

II. TECHNOLOGIES FOR DEPOLLUTION

MICROBIAL BIOREMOVAL OF DIVALENT TOXIC METALS

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Abstract. The problems of polymetallic wastewater treatment from mining enterprises as well as the accumulation of organic waste are acute worldwide. The application of any existing methods of wastewater purification is ineffective and impossible due to the huge volumes and high concentrations of metals. Similarly, modern methods are ineffective for the treatment of huge amounts of organic waste. Therefore, there is a necessity to develop novel environmental biotechnologies providing the simultaneous degradation of organic waste and detoxification of toxic metals. The purpose of the work was to theoretically substantiate and experimentally confirm the possibility of toxic divalent cations removal using dissimilatory sulfate reduction via anaerobic fermentation of ecologically hazardous model organic waste. Colorimetric and potentiometric methods were used for pH and redox potential measurement; volumetric and chromatographic methods – to control volume and composition of synthesized gas; permanganate method – to determine the concentration of dissolved organic carbon (DOC); photolorimetric method via the qualitative reaction with Nessler's reagent was used to determine the concentration of ammonium ions. The Co^{2+} and Ni^{2+} content in medium was determined by a colorimetric method with 4-(2-pyridylazo)resorcinol (PAR). Fermentation parameters were calculated with the use of mathematical and statistical ones. Modified Postgate B medium with different sources of carbon and energy (potatoes, alanine, and meat) was used for cultivation of dissimilatory sulfate reducing bacteria. The anaerobic microbiome obtained from the sludge of methane tanks showed high efficiency to remove Co^{2+} and Ni^{2+} from the liquid medium. The highest efficiency (100% in 9 days) was observed when alanine was used as a source of carbon and energy. The slowest metal precipitation process occurred using meat (20 days). Also, the use of a protein substrate did not provide the expected alkalization of the medium, which could significantly accelerate the process of metal precipitation. The precipitation of cobalt and nickel cations during the hydrogen fermentation of potato starch was complicated by acidification of the medium, but it was equally effective when the pH was adjusted. The proposed approach, the slow dissimilatory sulfate reduction, due to the sparingly soluble calcium sulfate as electron acceptor, can be used as a basis for the development of new biotechnologies for the treatment of wastewater contaminated with divalent heavy metals with the simultaneous treatment of ecologically hazardous compounds.

Key words: thermodynamic prediction, metal containing wastewater purification, environmental biotechnologies, metals detoxification, metal pollution, toxic metals, sulfate reduction, ammonification, environmentally hazardous compounds

INTRODUCTION

The pollution of water reservoirs and soil by heavy metals [1, 2] as well as the accumulation of huge amounts of organic waste [3] is one of the biggest environmental problems of modern mankind. The exploitation of metal-containing deposits is necessary for the economic development of any country, but is hazardous for the ecosystems. As a result of industrial activities, a large number of toxic elements where heavy metals make up to 75-80% are released [4]. There are dozens of such enterprises in North and South America [5, 6] as well as in Europe [7]. Artificial metal-containing rivers are formed as the result of intensive and usually irrational exploitation of mining and processing plants [8]. Dumped to the environment, they cause pollution of soil and water reservoirs [9].

The negative ecological impact of heavy metals takes place due to their toxicity, high persistence and non-degradability in the environment [10]. Some metals in low concentrations, such as manganese, nickel, zinc, iron, are necessary for the metabolism of living organisms, including human beings. The

increased concentration of these metals has a harmful effect and leads to intoxication. Lead and cadmium are not required for metabolic activity and can have toxic effects on the body at very low concentrations [11].

Currently, there are no effective physical, chemical, or biological methods for the treatment of polymetallic artificial rivers [12]. Conventional wastewater treatment technologies such as electrocoagulation, flocculation, co-precipitation, filtration, reverse osmosis, membrane bioreactor, electrodialysis, ultrafiltration, biosorption, solvent extraction, ion exchange, and wetland technology are not effective for the removal of heavy metals [13, 14]. The disadvantages of these methods are their low removal efficiency, high energy requirements, complexity in application, high cost, the need to use chemicals that can cause additional pollution [15, 16]. But the huge volumes of wastewater are the main reason that hinders the application of the existing methods [17]. Therefore, novel biotechnological methods are of great interest to science and industry, considering their environmental friendliness and cheapness.



Microbial treatment is a promising method of industrial metal-containing wastewater purification [18]. It is based on the processes of interaction of microorganisms (bacteria, fungi, microfauna, etc.) with heavy metals, among which the immobilization and accumulation are the most relevant [19]. It results in the precipitation of metals in the form of insoluble compounds (e.g. carbonates, hydroxides, sulfides) [20]. However, the existing biological methods are often ineffective, as they have a number of disadvantages. First, the high toxicity of heavy metals; second, the sensitivity of the applied microbiomes to these metals and their low competitiveness; third, the necessity to add additional nutrients to the environment, as well as to regulate various parameters: pH, temperature, humidity, redox potential [21].

