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Evaluation of Sulfate-Reducing Bacteria to Precipitate Mercury from Contaminated Groundwater

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Abstract

Several regions in the Republic of Kazakhstan are contaminated with mercury as a result of releases from industrial plants. Operations at an old chemical plant, "Khimprom", which produced chlorine and alkali in the 1970s-1990s, resulted in significant pollution of groundwater and surface water with soluble mercury compounds near a suburb of the city of Pavlodar. This mercury contamination poses a considerable risk to the local population and the environment. The objective of this study was to investigate the efficacy of using sulphate-reducing bacteria to precipitate mercury from contaminated groundwater, without forming methyl mercury, as an avenue to mitigate the contaminatation in groundwater at the Khimprom plant site. Sulfate-reducing bacteria were cultured from soil collected at the site and tested in laboratory experiments. The efficiency of two strains, IMV8a and IMV12 identified as similar to *Desulfotomaculum* spp., to precipitate soluble mercury was investigated. In experiments conducted at 4 °C and

at 28 °C using media with acetate as the carbon and energy source, mercury was precipitated from the media without forming methyl mercury. Acetate-utilizing sulfate-reducing bacteria might provide a basis for the biological removal of mercury from groundwater, however much additional research remains to assure it would be a safe process.

Keywords: mercury, sulphate-reducing bacteria, methyl mercury

1. Introduction

Since mercury is highly toxic and accumulates in organisms, particularly in fish that may be ingested by humans, it is one of the most important and well-studied pollutants. Factors that control the bioconcentration of mercury through the food chain in various environments are still not fully understood [1]. Elemental mercury (Hg^0) efficiently volatilizes to the environment and even remote areas show evidence of mercury pollution originating from industrial sources such as power plants and factories. Besides Hg^0, the main forms of mercury in water are soluble compounds of inorganic mercury (II) (chlorides, sulfates, or complexes of organic acids) and also organic mercury, mainly, MeHg. MeHg rather than inorganic mercury can be bioconcentrated because it is better retained by organisms at various levels in the food chain. The mercury cycle is one of the most well studied examples of microbial metabolism affecting the chemical form of a heavy metal. Microbial activity is connected with methylation of mercury, demethylation, oxidation and reduction of inorganic mercury [2] (Eq. 1).

$$\mathbf{CH_3Hg\ * \leftrightarrow Hg\,(II) \leftrightarrow Hg^0} \quad \mathbf{(1)}$$

The chemical transformation of mercury by bacteria is a secondary process of normal metabolism that is necessary for the bacteria to survive. The mechanisms of bacterial resistance are based on transformations of the mercury [3]. The key factor determining the concentration of mercury in biota is the concentration of MeHg in water, which is controlled by the relative efficiencies of methylation and

demethylation processes. It has been shown in pure culture experiments that sulfate-reducing bacteria (SRB) both methylate and demethylate mercury, while methanogenic bacteria only catalyze the demethylation of mercury, and acetogens neither methylate nor demethylate mercury [4].

Anoxic waters and sediments are important sources of MeHg, apparently as the result of the methylating activity of (SRB) [4-9]. Field observations and experiments with natural samples conducted by Gilmour and Henry [10] showed that methylation by *Desulfovibrio desulfuricans*, an SRB, increased with sulfate concentrations up to 200-500 μM and decreases at higher concentrations. At the same time King *et al*. [11] found that rapid accumulation of CH_3Hg was coupled with rapid sulfate-reduction in marine sediments rich in organic matter and dissolved sulphide.

Benoit *et al*. [12, 13] developed an equilibrium model for Hg complexation in sediments with sulfidic pore waters. The model indicated that $HgS^0_{(aq)}$ is the dominant dissolved neutral mercury complex determining lipid-solubility in sulfidic solutions at near neutral pH. The concentrations of neutral dissolved mercury complexes decreased with increasing sulfide concentration, which is consistent with observed patterns of MeHg production and accumulation in aquatic ecosystems. These results support the hypothesis that the passive uptake of neutral dissolved Hg-S complexes may control the bioavailability of Hg to methylating bacteria. In surface waters, MeHg may originate from anoxic layers or be formed through still unknown biological or chemical processes [1]. These observations have broad implications for understanding the control of CH_3Hg formation and for developing remediation strategies for Hg–contaminated groundwater.

