored. Three replicates were done for each bioregeneration experiment.

For other bioregeneration the K bacteria and pure strain of *Bacillus cereus* were selected. The tubes were incubated under the static conditions for 10 days. The liquid supernatant was sampled after 3, 7 and 10 days. The results were compared with the abiotic control experiments. The medium described above was also used for the abiotic control.

### 3. Results

#### 3.1. Chemical Regeneration of Composite

The treating of the composite with organic acids led to partial Cu(II) dissolution already after 2 days. The oxalic acid dissolved 24 % of adsorbed Cu(II) and the citric acid 34 %, Figure 1. Except the Cu(II), the oxalic and citric acids also dissolved  $253 \text{ mg Fe(III) L}^{-1}$  and  $81 \text{ mg Fe(III) L}^{-1}$  from the composite, respectively. When the hydrochloric acid was used for the regeneration, 95 % Cu(II) was released after 2 days. Unfortunately, together with Cu(II) also Fe(III) ( $333 \text{ mg L}^{-1}$ ) was liberated.

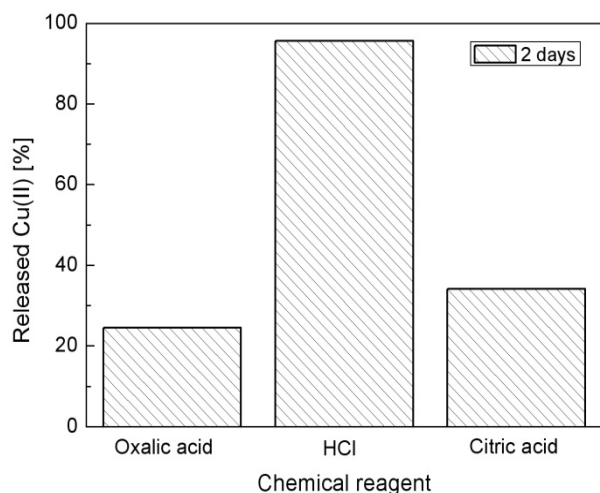


Figure 1. Effect of chemical leaching on sorbent regeneration after 2 days

#### 3.2. Regeneration of Composite by R and K bacteria

Already after 2 days of bioleaching R and K bacteria

dissolved more than 66 % of adsorbed Cu(II). During the first 7 days of bioregeneration there was no significant difference in the bacteria activity, Figure 2. After 9 days, the R bacteria dissolved 80 % Cu(II) from the composite. During the next days, the dissolution slowly decreased. For the K bacteria, the highest extraction was observed on the 7<sup>th</sup> day of the experiment.

Following the effect of bacteria on Fe(III) liberation from the composite loaded with Cu(II) the concentration of dissolved iron ions was low, especially in first 2 days. The dissolution of Fe(III) enhanced and the precipitation of Cu(II) was observed after 16 days of bioregeneration using the R bacteria, Figure 3a. Probably, when the Cu(II) dissolution was finished, the bacteria started to release Fe(III). Using the K bacteria, the Fe(III) dissolution increased on the 9<sup>th</sup> day of bioleaching, Figure 4a. On the 22<sup>nd</sup> day, due to higher concentration of liberated Fe(III), precipitation of Cu(II) occurred.

For both types of bacteria, liberation of Fe(III) from the as-prepared composite was much more higher than from the composite loaded with Cu(II), Figures 3b, 4b.

