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EFFECT OF THE HEAVY METALS PRECIPITATION ON THE ORGANIC MIXTURE`S SORPTION AND BUFFERING PROPERTIES DURING BIOREMEDIATION OF ACID MINE DRAINAGE BY MEANS OF MICROBIAL SULFATE REDUCTION

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ABSTRACT. A permeable reactive barrier with a total volume of 23 m^3 for treatment of acid mine drainage, generated in the abandoned uranium mine Curilo was constructed and put in operation in 2004. The largest part of dissolved heavy metals and uranium was retained in the anaerobic section for microbial sulfate reduction where the pollutants were precipitated mainly as sulphides and uraninite, respectively. The efficiency of this process was strongly dependent on the rate of biopolymers` hydrolysis, sorption and buffering pproperties of the used organic mixture. The changes of these properties influenced greatly on the spatial accumulation and the mobility fractions distributions of the retained pollutants that were determined after long permeable barrier's operation.

Keywords: *acid mine drainage, proton consumption, mobility fractions, sorption properties*

ВЛИЯНИЕ НА УТАЯВАНЕТО НА ТЕЖКИ МЕТАЛИ ВЪРХУ СОРБЦИОННИТЕ И БУФЕРИРАЩИ СВОЙСТВА НА ИЗПОЛЗВАНАТА ОРГАНИЧНА СМЕСКА ПРИ БИОЛОГИЧНОТО ПРЕЧИСТВАНЕ НА КИСЕЛИ ДРЕНАЖНИ ВОДИ ПОСРЕДСТВОМ МИКРОБНА СУЛФАТ РЕДУКЦИЯ

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РЕЗЮМЕ. Пропусклива реактивна бариера с общ обем 23 m^3 за третиране на кисели дренажни води беше конструирана в изоставената уранова мина Курило и пусната в действие през 2004 година. Основната част от разтворените тежки метали и уран бяха задържани в анаеробната секция за микробна сулфат редукция, където замърсителите бяха утаени главно като сулфиди и уранинит, съответно. Ефективността на този процес беше силно зависима от скоростта на хидролиза на биополимерите, сорбционните и буфериращи свойства на използваната органична смес. Измененията на тези свойства определиха пространственото разпределение и фракционен състав на замърсителите в секцията за микробна сулфатредукция, установени след продължителна работа на пропускливата бариера.

Ключови думи: кисели дренажни води, неутрализацинен потнециал, мобилни фракции, сорбционни свойства

Introduction

The passive systems are the only technical solution which was proven in practice as sufficient way for treatment of acid mine drainage containing dissolved heavy metals, toxic elements and in some cases radionuclides, at communally accepted price. That treatment relies on using of wide range of chemical and biological processes, which are carrying out in separate sections. In this way the selective removal of different classes of inorganic pollutants into the relevant sections of the passive system is possible to achieve. The passive systems could operate at short residence times and higher percent of the pollutants removal to be maintained at lower operation expenses if the scaling of each section takes into consideration the concentration of the relevant group of pollutants, the total discharge of the plume, as well as all sections of the barrier are consequently connected in the appropriate order. The application of the passive systems for water treatment into the practice would enable the environmental status in the mining areas to be improved significantly due to the neutralization of water acidity, hindering of the pollutants migration into the environment, and recovering of the heavily affected aquatic ecosystems. (Georgiev et al., 2010)

This article presents some results about how the sorption and buffering properties of the organic mixture was changed in time due to the precipitation of iron, heavy metals, and uranium at sulfate reducing condition.

Materials and methods

Pilot permeable barrier for treatment of acid mine drainage (AMD), generated in the abandoned uranium mine Curilo, was constructed and put in operation (Groudev et al., 2008). The passive system was consisted of an alkalizing drain, an anoxic section for microbial dissimilatory sulfate reduction (MSDR) and a surface-flow wetland. The alkalizing drain was filled with a mixture of crushed limestone and gravel pieces. Its total volume was 2.5 m^3. The section for MSDR had a volume of about 20.4 m^3 and was filled with a mixture of biodegradable solid organic substrates (cow manure, plant compost, straw), crushed limestone as well as zeolite saturated with ammonium phosphate. The surface flow wetland (a third section) had a total volume of about 5.2 m^3 and was filled with grey forest soil, plant compost, plant debris as well as zeolite saturated with ammonium phosphate. The wetland was biotope for some typical plants (narrow leaf cattail (*Typha angustifolia*), jointed rush (*Juncus articulatus)*, bulrush (*Scirpus spp.)*, etc.