Regions in the Republic of Kazakhstan are contaminated with mercury as a result of losses from chlor-alkali plants. Ingress of mercury into the environment has resulted in significant pollution of groundwater and surface water by soluble mercury compounds. Such contamination poses considerable risks to the populations of these regions. In particular, the Northern outskirt of Pavlodar city is contaminated with mercury as a result of activity of a chemical plant, Khimprom,

which produced chlorine and alkali in the 1970s to the 1990s. Contaminated ground water is moving towards the Irtysh River which is located 5 km from the plant. There are also high levels of mercury in the waters of Lake Balkyldak into which the Khimprom plant had disposed wastewater [14]. In 2004, Phase I of demercurization of the Khimprom plant, designed to contain and isolate the main sources of mercury pollution, was completed. However, the area surrounding the plant will continue to be a source of $HgCl_2$ ingress into groundwater. A 0.65 km^2 plume of mercury contaminated groundwater containing up to ten tons of mercury has spread 2.5 km from the site of the electrolysis workshop which was one of the main sources of contamination. This groundwater continues to be an environmental health risk and warrants consideration of remedial actions in order to prevent soluble mercury from entering into both the Irtysh River food chain and drinking water supplies.

A trench containing ground, discarded automobile tires placed in the flow of contaminated groundwater provided an efficient adsorbent of mercury [15]. The utility of such a system might be biologically enhanced, or it may be that treating a larger area of the contaminant plume becomes necessary. In the latter case, it would be advantageous to have a treatment suited to injection below the ground.

The objective of this study was to investigate the efficacy of using SRB to precipitate mercury, without forming MeHg, as an avenue to mitigate the contaminated groundwater at the Khimprom plant site near Pavlodar.

2. Materials and methods

Nine strains of SRB were isolated from the soils and sediments surrounding the Khimprom plant. The strains were isolated using a modified Postgate B medium [16] with either lactate or acetate as the electron donor.

The nine strains were tested for their ability to grow and produce H_2S in MPB media which contained lactate or acetate and 1000, 4000 or 10,000 μg l^{-1} $HgCl_2$. All strains grew under these conditions. Two SRB strains, IMV12 and

IMV8a, which grew in media with either lactate or acetate were selected from the collection for further work.

The ability of strains IMV12 and IMV8a to precipitate mercury was determined by measuring the amount of mercury remaining in the culture medium following growth. Mercury sulfide precipitants and cells were removed from the medium by filtration first through an ashless filter followed by filtration through a 0.45μm filter.

Colonization of support materials by SRB strains IMV12 and IMV8a was investigated. The bacteria were grown on modified MPB media and placed into bottles containing a sterile support material under anaerobic conditions. Claydite, synthetic polyester fiber and sand were used as support materials. Immobilization of bacteria on the support materials was allowed to proceed at 28^{o}C for one day.

Total mercury in water samples was determined on a Millennium Merlin cold vapor atomic fluorescent spectrometer (CVAFS) (P.S. Analytical Ltd., U.K.) using the manufacturer's procedure. MeHg was determined by extracting it from acidified solutions into toluene with subsequent re-extraction into an aqueous solution of sodium thiosulfate, digestion by a bromide-bromate mixture and its subsequent detection as total mercury. Sulfide was determined by iodine titration [17].

3. Results and discussion

3.1. Resistance of SRB to $HgCl_2$

Activity of SRB strains IMV8a and IMV12 in the presence of mercury was studied in MPB medium containing either lactate or acetate with 1,000, 4,000 and 10,000 μg l^{-1} $HgCl_2$. Bacterial activity was determined from the amounts of sulfide produced (Table 1).

Table 1. Amounts of sulfide (μg l^{-1}±S.D., n=3) detected after nine days growth by SRB, and uninoculated controls, in media with lactate or acetate in the presence various concentrations of mercury.