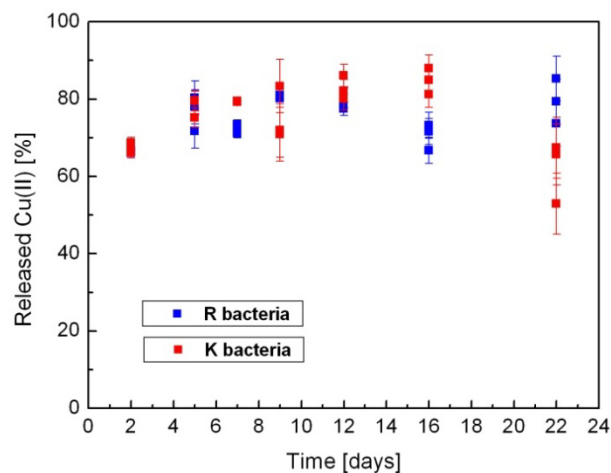


Figure 2. Effect of R and K bacteria on Cu(II) release from the composite material

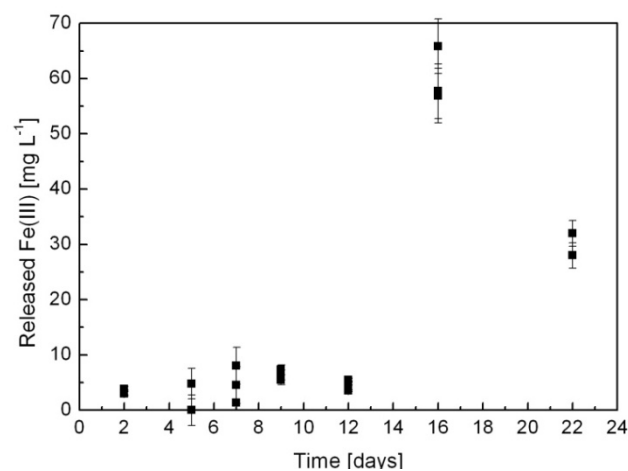
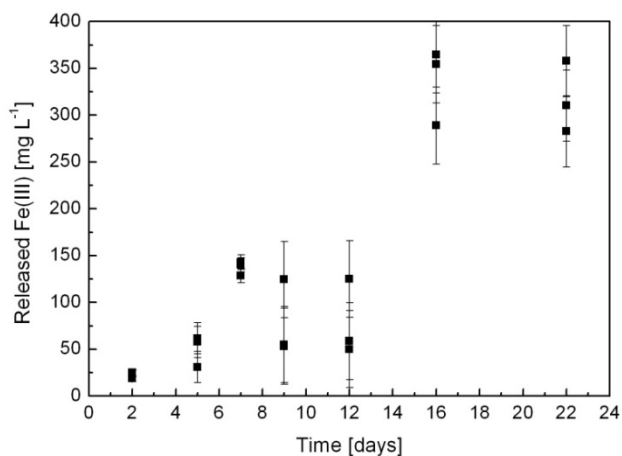
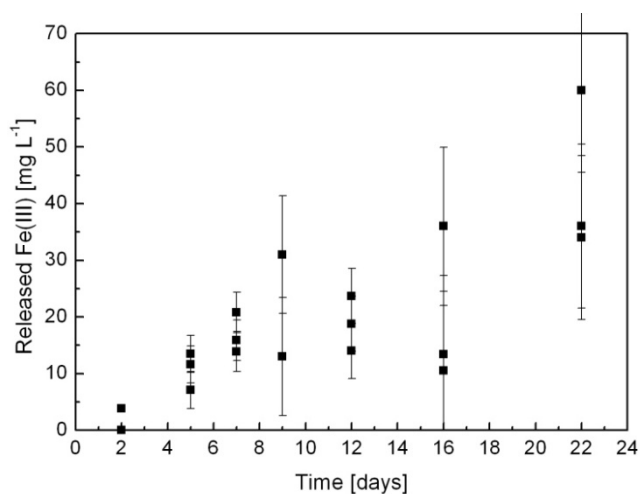