The large volumes of organic food waste, their rapid accumulation and uncontrolled decay is another global problem nowadays. It causes the pollution of the environment by pathogenic microorganisms and the formation of huge volumes of toxic liquid and gaseous products (alcohols, fatty acids, hydrogen sulfide, ammonia, etc.) [22]. Recycling, incineration, conversion of waste to energy, composting or landfilling can be distinguished among the most common methods of disposal of solid household waste [23]. However, for modern society, these methods are outdated and ineffective. It is imperative to consider the possibility of treatment and conversion of solid organic waste into valuable products such as energy, alternative fuels and other (biofertilizer etc.) [24]. This pathway is possible in the case of the application of microorganisms. They ferment organic compounds with the formation of end products, such as the hydrogen and methane, carbon dioxide and water. This group of processes is called biodegradation [25]. Microorganisms are the key to pollutants detoxification and environment protection of biological systems [26].

In the article, we propose a new combined approach for fast and effective detoxification of metals with the simultaneous anaerobic degradation of environmentally hazardous organic compounds with the production of hydrogen gas.

Thus, the purpose of our study was to investigate the dissimilatory sulfate reduction for the toxic divalent heavy metals removal (cobalt and nickel cations) and the simultaneous degradation of environmentally hazardous food waste via hydrogen fermentation.

MATERIALS AND METHODS

Thermodynamic prediction of microbial interaction with metals

Thermodynamic prediction was used as a theoretical basis to substantiate the possibility of removal of divalent heavy metals Co^{2+} and Ni^{2+} from model wastewater by anaerobic microbiome. This method makes it possible to choose the most effective mechanisms of the detoxification of soluble toxic cations Co^{2+} and Ni^{2+} via microbial sedimentation in the form of insoluble compounds of $\text{Co}(\text{OH})_2\downarrow$, $\text{Ni}(\text{OH})_2\downarrow$, $\text{CoCO}_3\downarrow$, $\text{NiCO}_3\downarrow$, $\text{CoS}\downarrow$, $\text{NiS}\downarrow$. The Pourbaix equations (the equations of the elements stability of in "pH-Eh" coordinates) was used as a basis for the thermodynamic prediction of microbial interaction with soluble heavy metals [27].

Divalent toxic metals removal via anaerobic fermentation of ecologically hazardous model waste

The sludge from methane tanks (Bortnytska aeration station in Kyiv, Ukraine) was used as the source of microbiome. Slightly soluble gypsum CaSO_4 (Klebrig, Czech Republic) was studied as the terminal acceptor of electrons for the slow sulfate reduction. Starch (potatoes), alanine, and natural proteins (meat) were used as the sources of carbon and energy as well as to study and compare the efficiency of their degradation by anaerobic microbiome with the simultaneous precipitation of toxic divalent heavy metals Co^{2+} and Ni^{2+} ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{NiNO}_3 \cdot 6\text{H}_2\text{O}$). The efficiency of the fermentation process was evaluated by the level of the decrease in the concentration of dissolved organic compounds (DOC) and the decrease in concentration of divalent cations in the medium.

Three variants of the experiment were performed to compare the effectiveness of the precipitation of Co^{2+} and Ni^{2+} by anaerobic microorganisms (Table 1).

Table 1. The design of the experiment.

Treatment	Substrate	Concentration Co^{2+} and Ni^{2+} , mg/L
Control 1	Potatoes	0
Control 2	Alanine	0
Control 3	Meat	0
Experiment 1	Potatoes	100
Experiment 2	Alanine	100
Experiment 3	Meat	100

Modified by us Postgate B medium was used for the cultivation of dissimilatory sulfate reducing bacteria to precipitate Co^{2+} and Ni^{2+} by H_2S to insoluble $\text{CoS}\downarrow$ and $\text{NiS}\downarrow$. The medium consists of (g/L): KH_2PO_4 – 0.5; NH_4Cl – 1.0; FeSO_4 – 0.1; CaSO_4 – 5.0. Tap water was used for the medium. Potatoes and meat were pre-cleaned and cut into 5 mm cubes. Substrates were added into the medium in the amount (g/L): alanine – 5.0; potato – 30.0; meat – 50.0. The flasks with volume 0.25 L were filled with the medium to the volume of 220 ml. The study of the divalent cations precipitation via sulfate reduction was tested by adding the solution of both Co^{2+} and Ni^{2+} to a final total concentration 100 mg/L of cations to culture medium at the beginning of cultivation. The fermentation flasks were closed with rubber stoppers with fittings to sample the aliquots of culture fluid and gas and to remove the synthesized gas. The process of the precipitation of Co^{2+} and Ni^{2+} was performed in the hermetically sealed vials with a total volume of 0.25 L at 32 °C during 30 days. The synthesized gas was removed by a plastic syringe (0.1 L volume) to measure its volume. The efficiency of cations precipitation was assessed by the decrease in their concentration in the medium. The efficiency of the fermentation process was evaluated by the yield of biogas (H_2 , CO_2) and the decrease in the concentration of soluble organic compounds. Statistical analysis was carried out using Exel and Origin Pro 7 software. All experiments were performed in triplicate.

The preparation of metals solution and determination of Co(II) and Ni(II) concentration

The solution of 30000 mg/L Co^{2+} and Ni^{2+} was used as the stock. It was prepared by $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{NiNO}_3 \cdot 6\text{H}_2\text{O}$ dissolution in distilled water in a volumetric flask. The Co^{2+} and Ni^{2+} concentration in medium was determined by a colorimetric method with 4-(2-pyridylazo)resorcinol (PAR). The method is based on the ability of PAR to form colored red complexes with cations of divalent metals including Co^{2+} and Ni^{2+} [28].