Since the summer of 2004, when the passive system was put in operation, a portion of AMD generated in the ore body is being treated permanently. The quality of the treated waters was monitored regularly by means of different sampling points located at the inlet and the outlet of each section of the passive system. Besides of it, periodical monitoring based on solid sampling taken from the second section of the permeable barrier was applied. The solid samples were taken from different depth and at different distance from the second section onset periodically. The ash content in the solid samples as well as their heavy metals concentration were determined by means of the samples burning and the acid dissolution (HCl) of generated ash, respectively (Page et al., 1982). The concentration of dissolved heavy metals and uranium was determined by means of Atomic Adsorption Spectrophotometry (AAS)/ Induced Coupled Plasma (ICP) and a photometric method (Savvin, 1961), respectively. The exchangeable mobility fraction of the retained pollutants in the solid samples was determined with accordance to wide accepted method (Tessier et al., 1979).

The sorption and buffering properties of the solid samples were determined after the preliminary removal of all limestone particles. The sorption property was studied by means of batch experiment conducted in a polypropylene vessel at 25 ˚C. Solid/ solute ratio of 1/30 was achieved by means of addition of needed amount of electrolyte solution (0.01M KCl). Solution of $ZnCl_2$ (130 μM) was added to the sample in the beginning of the experiment and the sorption of Zn ions was studied during the pH titration from the native-pH-value of the organic sample to the pH-end point (2.5) by means of HCl (0.1 M) addition. The acid consumption per each 0.5 unit of pH reduction was measured by means of automatic pH titration. When the relevant pH-point was reached, aliquot samples (10 ml) were filtered through 0.1 μm filter and the concentration of Zn ions was measured in the transparent solution (Li et al., 2001). The whole experiment was carried out by means of a titrator (Methrohm 718 Stat Titrino) and pH glass electrode Methrom (filled with 3M KCl).

The organic fractional analysis of the samples was performed after their preliminary drying till stable weight and the removal of all limestone particles. The concentration of dissolved monomers was determined by means of the sample's leaching with distilled water at room temperature and short agitation. The total amount of carbohydrates, i.e. crystalinne polysacharide (cellulose) and non-critsaline polysacharides (hemicellulose), was determined by sample leaching in 12 M H_2SO_4 for 12 h (Lowe, 1993). The concentration of non-crystaline polysacharides (hemicellulose) was studied by means of acid hydrolysis (with HCl solution) under anaerobic conditions in the Soxhlet apparatus at higher temperature. Lignine in the samples was selectively solubilized by means of alkaline solution containing NaOH, CuO, $Fe(NH_4)_2(SO_4)_2.6H_2O$ under anaerobic conditions. The whole analysis was carried out in the Soxhlet apparatus at 170° C for a period of 4 h (Kőgel and Bochter, 1985). The concentration of dissolved organic carbon (DOC), which was leached at the relevant conditions, was determined by means of its chemical oxidation at high temperature, followed by absorption of the produced CO_2 (in NaOH solution) and classical acid-base titration with phenolphthalein as indicator (Lurie, 1973).

The biodegradable fraction of the studied organic samples was calculated by well-known method (Chandler et al., 1980).

Results and discussion

The effective treatment of AMD by means of permeable barriers is strongly determined by the correct combination of all physical, chemical as well as biological processes that take part in the precipitation of different heavy metals, toxic elements, and radionuclides. It is achieved practically by the processes` spatial separation by means of different sections construction where the needed conditions could be maintained easily. For that reason, three sections were constructed in the permeable barrier that was put in operation for AMD treatment in the abandoned uranium mine Curilo. First section of the barrier was an alkalizing drain where the water acidity was consumed by limestone with it the section was filled up. In addition, the chemical neutralization enabled chemical oxidation of ferrous iron to ferric state to take place which was followed with the formation of ferric hydroxides. Despite the pollutants removal in the alkaline drain, the most effective precipitation of all dissolved heavy metals excepting manganese was measured in the section for microbial sulfate reduction. Manganese was removed most effectively by surface-flow wetland where aerobic conditions were maintained by means of oxygen diffusion from atmosphere as well as plant and algae photosynthesis. These conditions enabled the oxidation of residual concentrations of ammonia, hydrogen sulfide, and dissolved organic monomers to harmless compounds also to take place. So, all qualities of AMD being treated through the constructed pilot permeable barrier were turned below the relevant permissible levels for waters intended to use in industry/ agriculture. The second section of the permeable reactive barrier acted as a settlement tank where dissolved heavy metals, iron and uranium were precipitated as the relevant sulfides and uraninite (Groudev et al., 2008), respectively (Table 1). It was determined by the anaerobic condition that was maintained into the section and generation of hydrogen sulfide due to the growth and activity of sulfate reducing bacteria.

