Heavy metal immobilization in groundwater by in situ bioprecipitation: comments and questions about carbon source use, efficiency and sustainability of the process

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ABSTRACT

About 45% of the contaminated sites are dealing with heavy metal problems. An important groundwater remediation measure is the immobilization by inducing sulfate reducing bacteria to transform the sulfates, that are very often present in the same groundwater (due to the metal mining or processing activities), into sulfides. These sulfides will precipitate the metals as insoluble metal sulfides. At the moment several studies have demonstrated the feasibility of this *In Situ* Bioprecipitation Process (ISBP) as well at lab scale (batch and column tests) as at field scale. However some questions arise concerning the continuation of the process, the efficiency and the sustainability of the precipitates. The presented study will try to answer these questions. The study is based on more than 12 different studies, all done by the same authors, on different groundwaters and aquifer samples.

As carbon sources mostly molasses, lactate, acetate, compost, HRC® and MRC® are used to induce the ISBP. Economical reasons drive people to use cheap waste products. However some questions arise concerning the use of these carbons sources. Some of them (e.g. molasses) seem to have high metal complexing characteristics and keep the metals in an organic matrix more adsorbed to the aquifer compared to a real metal sulphide precipitate. Also the fermentation of molasses leads primarily to a pH decrease resulting in some heavy metal release from the aquifer. Acetate on the other hand induces only some sulfate reducers and the induction is a relatively slow process (in one case an induction time of 150 days was observed).

In some cases it was observed that the ISBP stopped after some time. The hypothesis of acetate intoxication will be explained. It seems that acetate, which is also a degradation product of fermentation of some carbon sources, can inhibit some sulfate reducers. Also the relation between slow release compounds and immediately available compounds will be explained.

An important question in the ISBP is related to the sustainability of the heavy metal precipitates. It could be observed that the interruption in the carbon source delivery also stopped the *in situ* bioprecipitation process. Depending on the metal also some release from the precipitate could be observed. It was shown that release can occur with Co and Ni. Aquifer, on which the metals precipitated during the ISBP, were treated in a sequential extraction process under anaerobic and aerobic conditions. It could be shown that especially under the anaerobic conditions the Zn and Cd precipitates changed from the leachable and the exchangeable fraction to the organic or Fe-Mn fraction (i.e. from the unstable to the stable fraction). The same could be observed for Co and Ni but still some fraction was present under a more available form. On the other hand it could be shown that large differences were observed concerning the heavy metal speciation on the aquifer in function of the used carbon source during the ISBP. The molasses treated systems showed a much more available heavy metal fraction on the aquifer compared to the lactate or HRC® treated groundwater. The different speciation forms based on the use of different carbon sources will be discussed in detail.

At the end also some general considerations on the use of the ISBP in different soil types (sand, clay, fractured rock, carbonate) and the influence of natural attenuation processes will be presented.

INTRODUCTION

The ISBP process was investigated for metals as Cu, Zn, Cd, Ni, Co, Fe, Cr, and As. The first field tests showed that ISBP is feasible as a strategy for sustaining groundwater quality (Geets et al. 2003; Ghyoot et al. 2004). However, some questions remain to be answered, especially about the pH increase due to molasses fermentation, the stability of the Ni and Co precipitates, the choice of electron donor, and the frequency of the electron donor injection.

In this paper an overview will be presented based on several other publications and many feasibility tests performed for different industrial clients. It is the result of studies for more than 20 different sites with different aquifer compositions, different concentrations of heavy metals and other components, different physico-chemical parameters etc. Although from our experience we can say that at appropriate redox potential and pH in nearly all aquifers we could induce the sulfate reducing process. The different parameters determining the quality, efficiency and sustainability of the ISBP-process will briefly be discussed.