	Lactate			Acetate		
$HgCl_2$ in Medium (μg l^{-1})	Strain IMV8a	Strain IMV12	control	Strain IMV8a	Strain IMV12	control
0	111±34.2	63±6.4	0	9±3.9	9±0.0	0
1,000	95±2.1	52±8.6	0	15±8.6	21±7.0	0
4,000	134±9.4	75±3.2	0	20±6.1	24±9.8	0
10,000	102±32.5	66±10.6	0±0.6	25±2.1	25±11.2	0

The strains grew well on lactate and produced up to 134 mg l^{-1} sulfide after 9 days of growth at 28°C. The strains did not grow as well on MPB medium with acetate, but still formed H_2S up to 25 mg l^{-1}**.** Some mercury would be expected to be precipitated by the sulfide present in the medium at the start of the incubation. Indeed, mercury concentrations measured in the filtrates and outflows of uninoculated controls were substantially lower than the nominal level. However, media prepared with 10,000 µg l^{-1} $HgCl_2$ still contained 1,500 $\pm$ 770 µg l^{-1} soluble mercury (Table 2).

Table 2. Total and methyl mercury in culture media filtrate after growth of SRB. Strains were grown with lactate and $HgCl_2$ for 12 days. Uninoculated media and media without mercury were run in parallel. Values for concentrations are averages ($\mu g\ l^{-1} \pm$ S.D.) for controls (n=8) and SRB cultures (n=4).

	Uninoculated control		SRB strain IMV8a		SRB Strain IMV12	
$HgCl_2$ ($\mu g\ l^{-1}$) in culture medium	total Hg	MeHg	total Hg	MeHg	total Hg	MeHg
0	1.6 $\pm$ 1.00	<0.5	2.2 $\pm$ 1.20	< 0.5	< 1.0	< 0.5
10,000	1500 $\pm$ 770	13.8 $\pm$ 23.45	2.4 $\pm$ 0.56	1.1 $\pm$ 0.40	11.5 $\pm$ 3.04	1.1 $\pm$ 0.40

Thus, activity of SRB in media made with the highest mercury concentration.

3.2. Precipitation of mercury

Experiments with SRB strain IMV12 were carried out in order to compare the efficiencies of SRB grown in MPB medium with either lactate or acetate to precipitate mercury. Substantially more mercury remained in the filtrate of cultures grown in the medium with lactate. The mercury detected in the filtrate is likely either an organic form of mercury or a soluble sulfide. When grown in media with acetate, soluble mercury was at the detection limit level of the analysis.

Soluble mercury was therefore either absent from filtrate or produced in a minimal quantity. Thus, growth on acetate limited formation of soluble mercury which may be related to the low concentration of sulfide and the physiology of SRB growing on acetate [11-13, 19, 20]. These results indicate that SRB strains IMV8a and IMV12 are capable of producing sulfide in amounts sufficient for precipitating mercury. Further, the amounts of sulfide produced are far in excess of what would be required to precipitate 1000 μg l^{-1} mercury, the highest concentration encountered at the site.

3.3. Production of MeHg by SRB

Since MeHg bioconcentrates, its formation during bioremediation must be avoided. Therefore, the potential for SRB strains IMV12 and IMV8a to produce MeHg was investigated. The strains were inoculated into modified MPB media containing lactate and 10,000 μg l^{-1} $HgCl_2$. The high mercury concentration was used to attain a high concentration of soluble mercury. Filtrates from the cultures and controls without bacteria were analyzed for MeHg and total mercury after 12 days (Table 2).

Total soluble mercury in the uninoculated controls, as previously, was 1500 $\pm$ 770 μg l^{-1}. Growth of strain IMV8a lowered the soluble total mercury concentration to 2.4 $\pm$ 0.56 μg l^{-1}, which is equivalent to levels measured in the medium without added mercury, and strain IMV12 lowered the concentration to 11.5 $\pm$ 3.04 μg l^{-1}. MeHg levels in the SRB cultures were low and slightly above the detection limit for the analysis. Relatively high and variable concentrations of MeHg were

detected in the uninoculated contols. The MeHg may have been formed by abiotic processes as recently described by Celo et al. [21]. Yeast extract is rich in B vitamins including methylcobalamin (B_{12}) which can serve as a methyl donor to mercury in abiotic reactions. The very low concentrations of MeHg detected in the SRB culture filtrates suggest that the strains do not have the capacity to methylate mercury. Both strains have been identified as Gram-positive SRB, similar to *Desulfotomaculum* spp. Little information is available on mercury methylation by Gram-positive SRB.