The control of metabolic parameters of the fermentation process

The metabolic parameters were determined as following [29]: pH, redox potential (Eh, mV), total concentration of dissolved organic compounds (DOC, mg/L), concentration of NH_4^+ ions (mg/L), the volume (mL) and concentration of the gas phase (H_2 , O_2 , N_2 , CH_4 , CO_2 in %), H_2 and CO_2 yield (L of gas/kg of substrate carbon content).

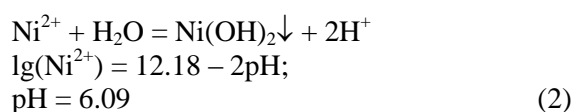
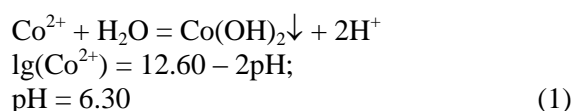
The pH and Eh were determined potentiometrically using the ionometer EZODO MP-103 with remote electrodes Ezodo and BNC connectors-models PY41 and PO50, respectively. The reliability of the measurements was confirmed by standard buffer solutions [29].

The determination of gas composition was performed by the gas chromatography method [30]. The concentration of dissolved organic carbon (DOC) was determined by a permanganate method [31]. The concentration of ammonium ions was determined by photopolarimetric method via the qualitative reaction with Nessler's reagent [32].

RESULTS

Theoretical basis of the research

Our approach is based on the thermodynamic calculations on the stability of soluble compounds of divalent metal cations. According to the thermodynamic calculations, their precipitation occurs with the pH increase. Soluble cations Co^{2+} and Ni^{2+} are precipitated into insoluble hydroxides or carbonates at the $\text{pH} \geq 6.0$:



Such metabolic pathway as dissimilatory sulfate reduction is used for the Co^{2+} and Ni^{2+} precipitation. Dissimilatory sulfate reduction is based on the use of sulfates by microorganisms as a terminal electron acceptor. Hydrogen sulfide or sulfide anions are the end products of sulfate reduction. They are universal compounds for the precipitation of a wide range of divalent cations of heavy metals. Divalent metal cations, for example Co^{2+} , Ni^{2+} are precipitated to insoluble $\text{CoS}\downarrow$, $\text{NiS}\downarrow$.



During the dissimilatory sulfate reduction, soluble sulfates are rapidly reduced with the formation of stoichiometric amounts of H_2S . But, even at low concentrations (0.01 – 0.1%), hydrogen sulfide and other sulfides (S^{2-} , SH^- etc.) are very toxic. This is why sulfate reduction is considered unsuitable for application in metal removal biotechnologies. The use of a sparingly soluble source of sulfates such as

gypsum, $\text{CaSO}_4 \cdot n\text{H}_2\text{O}$ is the solution of this problem. The gypsum was shown to have a very low solubility, only 10–20 mg/L. In this regard, microorganisms are assumed to reduce sulfate very slowly. The concentration of sulfides will be low and non-toxic, but enough for the complete precipitation of Co^{2+} and Ni^{2+} . We hypothesized that the release of H_2S would be very slow at the safe concentrations 10–20 mg/L. However, it will be sufficient for the irreversible precipitation of a wide range of metals (Co^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Sn , Cd^{2+} , etc.) to insoluble compounds ($\text{CoS}\downarrow$, $\text{NiS}\downarrow$, etc.).

The strategic proposal is in the ecologically safety slow dissimilatory sulfate reduction for the irreversible precipitation of divalent metal cations in the form of sulfides as well as $\text{Co}(\text{OH})_2\downarrow$ and $\text{Ni}(\text{OH})_2\downarrow$.

Dynamics of microbiome growth via anaerobic fermentation of a starch-containing substrate, alanine and protein polymers

The process of precipitation of divalent heavy metals Co^{2+} and Ni^{2+} occurred during the fermentation of different types of substrates: potatoes, alanine, meat. The progress of the fermentation process is shown in Fig. 1.

The main parameters of fermentation of various substrates were obtained (Fig. 2): potatoes (Fig. 2a, b), alanine (Fig. 2c, d), meat (Fig. 2e, f). The initial values of pH, redox potential, concentration of dissolved organic compounds and ammonium ions were approximately the same. The injection of metals Co^{2+} and Ni^{2+} into the medium up to a final concentration of 100 mg/L had almost no effect on the main parameters of the process.