Table 1. *Data about the chemical content of the organic mixture that was taken from the first one third of the section for microbial sulfate reduction to the relevant sampling periods*

Content, mg/kg	Period of sampling, months			
	10	16	22	28
Pb	26.7	46.6	62.5	78.6
Zn	362.8	586.7	1147.3	1488
Cu	481.5	1211	1650	1950
Ni	118.3	269.3	577.1	1565
Fe	2343	6204	6440	6632
Mn	810.1	473	670	895
Al	5170	7270	7540	8020
Co	98.4	298	512	890
U	45.3	60.3	178.4	215.3
S total	6239	6612	7970	10087
P	2450	1650	1560	1512

The pollutants precipitation in the section for microbial sulfate reduction was initiated by the attraction and sorption of the heavy metals` cations to the negative charged surface groups of the organic mixture. Despite the precise mixing of all organic components that were used for the preparation of that mixture, the charged groups were not distributed equally on its surface. It enabled zones with higher sorption potential to exist where the pollutants sorption was more prominent. As a result of this, zones oversaturated with some of the presented pollutants were created which enabled the process of pollutants removal to continue further to the minerals formation if suitable ligands were presented in. Hydrogen sulfide and its dissolved forms was such suitable ligand that enhanced to draw out the reaction to sulfides (MeS) and uraninite (UO_2) formation. As a result of this, the relative content of pollutants that were presented as an exchangeable fraction (free cations, dissolved complexes) in the solid samples taken in the summer were less than 5 % in comparison to the total content of the relevant pollutant (Table 2). However, the bacterial sulfate reduction as each biological process is strongly temperature dependent and as a result of this very low concentration of hydrogen sulfide was measured during the winter when the daily temperature in the barrier was in the range 1.3-3.1˚C. So, the sorption of dissolved pollutants was acting constantly into the section but the concentration of hydrogen sulfide was not sufficient which limited the process of pollutants precipitation. As a consequence, the relative content of exchangeable fraction of copper and lead, for example, were increased three fold in comparison to the values measured in samples that were taken in the summer. In spite of that, the concentrations of all pollutants, with exception of manganese, that were measured in outlet of the section for microbial sulfate reduction were still below the relevant permissible levels for waters intended for use in agriculture and industry (Groudev et al., 2008). These results revealed the real significance and importance of the sorption process towards inorganic pollutants removal during the treatment of acid mine drainage by means of passive systems.

Table 2. *Data about the exchangeable fraction content (In %) of heavy metals and uranium in the organic mixture that was taken from the first one third part of the section for microbial sulfate reduction to relevant the sampling periods*

Pollutant	Period of sampling, months			
	10	16	22	28
Pb	1.8	7.3	2.2	62.1
Zn	0.1	1.5	0.07	3.2
Cu	0.3	0.8	0.5	1.7
Ni	0.7	2.5	0.9	3.4
Fe	0.005	0.5	0.005	0.7
Mn	5.1	6.2	4.8	10.1
Co	2.0	6.1	2.2	5.4
U	6.4	12.4	7.1	15.7

The content of the biodegradable fraction of the used organic mixture decreased steadily during the applied period of monitoring (Table 3). It was a result of the preferential hydrolysis of biopolymers as cellulose, hemicelluose, and proteins – a process which enabled the growth and activity of the local microbocenose as well as the anaerobic conditions into the second section of the barrier to be maintained. The biopolymers biodegradation resulted in the steadily decreasing content of the volatile solids as well as the higher relative content of refractory biopolymers with more complex structure such as lignine to increase. As a result of this, the concentration of dissolved compounds that was measured in the pore waters of the first one third of the section was decreased below 70 mg Corg/ l which limited the growth and activity of sulfate reducing bacteria (Groudev et al., 2008). It determined the need to replace the exhausted organic mixture from the first one third of the section with fresh one which would enable to maintain the microbial activity as well as the proton consumption and pollutants sorption from drainage waters being treated through the barrier.

