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BIOTECHNOLOGICAL CLEANING OF POLLUTED WATERS BY MEANS OF A SYSTEM PRODUCING LARGE AMOUNTS OF BIODEGRADABLE ORGANIC MATTER

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ABSTRACT. Acid drainage waste waters polluted by heavy metals, arsenic and sulphates were treated by means of a laboratory-scale passive system consisting of an alkalizing limestone drain, a permeable reactive multibarrier (intended for microbial dissimilatory sulphate reduction, biosorption and additional chemical neutralization) and a constructed wetland arranged in a series. The main structure in this system was the multibarrier which was filled with a mixture of biodegradable organic substrates (mainly plant biomass, compost, cow manure) and was inhabited by different anaerobic microorganisms (mainly sulphate reducing bacteria and microorganisms metabolically connected with these bacteria). The treatment of the polluted waters by this system resulted in the removal of the heavy metals, arsenic and sulphates and in enrichment of the multibarrier effluents with soluble biodegradable organic compounds. The preliminary tests revealed that such waters will be suitable for electricity generation by means of a microbial fuel cell. The effluents from this cell were subjected to cleaning by means of the constructed wetland.

Key words: Passive systems, Multibarrier, Electricity generation

БИОТЕХНОЛОГИЧНО ПРЕЧИСТВАНЕ НА ЗАМЪРСЕНИ ВОДИ ПОСРЕДСТВОМ СИСТЕМА ПРОДУЦИРАЩА ГОЛЕМИ КОЛИЧЕСТВА БИОЛОГИЧНО РАЗГРАДИМА ОРГАНИЧНА МАТЕРИЯ

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РЕЗЮМЕ. Кисели дренажни отпадъчни води, замърсени с тежки метали, арсен и сулфати, бяха третирани посредством пропусклива реактивна мултибариера (предназначена за микробна дисимилативна сулфат редукция, биосорбция и допълнителна химична неутрализация) и изкуствено мочурище, свързани в серия. Главната структура в тази система беше мултибариерата, която беше запълнена със смес на биологично разградими органични субстрати (главно растителна биомаса, компост, говежда тор) и беше обитавана от различни анаеробни микроорганизми (главно сулфат – редуциращи бактерии и микроорганизми метаболитно свързани с тези бактерии). Третирането на замърсените води чрез тази система водеше до отстраняването на тежките метали, арсена и сулфатите и до набогатяване на изтичащите от мултибариерата води с разтворими биоразградими органични съединения. Предварителните експерименти показаха, че такива води са подходящи за генериране на електричество посредством микробна горивна клетка. Водите, изтичащи от тази клетка, бяха подложени на пречистване чрез изкуственото мочурище.

Ключови думи: Пасивни ситеми, Мултибариера, Генериране на електричество

Introduction

Acid drainage waters are persistent environmental problem at many active and abandoned mine sites. This phenomenon is connected with the oxidation of pyrite and other sulphide minerals as a result of which acidic waters containing sulphuric acid, dissolved heavy metals and solid iron precipitates are released to the environment. Toxic and radioactive elements such as arsenic and uranium are also solubilized from minerals containing these elements. In most cases the main role in these processes is played by different chemolithotrophic bacteria which are the prevalent inhabitants of these ecosystems (Hollberg and Johnson, 2001).

Several methods for treatment of acid mine waters exist, depending upon the volume of the effluents, the type and concentration of contaminants present. The most largely used method is connected with the chemical neutralization of the waters followed by the precipitation of metals. Such active treatment requires the installation of a plant with agitated reactors, precipitators, clarifiers and thickeners with high costs for reagents, operation, maintenance and disposal of the resulting metals laden sludge. The only alternative of such high-cost schemes are the passive treatment systems (Cambridge, 1995; Costello, 2003; Johnson and Hallberg, 2005; Groudev et al., 2007).

These systems have been developed on the basis of naturally occurring biological and geochemical processes in order to improve the quality of the influent waters with minimal operation and maintenance costs. The main advantage of these systems over chemical neutralization is that large volumes of sludge are not generated, the contaminants being precipitated mainly as sulphides.

A wide range of passive treatment systems is available currently. This paper contains some data about the treatment of acid drainage waters polluted by heavy metals, arsenic and sulphates by means of a laboratory-scale passive system initially consisting of an alkalizing limestone drain, a permeable reactive multibarrier (intended for microbial dissimilatory sulphate reduction, biosorption and additional chemical neutralization) and a constructed wetland arranged in a series. Howevevr, on the basis of the results obtained during this study, a second variant of the passive system was developed. In this variant the rich-in-dissolved biodegradable organic compounds effluents from the multibarrier were treated by a microbial fuel cell for electricity generation, and the effluents from this cell were subjected to purification by means of the constructed wetland.