3.4. Activity of SRB on support materials

One possible application of using SRB to remove mercury from groundwater would be in the form of a reactive barrier. The barrier would consist of a support material colonized by SRB producing sulfide that would precipitate mercury. Another possibility would be injecting bacteria into the groundwater to treat a more expansive area of a mercury plume. In that case, after an initial dispersion throughout the treatment area, the bacteria would need to colonize soil and sand particles and remain active. Therefore, the capacity of SRB strains IMV8a and IMV12 to colonize claydite, synthetic fiber and sand was investigated. Flasks containing media and the substrates were inoculated with the strains and incubated to allow time for the bacteria to adhere to the substrates. The medium was replaced with fresh medium and the activity of the strains was evaluated based on the production of sulfide. Strain IMV8a grown on claydite or synthetic fiber produced up to 10 and 20 mg l^{-1} H_2S, respectively (Figure 1).

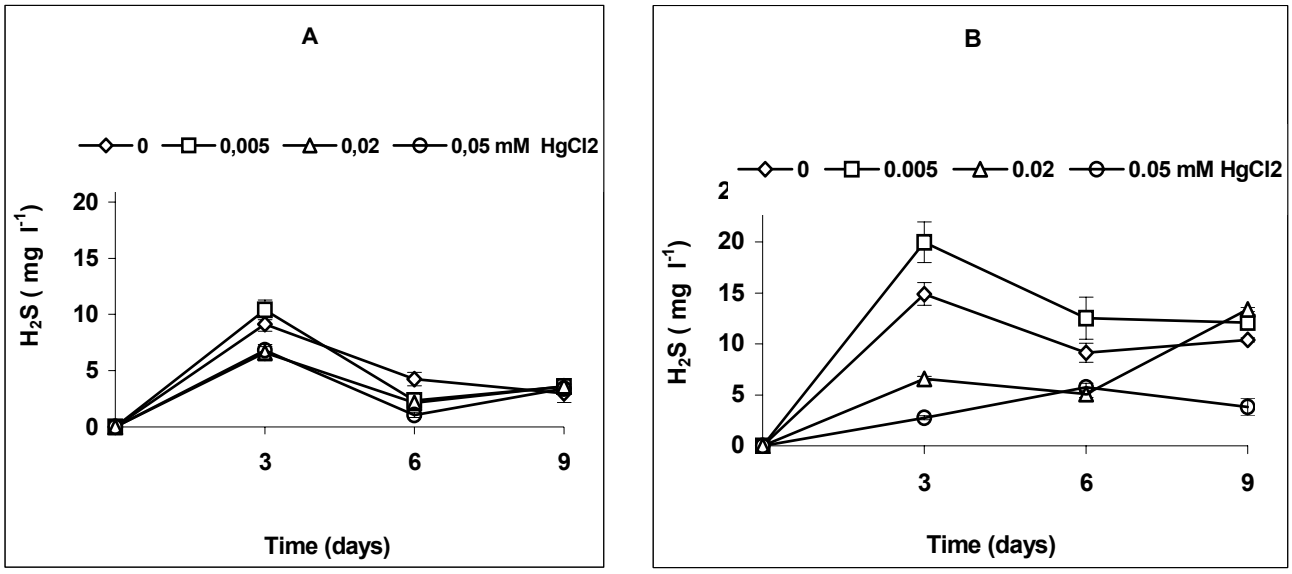

Figure 1. Production of H_2S (mg l^{-1}) by SRB strain IMV8a colonized claydite and synthetic fiber when growing in a media containing acetate. A – Claydite; B - Synthetic fiber.

Sulfide production appeared to be inhibited by increasing concentrations of mercury which was most evident with strain IMV8a on synthetic fiber. Sulfide production with strain IMV12 on claydite and synthetic fiber was similar to that of strain IMV8a, although inhibition due to increasing concentrations of mercury was not as apparent (Fig 2).