Table 3. *Data about the ash and volatile solids contents of the organic mixture that was taken from the one third part of section for microbial sulfate reduction to the relevant sampling periods*

Index	Initial value	Period of sampling, months			
		10	16	22	28
Ash content, %	34.2	36.5	37.4	40.5	41.4
Volatile solids, %	65.8	63.5	62.6	59.5	58.6
Biodegradable fraction	0.65	0.64	0.63	0.53	0.49

The total proton consumption of the organic mixture was measured before the start of barrier operation and how it was changed during the treatment of acid mine drainage. The total proton consumption of freshly prepared organic mixture was 0.529 g H^+/100 g sample (Table 4). That value could be used to evaluate the total power of all chemical processes that take part in the proton consumption in a biotope with higher organic content. For example, the exchange between basic cations adsorbed on the organic surface and protons from solutions is a process that takes place in the whole pH range (8.5-2.5) and as higher is free protons concentration as the process is more intensive. Another chemical process – hydroxyl displacement from sesquioxides by organic anions took place at pH range lower than 7.0. Both processes were responsible for a first peak formation in the pH range 7.7-5.0 where more than 47 % of the total protons consumption was measured (Fig. 1).

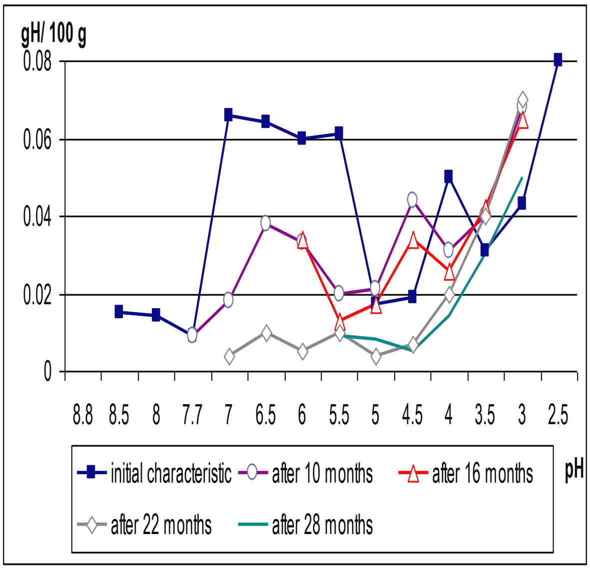

Fig. 1. Changes in the proton consumption of the organic mixture degraded at anaerobic conditions by means of microbial sulfate reduction

The peak's shape was determined mainly by the higher percent of caw manure that was used during the organic mixture preparation. It is well-known that the enzyme hydrolysis of biopolymers that took place in the stomach of ruminant animals resulted in the higher surface area of the processed herbage, higher percent of the biopolymers degradation as well as higher amount of liberated organic monomers. The second plateau of proton consumption was formed in the pH range 4.5-2.5 and it constituted more than 38% of the measured total proton consumption. It was determined by the protonation process of organic acids which typical pK values are in the range 1-5 (Stumm, Morgan, 1996).

Table 4. *Data about the changes in the proton consumption (in g H^+/ 100 g sample) of the organic mixture that was taken from the first one third of section for microbial sulfate reduction to the relevant sampling periods*

End point of pH titration	Initial value	Period of sampling, months			
		10	16	22	28
Initial pH	8.8	7.43	5.68	6.92	5.22
8.8	–	–	–	–	–
8.5	0.015	–	–	–	–
8.0	0.014	–	–	–	–
7.5	0.009	–	–	–	–
7.0	0.066	0.009	–	–	–
6.5	0.064	0.018	–	0.004	–
6.0	0.060	0.038	–	0.01	–
5.5	0.061	0.033	0.034	0.005	–
5.0	0.017	0.020	0.013	0.01	0.009
4.5	0.019	0.021	0.017	0.004	0.008
4.0	0.050	0.044	0.034	0.007	0.005
3.5	0.031	0.031	0.026	0.02	0.014
3.0	0.043	0.040	0.042	0.04	0.03
2.5	0.080	0.068	0.065	0.070	0.05
Total H+ consumption	0.529	0.322	0.231	0.172	0.116