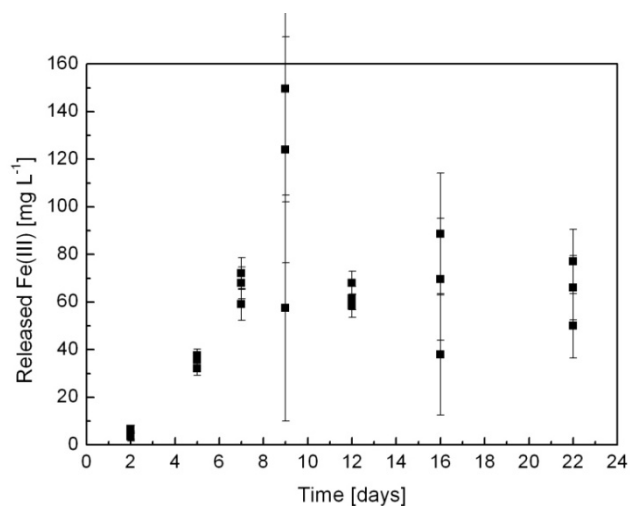
Figure 3a. Effect of R bacteria on Fe(III) dissolution from the composite material after the Cu(II) sorption



**Figure 3b.** Effect of R bacteria on Fe(III) dissolution from the composite material before the Cu(II) sorption



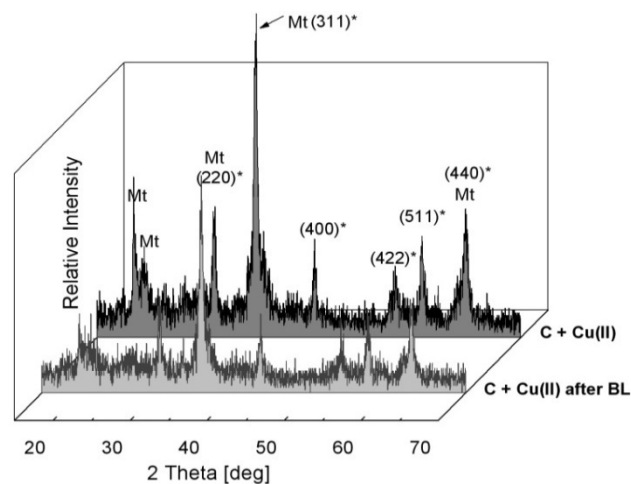
**Figure 4a.** Effect of K bacteria on Fe(III) dissolution from the composite material after the Cu(II) sorption



**Figure 4b.** Effect of K bacteria on Fe(III) dissolution from the composite material before the Cu(II) sorption

Following the results it can be concluded, that the Fe(III) dissolution is restricted by the presence of Cu(II), what

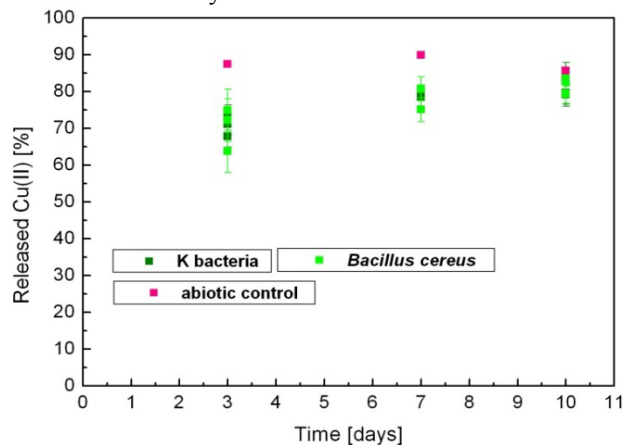
means, that the copper cations were adsorbed preferentially by maghemite particles. This can be also seen from the XRD pattern of composite before and after the bioleaching by K bacteria, Figure 5. The bacteria released the Cu(II) cations adsorbed on the iron oxides without expressive destruction of the composite structure and the regenerated sorbent could be used repeatedly for removal of metal cations.