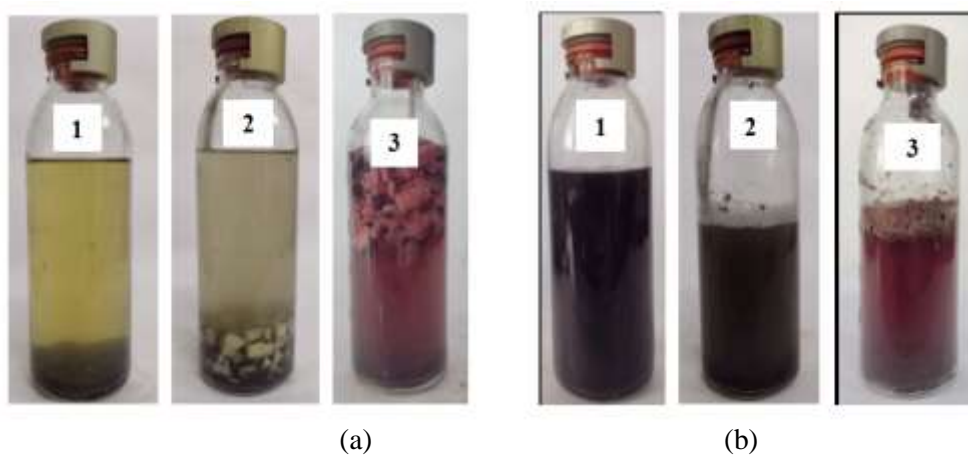
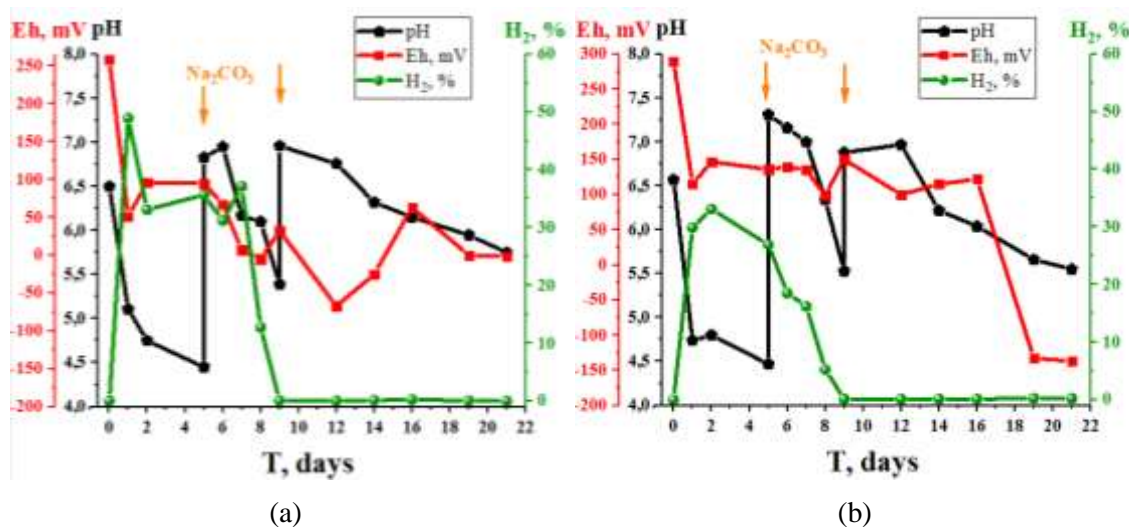


Fig. 1. Fermentation process: 0 day (a), 14 days (b); substrates: alanine (1), potatoes (2), meat (3)