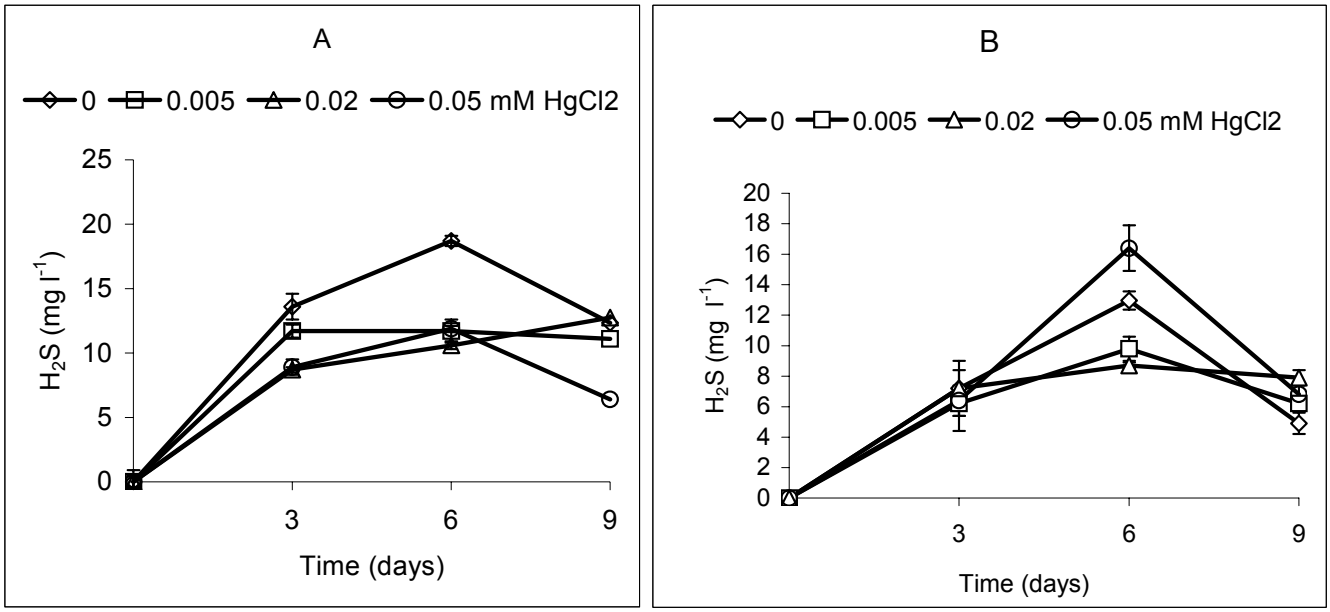

Figure 2. Generation of H_2S (mg l^{-1}) by SRB strain IMV12 colonized synthetic fiber and claydite when growing in a media with acetate. A – Claydite; B - Synthetic fiber.

Strains IMV8a and IMV12 colonized onto sand and exposed to 10,000µg l^{-1} $HgCl_2$ generated concentrations of sulfide comparable with the concentrations attained when the strains were on claydite and synthetic fiber.

Sulfide production during these experiments suggests the presence of $HgCl_2$ in the media did not prevent colonization of claydite, synthetic fiber or sand by the bacteria. A dark film developed on all three support materials which may have contained mercury sulfide in addition to ferrous sulfide. The results suggest the three support materials tested would provide suitable support for colonization by SRB in a reactive barrier. Further, the colonization of sand suggests the strains would also colonize the soil if injected into a mercury contaminated plume.

3.5. Effect of temperature on precipitation of mercury by SRB

Strains IMV8a and IMV12 produced maximum sulfide concentrations of 32 and 38 mg l^{-1}, respectively, in reactors run at 18-20^{0}C (Fig. 3).