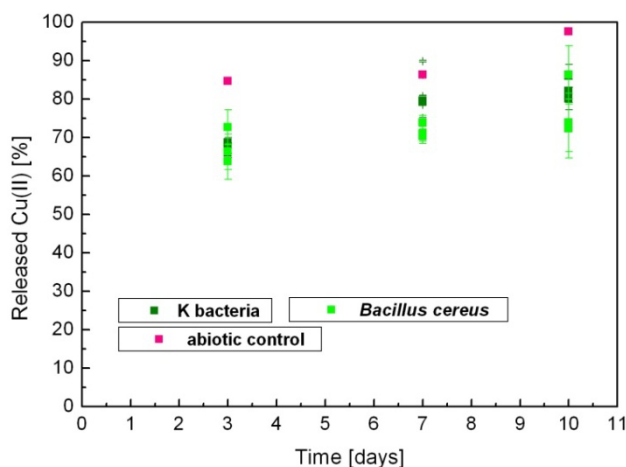
**Figure 5.** X-ray diffraction patterns of composite material loaded with Cu(II) before and after the bacterial regeneration with marked reflections of maghemite, montmorillonite - Mt and goethite - G; \* - adsorbed Cu(II)

### 3.3. Bacterial Regeneration of Montmorillonite and composite by K bacteria and *Bacillus cereus* sp.

Already after 3 days of bioleaching the K bacteria dissolved 71 % of adsorbed Cu(II) from the montmorillonite and 67 % Cu(II) from the composite, Figures 6, 7. After 10 days of regeneration, the higher dissolution, 83 %, was observed for the composite, Figure 7, leaving the sorbents stable. Similar results were obtained for release of Cu(II) by *Bacillus cereus* from both sorbents, only, slight decrease (to 77 %) in recovery of Cu(II) from the composite was observed after 10 days.



**Figure 6.** Effect of K bacteria and strain of *Bacillus cereus* on montmorillonite regeneration during the selected days in comparison with abiotic control



**Figure 7.** Effect of K bacteria and strain of *Bacillus cereus* on composite regeneration during the selected days in comparison with abiotic control

An important consideration is the regeneration of the sorbent for subsequent reuse. The regenerated composite material, after 5 days of bioleaching by K bacteria, was repeatedly used for the Cu(II) sorption from the model solution. Almost 72 % Cu(II) was removed, what was approximately 20 % less in comparison with the as-prepared material, but still enough from the environmental point of view.

### 3.4. Abiotic Control

In the control experiments – abiotic control, made in parallel with the regeneration experiments, the sorbents were treated by medium used for the bioleaching without the bacteria. Comparing the Cu(II) release, higher amounts were obtained, Figures 6, 7. Almost 86 % of adsorbed Cu(II) was dissolved from the montmorillonite and 98 % from the composite after 10 days. Lower dissolved amounts of Cu(II) during the bioregeneration in comparison with abiotic control could be caused by reverse sorption of dissolved Cu(II) by bacteria, what was also observed in the additional experiments with Cu(II) release by bacteria.

In spite of that this study showed the environmental friendly method of sorbents regeneration. On the other hand it was found that heterotrophic indigenous bacteria could be used as sorbents or components of materials for sorption of heavy metals cations from waste waters.

## 4. Conclusion

The results obtained from the introductory experiments of bioleaching of sorbents loaded with Cu(II) by heterotrophic indigenous bacteria were presented and compared with the chemical methods of regeneration.

1. Treating by inorganic and organic acid was not appropriate for the sorbents regeneration; except of Cu(II), high amount of Fe(III) was also dissolved from the composite surface in short time period.

2. During the bacterial treatment almost 82 % Cu(II) was released from the montmorillonite and 78 % from the composite, what was lower amount than was obtained from the abiotic control, due to reverse sorption of dissolved Cu(II) by bacteria.
3. No significant difference in activity of K bacteria and bacterial strain of *Bacillus cereus* was observed.
4. The bioregenerated sorbent had lower value of adsorption capacity than as-prepared, but still sufficient for removal of heavy metal ions.

Utilization of bacteria for the regeneration of sorbents represents ecological method, easy applicable for in-situ processes. Therefore our study will continue with the aim to specify the conditions for the enhancement of the bioregeneration. The observed reverse sorption of Cu(II) by heterotrophic indigenous bacteria could be also, in combination with other sorbents, effective for waste waters treatment.

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