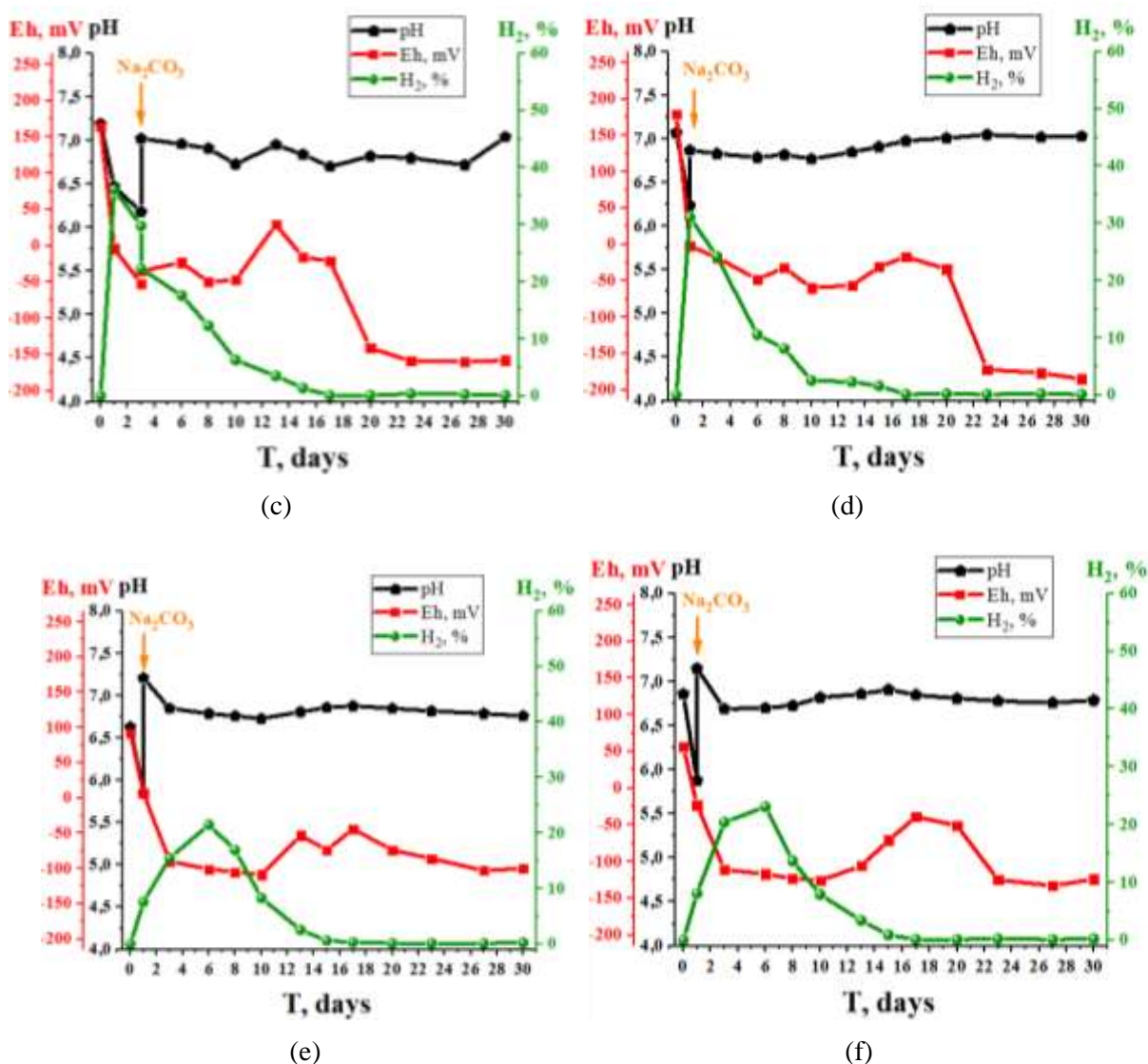


Fig. 2. The metabolic parameters of the fermentation: alanine without metals (a) alanine with metals (b); potatoes without metals (c) potatoes with metals (d); fermentation of meat without metals (e) and with metals (f).

The pH values of the medium during potatoes (Fig. 2a, b) fermentation decreased from 7.2 to 5.2 – 5.6. Therefore, the neutralization of the medium was carried out by the saturated Na₂CO₃ solution. The Eh values were within +20...+130 mV and finally decreased to –190 mV. The DOC was in the range of 150-270 mg/L. The concentration of NH₄⁺ in the medium at the end of cultivation was 1000 mg/L. The maximum concentration of hydrogen was 57.8% on the first day of cultivation, after which it decreased to 0.1%. After 12 days of cultivation, the process of methanogenesis began. The concentration of methane at the end of cultivation was 49%. The total volume of the synthesized gas for the fermentation of potatoes in the variants without metals and with

metals was 975 and 867 ml, respectively. The gas mixture consisted of H₂ and CO₂. The gas yield was calculated as the amount of H₂ and CO₂ (L) synthesized from 1 kg of DOC. Thus, the H₂ yield was 438.7 and 305.8 L/kg DOC, the CO₂ yield was 2538.7 and 2838.8 L/kg DOC for the fermentation of potatoes in the variants without metals and with metals, respectively.

During the fermentation of alanine (Fig. 2c, d), the pH value was in the range of 6.5 – 7.1. The Eh values were in the range of –50 to 0 mV. After 19 days the decreased to –190 mV. The concentration of DOC decreased from 405 to 140 mg/L. The concentration of ammonium nitrogen in the medium before the 21st day of cultivation was 4050 mg/L,

after which it decreased to 580 mg/L. The maximum concentration of hydrogen was 40.5% on the first day of cultivation, after which it decreased to 0.1%. After 18 days, the hydrogenic phase to the methanogenic phase. The final concentration of methane was 87.3%. The total volume of synthesized gas for the fermentation of alanine in the variants without metals and with metals was 433 and 191 mL, respectively. The H₂ yield was 374.6 and 137.1 L/kg DOC, the CO₂ yield was 578.9 and 255.3 L/kg DOC for the fermentation of alanine in the variants without metals and with metals, respectively.

The pH value of the medium at the meat fermentation (Fig. 2e, f) decreased from 7.3 to 5.96 on the first day of cultivation. Therefore, the neutralization of the medium was carried out by adding of saturated Na₂CO₃ solution. Then the pH value gradually increased to 6.8 – 7.1. The value of Eh decreased from +100 to –100 mV in the next 3 days and was in the range of –100 to –50 mV. The concentration of DOC substances was in the range of 440 – 540 mg/L. The concentration of NH₄⁺ in the

medium during cultivation was 7000 – 13000 mg/L. The maximum concentration of hydrogen was 26.8% on the first day of cultivation, after which it decreased to 0.07%. After 14 days, the process of methanogenesis began. The concentration of methane was 19% at the end of cultivation. The total volume of the synthesized gas for the fermentation of meat in the variants without metals and with metals was 599 and 670 mL, respectively. The H₂ yield was 371.3 and 338.3 L/kg DOC, the CO₂ yield was 618.8 and 563.8 L/kg DOC for the fermentation of meat in the variants without metals and with metals respectively.

The removal of metals due to the dissimilatory sulfate reduction

Compounds of alanine, potatoes and meat served as the electron donors, and the sparingly soluble calcium sulfate (gypsum) served as the terminal electron acceptor.

The dynamics of metals precipitation is shown on Fig. 3.