DISCUSSION

The *sulfate concentration* is important in relation to the electron donor. If the sulfate concentration is low (< 100 mg SO_4^{2-}/l the sulfate reducing process will not start unless a sulfate reducing inoculum is added. Further at low sulfate concentration the sulfate reduction could only be started if hydrogen was added as electron donor. Diels et al. (2005d) showed the removal of Zn from a groundwater at a sulfate concentration of 74 mg SO_4^{2-}/l. In two conditions the sulfate reducing strain *Desulfovibrio desulfuricans* Ds8301 was added and only than Zn removal occurred. The same paper shows an ISBP-process at a sulfate concentration of 506 mg SO_4^{2-}/l. In this case the Zn is also removed without addition of Dd8301. On the other hand it was also shown that at a carbon concentration of 5 times the necessary carbon concentration the ISBP-process stopped. In the beginning we supposed that it was due to the fact the methanogenic conditions occurred. However when we looked to the redox potential we observed a much lower potential which presumably indicates that the sulfate reduction process in inhibited by the high acetate concentration. It was already observed (Caplice et al. 1999) that high acetate concentrations can inhibit the sulfate reduction process. The direct antimicrobial effects of organic acids including lactic, acetic and propionic acids which may be produced by lactic acid bacterial fermentation of foods, are well known. The antagonism is believed to result from the action of the acids on the bacterial cytoplasmic membrane which interferes with the maintenance of membrane potential and inhibits active transport, and may be mediated both by dissociated and undissociated acids. The antimicrobial activity of each of the acids at a given molar concentration is not equal. Acetic acid is more inhibitory than lactic acid and can inhibit yeasts, moulds and bacteria. This is one of the supposed reasons of the temporarily decrease in the sulfate reduction process observed in reactors and passive treatment systems. On the other hand to high concentrations of electron donor can also lead to methanogenic conditions. In some cases we observed high gas production and no metal precipitation process anymore. So the *carbon source concentration* plays also an important role. This is especially difficult in the case of carbon source injection. Temporarily high concentrations of electron donor can be present in the injection well and will lead to methane production. High concentrations of molasses could induce a pH decrease due to fermentation processes. A lowering of pH can lead to metal release from the aquifer into the groundwater.

High molasses concentrations also lead to the complexation of chromium (III) and hence its solubilization (Diels et al. 2005a). It turned out that chromium (VI) can be very easily reduced by several electron donors. However in some cases the chromium (III) stayed in solution due to complexation with some compounds of the carbon source. It could be indicated that increasing concentrations of molasses (but also protamylasse) decreased the precipitated chromium (III) concentration especially at a rather low pH of 6. Chromium (III) precipitation could be improved by increasing the pH up to 8.

Janssen et al. (2004) mentioned the need of SRB medium to induce the SRBs.

Vanbroekhoven et al. (2005) showed also that molasses induced the ISBP-process very fast, but failed in the following weeks in maintaining the sulphate reduction. Even metal concentrations quickly increased in the column's effluent. The HRC® and lactate gave rise to a more continuously process. However also a difference could be observed between the HRC® induced process and the lactate induced process. In the last one besides lactate also a mixture of nitrogen and phosphorus were added and this supported very strongly the ongoing of the process indicating that nutrients and perhaps on a long term also micro-elements are necessary to keep the process on track.

A wide range of *electron donors* has been proved to be useful in the process, varying from expensive and pure substrates such as ethanol (Kalyuzhnyi et al., 1997), lactate (Hammack et al., 1992), and hydrogen (van Houten et al., 1994) to economically more favorable waste products with or without enrichment with pure substrates or inoculating with monocultures or media (manure, sludge, soil) containing SRBs (Maree, et al., 1987, Annachhatre et al., 2001, Prasad et al., 1999). Acetate, a rather cheap carbon source can also be used. However only few SRBs, like *Desulfobacca (Oude Elfereink et.al.,1999)*, *Desulfobacter (Skerman et. al., 1980)* and *Desulfovibrio* can assimilate this carbon source. In that way it could be indicated that using acetate as single electron donor lead to very long induction periods. In some cases more than 150 days were necessary to start the sulfate reduction process. Therefore it is suggested to *combine electron donors* that easily induce the process as lactate and molasses with cheap carbon sources as ethanol and acetate. Several carbon sources are used in a two step system. Molasses (and other waste products) or polylactate (on the market under the name of HRC® or MRC®) must be first fermented to small volatile fatty acids and hydrogen gas. The hydrogen gas and in some cases also some fatty acids serve as electron donor. In this way the HRC® or MRC® are mentioned as *slow release compounds* and can be used as a long lasting electron donor source. In experiments where we added molasses and HRC® it turned out that the molasses did not work for a long time. Pulles et al. (2004) mentioned that the sulfate reduction process is not always stable. It starts quickly and drops down and starts again. Presumably some sugars in the molasses are degraded too fast and than it takes a while to generate new useful electron donors. And care must be taken that this process will not start already in the stock recipient. The polylactate however lasted much longer (evaluation is still going on). Slow release compounds have the advantage of removing very efficiently all the oxygen and decreasing in that way the redox potential and have no need for regular injection. The slow release compounds seem to last for at least 6 months (study is going on).