The change of proton consumption of the organic mixture was a parameter studied on the solid samples taken from the second section of permeable barrier during the applied sampling campaigns – after 10, 16, 22, and 28 months, respectively, since the start of the barrier operation. It is interesting to note that the second and forth sampling campaigns were realized during the winter when the daily temperature in the second section was in the range 1.3-3.1 ˚C. Because of the lower temperature, the rates of all microbial processes (ammonification of organic nitrogen, decarboxylation of organic acids, microbial sulfate reduction) leading to proton consumption and the acidity neutralization were decreased significantly. As a result of this, pH of the solid samples taken in winter was buffered only by the processes of chemical neutralization carried out by the presented limestone and the constituents of the organic mixture. As a result of this, pH of the solid samples taken in winter was with 1.5 units lower in comparison with the pH of samples taken in summer (Table 4). It was also evidence that most of limestone surface was armored by minerals of precipitated iron and heavy metals after almost 2.5 years of the barrier operation which decreased its ability to neutralize water acidity. From the presented results (Table 4, Figure 1), a tendency of gradually decrease of the total proton consumption of organic mixture that had been used in water treatment was easily visible. It was determined by the reservoirs of basic cations and easily dissolved organic anions exhaustion in the initially prepared organic mixture as well as the loss of its active surface due to the continual precipitation of iron and heavy metals. As a result of this, the protons consumption in the pH range 7.0-5.5 diminished in power gradually and after 28 months of the barrier operation the buffering properties of the mixture in this range was almost negligible. At the same time, the proton consumption in the pH range 3.5-2.5 was almost unchanged probably due to the attraction and adsorption of protons on the negative surface charged groups of the newly-formed minerals (sulfides and hydroxides mainly) as well as the dissolution of these with non-crystalline structure.

Zinc is one of the heavy metals which are used widely to study the cation sorption on the surface of organic as well as inorganic compounds in wide pH range (Diatta, Kocialkowski, 1998; Toner et al., 2005; Balkaya, Cesur, 2008). It is determined by its properties to migrate as free cation or as stable complexes with suitable net charge in dependence on the local pH. As a result, three distinct zones of zinc sorption on the initially prepared organic mixture was formed (Table 5, Fig. 2). First zone was in the pH range 8.8-7.5 where zinc sorption was more than 50 %. At that pH zinc formed stable complexes with net negative or zero charge with some inorganic and organic ligands presented in solution (Stumm, Morgan, 1996). Simultaneously, a pH-dependent net negative charge on the organic mixture surface had already existed due to the deprotonation of presented carboxylic functional groups. The second zone of zinc sorption was in the pH range 6.5-5.5 where more than 80 % of the element was sorbed. Zinc existed in that pH range as free cations as well as hydroxy complex which are adsorbed easily to the net negative charged positions. The third zone was in the pH range 4.5-3.5 where less than of 50% of added zinc was adsorbed. Zinc adsorption dropped significantly below pH 3.0 due to its presence as free cation, the higher concentration of H^+ in solution, and alteration of the organic surface net charge from negative to positive.

On the analogy of buffering properties, the sorption feature of the organic mixture were altered significantly due to the heavy metals accumulation into the second section of permeable reactive barrier. The sampling campaigns conducted through the summer (first and third, respectively) revealed the greatly improvement of the organic sorption properties in a distinct pH range in comparison with the properties that was measured before the start of permeable barrier operation. For example, zinc sorption was more than 90% in the pH range 7.5-6.0 after 10 months of the start of barrier operation in comparison to 84-27 % before that. Even pH below 4.5 more than 55 % of added zinc was sorbed by the organic mixture. These results showed that deep transformations have taken place in the organic as well as inorganic part of the initially prepared organic mixture. Higher daily temperature in summer enabled the higher activity of all microbial groups that take part in the organic degradation at anaerobic conditions. The bacterial hydrolysis of biopolymers and the ammonification of amino groups were ones of the most important microbial processes that were linked directly to the studied sorption properties. The bacterial hydrolysis of cellulose and hemicellulose was responsible for the gradual liberation of monosugars from their structure which enabled the growth and activity of all microbial groups of the microbocenose to be maintained in the second section in a long-term period. The ammonification of amino group turned its partly positive charge to the net negative due to the releasing of ammonia as an end product into the environment. By this way, the end products of both processes were compounds enriched with functional groups (O-H, COO-H) which attracted and sorbed heavy metals cations preferentially (Beech & Cheung, 1995) Also, the processes were responsible for the steadily degradation of the organic matrix which resulted in the steadily increasing of its active sorption surface.