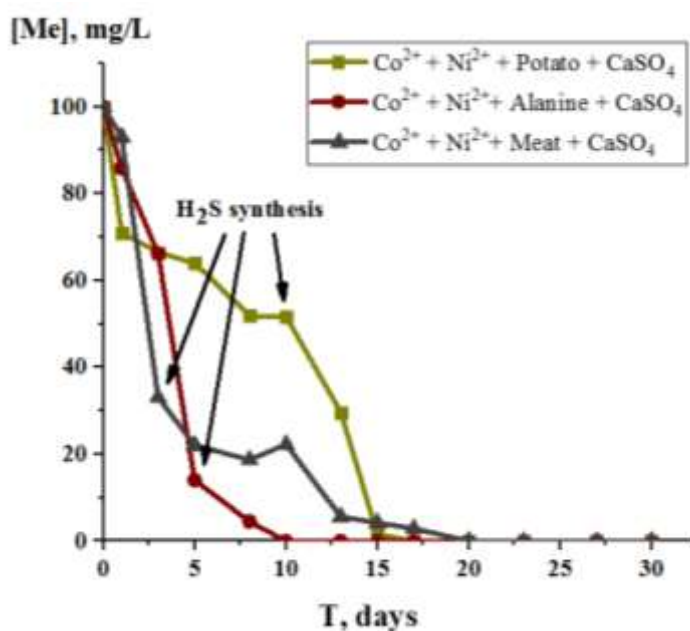


Fig. 3. Dynamics of metals precipitation using alanine, potatoes, and meat at the dissimilatory sulfate reduction.

The fermentation of alanine resulted in the complete precipitation of metals occurred after 9 days of cultivation. After 5 days, the medium in the bottle darkened indicating the formation of a precipitate of metal sulfides, and the concentration of metals decreased to trace concentration 5.5 mg/L.

The duration of Co²⁺ and Ni²⁺ precipitation in the medium with meat was 20 days. At the same time, the expected alkalinity was not observed, which should have occurred as a result of the ammonification processes and contributed to the acceleration of the precipitation of metals.

During potatoes fermentation, complete precipitation of metals occurred after 19 days of cultivation. A darkening of the medium was also observed on the 14th day, which indicated a decrease in the concentration of metals due to the formation of sulfides. During the fermentation of potatoes in this version, there was a problem of strong acidification of the medium, and, as a result, the need to adjust the pH to provide the efficient metal precipitation.

DISCUSSION

The advantages of our research are as follows. In our work, we used environmentally hazardous multicomponent waste as components of environmental biotechnologies: solid food waste is electron donor; the sludge from methane tanks is the source of effective microbiome; an ultra-cheap mineral – slightly soluble gypsum CaSO_4 is the electron acceptor. To increase the efficiency of the fermentation process, we did not use additional substrates or enzymes, which were currently common in the available literature. We used a diversified microbial community that consisted of different physiological groups of microorganisms, including aerobic and anaerobic ammonifiers and sulfate reducers capable of performing all stages of conversion of organic matter into the end products H_2 and CO_2 and S^{2-} (H_2S , SH^-). Similar studies are not available in scientific sources. This is the basis and perspective for the industrial approach of precipitation of toxic heavy metals.

In the literature, there are few variants of precipitation of heavy metals with the use of sulfate reduction. For example, dissimilatory sulfate reducing removal of Cu^{2+} , Fe^{2+} , Ni^{2+} and Zn^{2+} from mine drainage in an up-flow sludge blanket reactor in anaerobic condition. The bioreactor was operated for 500 days with ethanol as carbon and electron source. The effectiveness of metals removal was 99%. The sulfate reducing bacteria species identified in the column reactor were *Desulfovibrio desulfuricans* and *Desulfomicrobium baculatum* [33]. The effectiveness of zinc removal by sulfate-reducing bacteria *Desulfovibrio desulfuricans* was also proven [34]. The removal of zinc was 100% in 24 hours, which was much more effective than the application of the indigenous bacteria isolated from the mine area soil sample. The strain *Brevibacillus laterosporus* ZN5 was studied in the work [35], which could induce different carbonate precipitations of Pb(II) through ammonification and nitrate assimilation processes without the addition of urea. The minimum inhibitory

concentrations of Pb(II) to strain ZN5 in two processes were 150 and 100 mg/L, respectively. Strain could rapidly remove 94% and 70% of Pb(II) at an initial concentration of 15 mM through two processes, respectively. It was found that *E. huaxiensis* KHED8 was able to remove 89%, 90%, and 82.45% of Fe, Cd, and Cr ions, respectively, and the two isolates of *S. marcescens* subsp. *sakuensis* KH5M10 and KHCL12 were able to remove 90%, 95%, and 85.62% of Fe, Cd, and Cr, respectively [36]. In the article [37], the efficiency of the precipitation of heavy metals under the conditions of sulfate reduction by different variants of anaerobic biomass obtained from bioreactors was investigated. The removal efficiency of soluble heavy metals was in the order of $\text{Cu} > \text{Fe} > \text{Ni} > \text{Pb} > \text{Cd} > \text{Zn}$. At an initial metal concentration of 10 mg/L, the metal removal efficiency was about 95% for all biomass variants. When the concentration of metals increased to 50 mg/L, the indicators decreased significantly. At this initial metal concentration, over 77% removal of all metals was achieved with the biomass from a lab-scale upflow anaerobic-packed bed reactor (UFAR). Biomass from a lab-scale anaerobic-packed bed reactor (APR) resulted in 60% removal of cadmium and over 70% removal with other metals. Removal of copper, iron, lead and zinc was obtained using COS biomass of more than 65%, while removal of cadmium was only 46%. In the case of nickel – 60%. A sulfate-reducing bacterium named *Pseudodesulfovibrio cashew* SRB007 was isolated and purified from deep-sea cold seepage [38]. The minimum inhibitory concentration of heavy metals on *P. cashew* SRB007 was 2.5 mM, 2.5 mM, 2.0 mM, and 0.10 mM for Co^{2+} , Ni^{2+} , Cd^{2+} , and Hg^{2+} , respectively. The efficiency of heavy metal removal was 91.5%, 90.2%, 96.2% and 89.8% for Co^{2+} , Ni^{2+} , Cd^{2+} and Hg^{2+} , respectively, at the end of the fourth day. Deposition took place with the formation of characteristic precipitates of metal sulfides $\text{CoS}\downarrow$, $\text{NiS}\downarrow$, $\text{CdS}\downarrow$ and $\text{HgS}\downarrow$, which were determined using energy dispersive spectrometry.