Materials and Methods

The laboratory-scale unit consisted of an alkalizing limestone drain, a permeable reactive multibarrier and a constructed wetland arranged in a series. The alkalizing drain was cylindrical plastic column 50cm high, with an internal diameter of 20 cm and a total volume of 15.7 l. 20 kg of limestone (with 98.1% $CaCO_3$) crushed to minus 10 mm particle size were put in this column. The permeable reactive multibarrier was also a plastic cylindrical column but 80 cm high, with an internal diamenter of 30 cm, and volume of 56.5 l. The column was filled with organic matter consisting of spent mushroom compost, fresh leaf compost, animal manure and sawdust. These components contained their own viable indigenous microflora. Iron shaving were mixed with the organic matter to react with the excess of hydrogen sulphide produced in situ by the anaerobic sulphate-reducing bacteria. The decrease of the concentration of the hydrogen sulphide was necessary to avoid its inhibitory effect on the sulphate-reducing bacteria and other microorganisms inhabiting the multibarrier. Apart from the natural microflora of the organic substrates, inoculum containing such microorganisms was initially introduced into a nutrient solution containing (in g/l) : Na_2SO_4 5.92, NH_4Cl 0.30, K_2HPO_4 0.15, $MgSO_4.7H_2O$ 0.50 and $CaCl_2.2H_2O$ 0.10. The initial pH of the inoculated nutrient solution was adjusted to 4.5 and the multibarrier was filled with such solution. The inoculated multibarrier was put into a thermostate at 32^0C for a period of 45 days. Sampling and analyses were carried out regularly using aliquot samples whose volume was replaced by distilled water.

The constructed wetland was a plastic vessel with rectangular form and a volume of 180 l (120 cm long, 60 cm wide and 25 cm deep). The bottom of the vessel was covered with a 20 cm layer consisting of a mixture of compost, soil with a high organic content, silt and sand. This layer was used to support the growth of the higher plants which were planted in the vessel, as well as a source of organic substances for the growth of the heterotrophic microorganisms which developed in this system. Water-torch (*Typha latifolia*) sod mast were placed in this layer, spaced evenly in the vessel, together with other emergent vegetation, mainly such related to the genus *Zygnemophyta*, as well as the water clover (*Marsilea*) were placed in the vessel which was filled with tap water. Fertilizers containing nitrogen, phosphorus and potassium in suitable forms were added to the vessel and artificial light was applied to enhance the growth of the vegetation. The vessel was maintained at 18-20^0C in the course of two months. A stable biocenose developed during this period. Apart from the above-mentioned plants, the biocenose contained various microorganisms, protozoa, insects and other invertebrate organisms.

The microbial fuel cell was plexiglass rectangular container already described (Groudev et al., 2014). The feed stream ,i.e. the multibarrier effluents, was supplied to the bottom anodic section of the container at rates varying in range of 10-50 ml/h and the effluent passed through the cathodic section and exited at the top continuously. Air was injected during the treatment to the cathodic section.

Elemental analysis of the water samples was performed by atomic absorption spectrometry and inductively coupled plasma spectrometry. The isolation, identification and enumeration of microorganisms were carried out by the classical physiological and biochemical tests (Karavaiko et al., 1988) and by the molecular PCR methods (Sanz and Köchling, 2007).

Results and Discussion

The pH of the mine waters was increased in the alkalizing limestone drain from the highly acidic values of 1.7-2.1 to values higher than 2.5-3.5 which facilitated their further treatment in the permeable reactive multibarrier (Table 1).

Table 1.
Data about the mine waters before and after treatment by the permeable reactive multibarrier

Parameters	Before treatment	After treatment	Permissible levels for waters used in agriculture and industry
pH	2.51 – 3.74	6.71 – 7.65	6 – 9
Eh, mV	(+384) – (+572)	(-97) – (-245)	-
Diss. O_2, mg/l	0.2 – 0.8	0.1 – 0.2	2
Solids, mg/l	41 – 148	23 - 104	100
Chemical oxygen demand (COD),mg/	21 - 53	320 - 710	40
SO_4^{-2} mg'/l	392 - 1980	170 - 640	400
Cu, mg/l	3.7 - 41	< 0.5	0.5
Zn, mg/l	1.7 - 32	< 0.5	10
Cd, mg/l	0.01 – 0.17	< 0.01	0.02
Ni, mg/l	0.9 – 7.1	< 0.5	0.5
Co, mg/l	0.6 – 4.8	< 0.5	0.5
Pb, mg/l	0.08 – 0.32	< 0.1	0.2
Fe, mg/l	95 – 1430	< 1 - 12	5
Mn, mg/l l	6.8 - 77	< 0.5 – 3.7	0.8
As, mg/l	0.05 – 0.32	< 0.01 - 0.1	0.2

It was found that an efficient removal of pollutants from the water being treated was achieved in the multibarrier even at dilution rates as high as 0.3-0.5 h^{-1}. The residual concentrations of pollutants in most cases were decreased below the relevant permissible levels for waters intended for use in agriculture and/or industry. This was due to different biological, chemical and physico-chemical processes but the main role was played by the microbial dissimilatory sulphate reduction. This conclusion was made on the basis of the data about the generation of hydrogen sulphide, the significant decrease of the concentration of sulphate ions and of the levels of redox potential (Eh), as well as about the increase of the number of sulphate-reducing bacteria, the level of the pH and the content of insoluble sulphides of the heavy metals and arsenic in the multibarrier. These heavy metals and the arsenic were precipitated mainly as very fine particles of the relevant sulphides. However, portions of these pollutants were precipitated mainly as hydroxides and carbonates or were removed as a result of their sorption by the organic matter in the multibarrier.