Table 5. *Data about the Zn $^{2+}$ sorption (in %) on the organic mixture that was taken from the first one third part of section for microbial sulfate reduction to the relevant sampling periods*

End point of pH titration	Initial value	Period of sampling, months			
		10	16	22	28
8.8	50.8	–	–	–	–
8.5	52.9	–	–	–	–
8.0	75.3	–	–	–	–
7.5	84.4	97.4	–	–	–
7.0	27.2	96.8	–	–	–
6.5	87.9	95.2	–	15.5	–
6.0	86.1	92.7	–	81.2	–
5.5	79.3	73.6	83.3	85.7	–
5.0	16.0	74.4	75.6	76.9	35.4
4.5	45.0	61.3	71.7	65.4	16.8
4.0	45.4	57.4	19.2	49.0	0
3.5	39.0	40.6	0	25.5	0
3.0	21.6	27.0	0	15.6	0
2.5	16.7	20.6	0	0	0

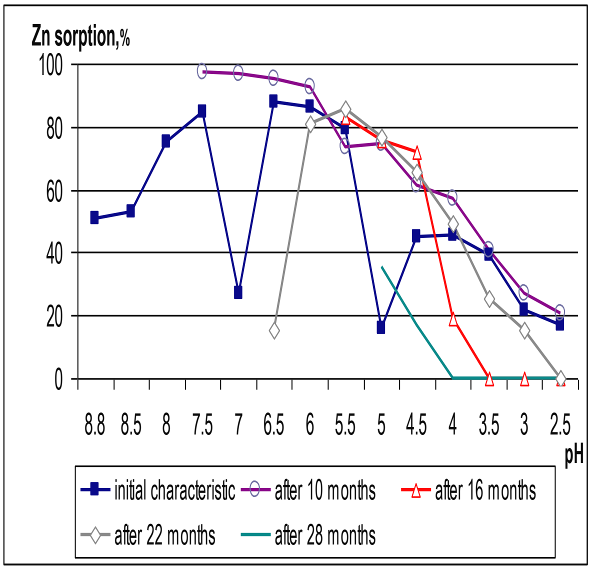

Fig. 2. Changes of Zinc sorption on the organic mixture that was used for treatment of AMD by means of microbial sulfate reduction

The areas with higher sorption capacity were the zones with more prominent net negative surface charge. The heavy metals cations were attracted to, sorbed and precipitated on them preferably. So, the newly formed minerals (oxides and sulfides) of heavy metals and uranium were constituted at low temperature and pressure and as a result of this they were characterized with a lower degree of crystallinity and higher surface area in the very beginning (Van den Hoop et al., 1997). As a result of this, the surface of these minerals took part actively in the processes of protonation/ deprotonation, adsorption/ desorption of cations from waters being treated which altered the sorption properties of the organic mixture as a whole. On the other hand, the second and forth sampling campaigns were conducted through the winter when the temperature inside the barrier was not suitable for higher microbial activity. As a result of this, the most of removed pollutants were presented as an exchangeable fraction mainly which determined their easily resolubilization at any significant change in the content of pore solution. For that reason, zinc sorption at pH below 4.5 was extremely low and if pH dropped further the solid sample acted as a source of zinc ions due to carrying out of an exchangeable reaction as well as dissolution of zinc-bearing minerals. It determined the lower value of proton consumption as well as lower sorption properties of the spent organic mixture.

The presented results shown that after 2.5 years of permeable barrier operation, the sorption and buffering properties of the used organic mixture from the first one third of the section for microbial reduction was almost completely exhausted. It determined a need of the mixture's replacement with new one with similar content and properties. By this way, the studied positive effects (i.e. the proton consumption and effectively heavy metals removal) on the local aquatic ecosystem (Georgiev et al., 2010) as a result of the permeable barrier operation could be maintained in a long-term period.

Conclusions

Organic mixture based on the higher ratio of caw manure determined its excellent buffering properties in the pH ranges 7.7-5.0 and 4.5-2.5, respectively, due to the higher content of basic cations as well as the higher content of organic acids. Both components took active part in chemical as well as biological processes of proton consumption during the treatment of acid mine drainage.

Heavy metals` sorption in the section for microbial sorption was strongly dependent on pH which had crucial effect on the organic mixture net surface charge as well as on the chemical form by which the relevant pollutant was presented. The sorption of the heavy metals on the organic mixture's surface was the main process of the pollutant removal in the section for microbial sulfate reduction during the winter when the daily temperature determined lower activity of all biological process that took part in the biodegradation of biopolymers.

After 2.5 years of permeable barrier operation, the sorption and buffering properties of the used organic mixture from the first one third of the section for microbial reduction was almost completely exhausted due to the consumption of its biodegradable fraction as well as its surface covering with precipitates of iron and other heavy waters from acid mine drainage being treated.

The exhausted organic mixture's replacement from the first one third of the section for microbial sulfate reduction with new one with similar content and properties would enable the long-term operation of the constructed pilot permeable barrier for acid mine drainage treatment at the relevant residence times.

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