The methods described in the literature are usually quite cumbersome, require complex technical support, can additionally pollute the environment, are not sufficiently effective and cannot be applied, considering the scale of the waste water generated. This indicates that this approach needs additional research.

CONCLUSIONS

The study confirmed the effectiveness of the slow ecologically safe sulfate reduction process for the irreversible precipitation of divalent metals, in

particular Co^{2+} and Ni^{2+} . It was shown the possibility of the microbial reduction of low-soluble gypsum for the slow production of non-toxic amounts of H_2S for the further complete precipitation of Co^{2+} and Ni^{2+} . This approach can be applied for the development of new biotechnologies for the treatment of wastewater contaminated with heavy metals. The use of sparingly soluble gypsum as an electron acceptor provides the release of hydrogen sulfide in amounts that are safe for ecosystems, but quite sufficient for complete precipitation of metals. The use of food waste (meat, potatoes) as the sources of carbon and energy ensures a long and stable process of sulfate reduction, and also contributes to the treatment of these ecologically hazardous compounds.

REFERENCES

1. Kumar V. et al., Global evaluation of heavy metal content in surface water bodies: A meta-analysis using heavy metal pollution indices and multivariate statistical analyses. *Chemosphere*, 124364, 2019.
2. Weissmannová, H.D., J. Pavlovský, Indices of soil contamination by heavy metals – methodology of calculation for pollution assessment (minireview). *Environ Monit Assess*, Vol. 189, 2017, 616.
3. Vongdala, N. et al., Heavy Metal Accumulation in Water, Soil, and Plants of Municipal Solid Waste Landfill in Vientiane, Laos. *Int. J. Environ. Res. Public Health*, Vol. 16, 2019, 22.
4. Gongadze A. et al., Accumulation and distribution of heavy metals in soils from the explosions by mining-processing poly-metallic plant, *Int. Multidisciplinary Sci GeoConf SGEM*, Sofia, Vol. 18, Iss. 3.2, 2018, 11-18.
5. Arce-Rodríguez A. et al., Pristine but metal-rich Río Sucio (Dirty River) is dominated by Gallionella and other iron-sulfur oxidizing microbes, *Extremophiles*, Vol. 21, 2017, 235–243.
6. Pilipović A. et al., Growth, physiology, and phytoextraction potential of poplar and willow established in soils amended with heavy-metal contaminated, dredged river sediments, *Journal of Environmental Management*, Vol. 239, 2019, 352–365.
7. Strzebońska M., E. Jarosz-Krzemińska, E. Adamiec, Assessing Historical Mining and Smelting Effects on Heavy Metal Pollution of River Systems over Span of Two Decades, *Water Air Soil Pollut*, Vol. 228, Iss. 141, 2017.
8. Heiman J.A. et al., Metal Accumulation in American Sycamores in a Mining-Contaminated River in Southeastern Missouri, *Water Air Soil Pollut*, Vol. 233, Iss. 125, 2022.
9. Zeng G. et al. Heavy Metal Accumulation and Release Risks in Sediments from Groundwater–River Water Interaction Zones in a Contaminated River under Restoration, *ACS Earth and Space Chemistry*, Vol. 4, Iss. 12, 2020, 2391–2402.
10. Leon Garcia G. J., D. Meza Figueroa et al., Study of Heavy Metal Pollution in Arid and Semi-Arid Regions Due to Mining Activity: Sonora and Bacanuchi Rivers, *International Journal of Environmental Sciences & Natural Resources*, Vol. 11, 2018, 1-11.
11. Zhang, G., J. Bai, et al., Heavy metal fractions and ecological risk assessment in sediments from urban, rural and reclamation-affected rivers of the Pearl River Estuary, China. *Chemosphere*, Vol. 184, 2017, 278–288.
12. Yuhu L., Environmental problems in the mining of metal minerals, *Conf. Series: Earth and Environmental Science*, Vol. 384, 2019.
13. Bankole M.T., A.S. Abdulkareem, et al., Selected Heavy Metals Removal from Electroplating Wastewater by Purified and Polyhydroxybutyrate Functionalized Carbon Nanotubes Adsorbents, *Sci Rep* Vol. 9, 4475, 2019.
14. Yadav M., R. Gupta, R. K. Sharma, Green and Sustainable Pathways for Wastewater Purification, *Advances in Water Purification Techniques*, 2019, 355–383.
15. Bolisetty S., M. Peydayesh, R. Mezzenga, Sustainable technologies for water purification from heavy metals: review and analysis, *Chemical Society Reviews*, Vol. 48, 2019, 463-487
16. Shrestha R., S. Ban, et al., Technological trends in heavy metals removal from industrial wastewater: A review, *Journal of Environmental Chemical Engineering*, Vol. 9, Iss. 4, 105688, 2021.
17. Sikder M. T., Y. Kihara, et al., River Water Pollution in Developed and Developing Countries: Judge and Assessment of Physicochemical Characteristics and Selected Dissolved Metal Concentration, *CLEAN – Soil, Air, Water*, Vol. 41, Iss. 1, 2012, 60–68.
18. Choudhary M., R. Kumar, et al., Bioremediation of Heavy Metals by Microbes, Bioremediation of Salt Affected Soils: An Indian Perspective, 2017, 233–255.
19. Kosolapov D. B., et al. Microbial Processes of Heavy Metal Removal from Carbon-Deficient Effluents in Constructed Wetlands. *Engineering in Life Sciences*, Vol. 4, Iss. 5, 2004, 403–411.