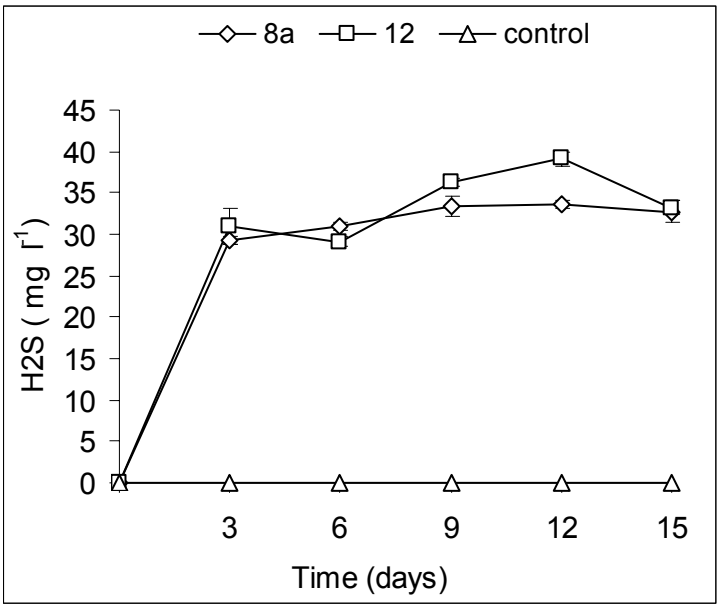

Figure 3. Sulfide concentration (mg l^{-1}) content in the out flow of reactors with SRB colonized synthetic fiber run at 18 – 20^{0}C. The concentration of $HgCl_2$ in the solution entering the reactors was 1000 μg l^{-1}.

These concentrations are lower than what was achieved in batch cultures without synthetic fiber and likely reflect the replenishment of the media. Sulfide was not detected in uninoculated control reactors. The concentrations of sulfide formed in these reactors were still sufficiently high to precipate mercury from the media (Fig. 4).

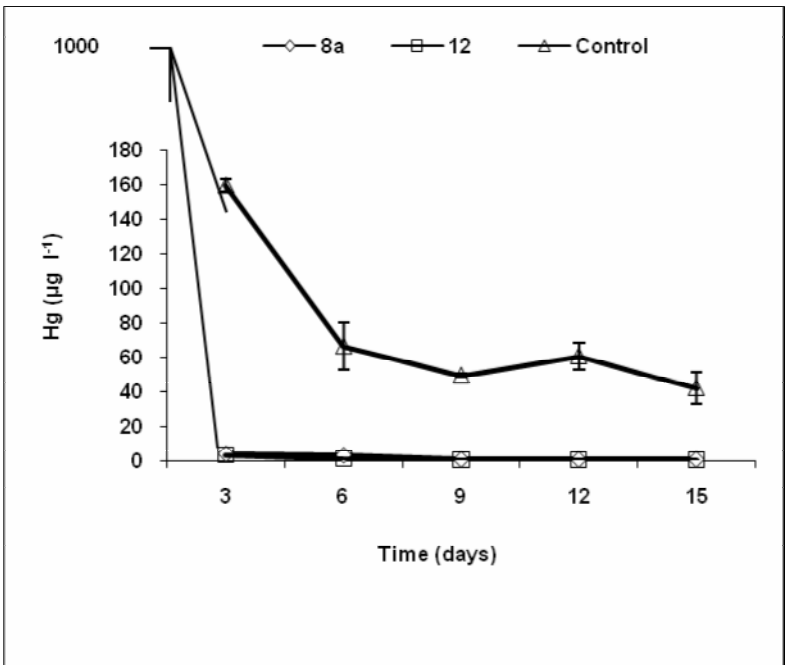

Figure 4. Total mercury content (µg l^{-1}) in outflow the outflow of reactors with SRB colonized synthetic fiber run at 18 – 20°C. The concentration of $HgCl_2$ in the inflow solution entering the reactors was 1000 µg l^{-1}. A and B are at different scales of total mercury.

Mercury concentrations in the outflows of the reactors dropped to near 0.5mg l^{-1}, 100 times lower then with the control reactor run without bacteria. The results of the experiment carried out at 4°C suggest that SRB colonized synthetic fiber produced lower amounts of H_2S at 4°C than at 28° C, but even this amount of H_2S is enough to precipitate mercury effectively.

During SRB growth in a medium with $HgCl_2$, sulfide produced by the bacteria will precipitate mercury in the medium as mercury sulfide. Since the stoichiometery of mercury precipitation with sulfide is 1 mole: 1 mole, 1.60 mg l^{-1} sulfide would need to be attained in order to precipitate 10,000 µg l^{-1} mercury from solution, the highest concentration used in this study. The amounts of sulfide produced by SRB in these studies were significantly higher. Thus, the SRB strains were capable of growth at 4°C and 18-20°C in a medium with acetate and produced H_2S in an amount sufficient for precipitating soluble mercury as mercury sulfide. Ground water is expected to have low temperatures so activity at low temperatures

would be required for any treatment. These bacteria can be recommended for additional studies to determine their use in biological treatment of mercury-contaminated groundwater. The potential of these SRB to produce only low amounts of H_2S, still sufficient to precipitate soluble mercury in groundwater, is advantageous as the process of bioremediation would not entail formation of considerable amounts of H_2S.