At the same time, the concentrations of organic compounds in the waters treated by the multibarrier (expressed in the Table 1 as a chemical oxygen demand) increased considerably with respect to these concentrations before treatment. This was connected with the much higher concentrations of different saprophytic microorganisms in the multibarrier (Table 2).

Table 2.
Microflora of the mine waters before and after their treatment by the permeable reactive multibarrier

Microorganisms	Before treatment	After treatment
	Cells/ml	
Fe^{2+} - oxidizing chemolithotrophs (pH 2)	10^4 - 10^8	0 - 10^1
Aerobic heterotrophic bacteria	10^1 – 10^4	10^1 – 10^3
Cellulose – degrading aerobes	ND – 10^1	0 – 10^1
Anaerobic heterotrophic bacteria	ND – 10^2	10^4 - 10^8
Cellulose – degrading anaerobes	ND – 10^1	10^4 – 10^6
Sulphate – reducing bacteria	ND - 10^8	10^6 - 10^8
Fe^{3+} - reducing bacteria	ND - 10^8	10^2 – 10^4
Bacteria fermenting sugars with gas production	ND	10^3 – 10^7
Ammonifying bacteria	ND	10^2 – 10^4
Denitrifying bacteria	ND	10^2 – 10^3
Methane – producing bacteria	ND	10^2 – 10^4

Note: ND = not detected

These saprophytes degraded the biopolymeric organic compounds and provided the sulphate-reducing bacteria with suitable monomeric organic sources of carbon and energy. The concentration of ammonium ions in the multibarrier was also increased due to the ammonification of the organic matter. The concentration of phosphate ions were also increased mainly due to the solubilization of a portion of the phosphate present in the spent mushroom compost. The organic substrates in the multibarrier were slowly degradable and supported microbial growth over the long experimental period of about 14 months. It was found that the substrate utilization during this period was about 40%.

The multibarrier effluents during the first 3-4 months since the start of this experiment were subjected to the treatment for the removal of the dissolved organic compounds, ammonium and phosphate ions by the constructed wetland. This treatment was very successful and the concentrations of these components in the wetland effluents were decreased below the relevant permissible levels. At the same time, the preliminary tests revealed that the multibarrier effluents were very suitable for electricity generation by means of microbial fuel cells. It was also found that these effluents contained some electrochemically active microorganisms. Some of these microorganisms were able to transfer electrons from the organic substrates in the multibarrier directly to the anode of the microbial fuel cell used in this study. Some other microorganisms present in the multibarrier effluents, mainly sulphate-reducing bacteria (Table 3), were able to perform the electron transfer by means of secreted metabolites, such as the hydrogen sulphide. For that reason, some multibarrier effluents containing both soluble organic substrates and the relevant microorganisms were directly used for electricity generation in the microbial fuel cell constructed for this purpose. However, apart from this way of treatment, in some cases the multibarrier effluents were additionally inoculated with some electrochemically active microorganisms and then were directed to the microbial fuel cell.

Table 3.
Sulphate – reducing bacteria in the effluents from the permeable reactive multibarrier

Sulphate – reducing bacteria	Cells/ml
Desulfovibrio (mainly D. desulfarions)	10^5 – 10^7
Desulfobulbus (mainly D. elongatus)	10^3 – 10^7
Desulfococcus (mainly D. postgatei but also some non – indentified species)	10^2 – 10^5
Desulfobacter (D. multivorans)	10^2 – 10^6
Desulfotomaculum (D. nigrificans and some non-identified species)	10^1 – 10^3
Desulfosarcina (D. variabilis)	10^3 – 10^5
Desulfomonas (mainly non – identified species)	10^2 – 10^4

The preliminary test revealed that such waters are suitable for electricity generation by means of the microbial fuel cell used in this study (Table 4).

Table 4.
Data about the electricity generation in the microbial fuel cell (MFC)

Parameters	Values
Influents in the MFC; COD, mg/l.d	300 - 700
pH	6.90 – 7.35
Eh,mV	(-210) – (-235)
Temperature, ^{0}C	28 - 32
Effluents from MFC; COD, mg/l.d	15 – 250
COD used in the MFC, mg/l.d	255 - 550
% from the initial	64 – 95
Current density, mA/cm^2	0.077 – 0.180
Voltage of the open circuit, mV	82 - 175
External resistance, ohm	20 - 100
Power, mW/m^2	230 - 930

The effluents from this cell still contained some dissolved organic compounds in total concentrations higher than the relevant permissible levels. However, these residual dissolved organic compounds were efficiently decreased to total concentrations below the permissible level by means of treatment in the constructed wetland.

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