20. Pohl A. Removal of Heavy Metal Ions from Water and Wastewaters by Sulfur-Containing Precipitation Agents. *Water Air Soil Pollut* Vol. 231, 2020, 503
21. Bernard E. Igiri, Stanley I. R. Okoduwa, et al., Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review, *Journal of Toxicology*, Vol. 2018, 2568038, 2018.
22. Abdel-Shafy H. I., M. S. M. Mansour, Solid waste issue: Sources, composition, disposal, recycling, and valorization, *Egyptian Journal of Petroleum*, Vol. 27, Iss. 4, 2018, 1275-1290.
23. Nanda S., F. Berruti, Municipal solid waste management and landfilling technologies: a review, *Environ Chem Lett*, Vol. 19, 2021, 1433–1456.
24. Munir M. T., S. S. Mansouri, Resource recovery from organic solid waste using hydrothermal processing: Opportunities and challenges. *Renewable and Sustainable Energy Reviews*, Vol. 96, 2018, 64–75.
25. Bayard, R., H. Benbelkacem, et al. Characterization of selected municipal solid waste components to estimate their biodegradability. *Journal of Environmental Management*, Vol. 216, 2018, 4–12.
26. Dadrasnia A., M. M. Usman, et al., Microbial Aspects in Wastewater Treatment, A Technical Review *Environmental Pollution and Protection*, Vol. 2, No. 2, 2017.
27. Pourbaix M., Atlas of electrochemical equilibria in aqueous solutions, Houston: NACE International, Mater. Sci. Forum, 1974, 43–54.
28. Prekrasna I. P., O. B. Tashyrev, Copper resistant strain *Candida tropicalis* RomCu5 interaction with soluble and insoluble copper compounds, *Biotechnol. acta*, Vol. 8, No. 5, 2015, 93–102.
29. Havryliuk, O., V. Hovorukha, et al., Anaerobic Degradation of Environmentally Hazardous Aquatic Plant *Pistia stratiotes* and Soluble Cu(II) Detoxification by Methanogenic Granular Microbial Preparation, *Energies*, Vol. 14, 2021, 3849.
30. Acree W.E., Basic Gas Chromatography (McNair, Harold M.; Miller, James M.), *J. Chem. Educ.* 1998, 75.
31. Suslova, O., V. Govorukha, et al., Method for Determining Organic Compound Concentration in Biological Systems by Permanganate Redox Titration, *Int. J. Bioautom.* Vol. 18, 2014, 45–52.
32. Qiu, X., G.-P. Liu, Y.-Q. Zhu Determination of Water-Soluble Ammonium Ion in Soil by Spectrophotometry, *Analyst*, Vol. 112, 1987, 909–911.
33. Sahinkaya E., A. Yurtsever, et al., Biotreatment of As-containing simulated acid mine drainage using laboratory scale sulfate reducing upflow anaerobic sludge blanket reactor. *Minerals Engineering*. Vol. 75, 2015,133–139.
34. Hwang S. K., E. H. Jho, Heavy metal and sulfate removal from sulfate-rich synthetic mine drainages using sulfate reducing bacteria, *Science of The Total Environment*, Vol. 635, 2018, 1308–1316.
35. Dhir B., Biotechnological Tools for Remediation of Acid Mine Drainage (Removal of Metals from Wastewater and Leachate), *Bio-Geotechnologies for Mine Site Rehabilitation*, 2018, 67–82.
36. Ka-ot A. L., S. R. Joshi, Application of acid and heavy metal resistant bacteria from rat-hole coal mines in bioremediation strategy, *Journal of Basic Microbiology*, 2021, 1-9.
37. Gopi Kiran M., K. Pakshirajan, G. Das, Heavy Metal Removal Using Sulfate-Reducing Biomass Obtained from a Lab-Scale Upflow Anaerobic-Packed Bed Reactor, *Journal of Environmental Engineering*, Vol. 142, Iss. 9, 2016.
38. Zheng R., S. Wu, C. Sun, *Pseudodesulfobrio cashew* sp. Nov., a Novel Deep-Sea Sulfate-Reducing Bacterium, Linking Heavy Metal Resistance and Sulfur Cycle, *Microorganisms*, Vol. 9, Iss. 2, 2021, 429.

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