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References

1. M.M. Morel, M.L. Kraepiel, M. Amyot, The chemical cycle and bioaccumulation of mercury, Annu. Rev. Ecol. Syst. 29 (1998) 543-566.
2. T. Barkay, I. Wagner-Dobler, Microbial transformations of mercury: potentials, challenges, and achievments in controlling mercury toxicity in the environment, Adv. Appl. Microbiol. 57 (2005) 1-52.
3. T. Barkay, S.M. Miller, A.O. Summers, Bacterial mercury resistance from atoms to ecosystems, FEMS Microbiol. Rev. 27 (2003) 335-384.
4. K.-R. Pak, R. Bartha, Mercury methylation by interspecies hydrogen and acetate transfer between sulfidogens and methanogens, Appl. Environ. Microbiol. 64 (1998) 1987-1990.
5. G.C. Compeau, R. Bartha, Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment, Appl. Environ. Microbiol. 49 (1985) 498-502.
6. G.C. Compeau, R. Bartha, Effect of salinity on mercury methylation activity of sulfate-reducing bacteria in estuarine sediments, Appl. Environ. Microbiol. 53 (1987) 261-265.
7. R. Devereux, M.R. Winfrey, J. Winfrey, D.A. Stahl, Depth profiles of sulfate-reducing bacterial ribosomal RNA and mercury methylation in an estuarine sediment, FEMS Microbiol. Ecol. 20 (1996) 23-31.
8. S.M. Ulrich, T.W. Tanton, S.A Abdrashitova, Mercury in the aquatic environment: a review of factors affecting methylation.Crit. Rev. Environ. Sci. Technol. 31 (2001) 241-293.
9. S.-C. Choi, T. Chase, R. Bartha R, Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS, Appl. Environ. Microbiol. 60 (1994) 1021-1034.
10. C.C. Gilmour, E.A. Henry, Mercury metyhlation in aquatic systems affected by acid deposition, Environ. Pollut. 71 (1991) 131-169.

11. J.K. King, J.E. Kostka, M.E. Frischer, F.M. Saunders, Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and on marine sediments, Appl. Environ. Microbiol 66 (2000) 2430-2437.
12. Benoit J.M., Gilmour C.C., R.P. Mason, A. Heyes, Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pure waters . Environ. Sci. Technol. 33 (1999) 951-957.
13. J.M. Benoit, C.C. Gilmour, R.P. Mason, Aspects of bioavailability of mercury methylation in pure cultures of *Desulfovibrio propionicus*, Appl. Environ. Microbiol. 67 (2001) 51-58.
15. T.W. Tanton, V.V. Veselov, M.A. Ilyushchenko, V.Yu. Panichkin, Risk assessment from mercury contamination of northern industrial site of Pavlodar city, National Acad. Sci. Republic of Kazakhstan. 4 (2003) 78-81
16. X. Meng, Z. Hua, D. Dermatas, W. Wang, H.Y. Kuo, Immobilization of mercury(II) in contaminated soil with used tire rubber, J. Hazard. Mater. 57 (1998) 231-241.
17. J.R. Postgate, The Sulphate-Reducing Bacteria, second ed., Cambridge Univ. Press, Cambridge, 1984.
18. Chemical analysis of industrial wastewater. ed. Yu. Lurie, Khimiya Publishers, Moscow, 1974, 335 p. (Ru)
19. J.M.Benoit, C.C.Glimour, R.P.Mason, The influence of sulfide on solid-phase mercury bioavailability for methylation by pure cultures of *Desufobulbus propionicus* (1pr3), Environ. Sci. Technol. 35 (2001) 127-132.
20. J.M. Benoit, C.C.Gilmour, R.P.Mason, Aspects of Bioavailability of Mercury for Methylation in Pure Cultures of Desulfobulbus propionicum(1pr3), Appl. Environ. Microbiol. 67 (2001)51-58.
21. V. Celo, D.R.S. Lean, S.L. Scott, Abiotic methylation of mercury in the aquatic environment, Sci. Tot. Environ. 368 (2006) 126-137.