Sulfate reducing bacteria can be induced at a *pH between 4 and 8*. At neutral pH SRBs from different origin could be detected (Groudev et al. 2005). Temminghof (2005) mentioned that at a location in The Netherlands (Dieren) with an initial pH of 3.9 the ISBP process could only be started after addition of an SRB inoculum. At low pH only *Desulphotomaculum* and *Desulphosporosinus* could be detected (Geets et al. 2005). It could be indicated by Johnson (personnal communication) that especially at low pH always *Desulphosporosinus* and *Desulphotomaculum* were observed.

Molecular biology is an excellent branch that can be used to identify the SRBs and study their diversity. Either 16S rRNA gene based primers can be used, either *dsr*-based (dissimilatory sulfite reductase) primers. It could be concluded that only by using *dsr*-based DGGE a real biodiversity could be observed (Geets et al. 2005).

A *redox potential < -200 mV* is necessary to induce the SRBs. Temminghof (2005) used Na_2S to reduce the Eh. Diels et al. (2005b) used other redox manipulating compounds to decrease the Eh especially in the case of low pH.

Competition between sulfate reducing bacteria and iron reducing bacteria is also of high importance. The energy benefit of sulfate reducing bacteria is only -0.10 kcal/mol e, while this is -10.49 kcal/mol e

For iron reducing bacteria. The ΔG for chromate reducing bacteria is also -10.76 kcal/mol e. It was very often observed that when the redox potential could not be decreased to low values denitrifying or iron reducing bacteria were observed to be active. At very low metal concentrations denitrifying conditions, induced for instance with acetate as electron donor, lead to very good Cd and Zn removal. This process turned out to be a rather natural attenuation process that can occur in diffusely polluted areas (Seuntjens 2005) when some organic carbon degradation products are available. Also under conditions of iron reduction some metals seem to be removed from the groundwater due to the co-precipitation processes. Especially arsenic can be removed or immobilized in that way.

The *soil composition* influenced in some way the ISBP-process. Comparative tests on a same groundwater from sandy and clay layers of a same site indicated that both aquifer compositions lead to the induction of the metal precipitation process. However the sandy aquifer seemed to be induced at a much faster rate than the clay aquifer. This is presumably due to the limited diffusion of electron donor in the clay system. At the moment tests are going on a gravel aquifer.

In order to define the stability of the immobilized metals it is important to analyze the metal precipitates by *sequential extraction* (Tessier et al. 1979). A detailed study is presented by Diels et al. (2005c). A summary of some results is given below. An aquifer from a non-ferrous contaminated site is used in a column study. Groundwater from the site was pumped during two years over the aquifer loaded columns. The columns were treated with different electron donors including molasses, and lactate. Besides lactate also lactate containing additional nutrients N and P (a mixture of ammonium nitrate and orthophosphate) was added in order to avoid nutrient limitation for the SRB population. Also a column without electron donor was operated. This column was representative for the Natural Attenuation process (NA). In the electron donor amended columns redox potential decreased to -250 mV, the initial pH of about 4 went up to 6 and most of the metals were removed. In the NA column nearly no metals were removed. Different carbon sources like molasses, HRC® and lactate (with of without N/P nutrients) promoted sulfate reduction within 8 weeks, with average sulfate-removal efficiencies of 50%. At the same time, substantial removal of Cd, Zn (at least 75%) and Co (at least 45%) took place, whereas attenuation of Ni was getting into its stride. In the following weeks, molasses failed in maintaining sulfate reduction, and metal concentrations quickly increased in the column's effluent. A few weeks later (after 15 weeks) the sulfate reduction process in the HRC® amended column also showed a decreasing efficiency towards metal removal, whereas the ISBP process seemed to be stable in the lactate amended columns (Vanbroekhoven et. al. 2005). At T2, after two years of operation, the columns were stopped and samples were taken at four places (C1, C2, C3 and C4) in the column. C1 is the inlet of the column and C4 is the outlet, and C2 – C3 intermediate. Different extractions were made in order to define the speciation of the metal: Leachable fraction (extraction with water), exchangeable fraction (extraction with $MgCl_2$), carbonate fraction (extraction with sodium acetate), Fe-Mn oxide fraction or reducible fraction (extraction with $NH_4OH.HCl$), organic or oxidizable fraction (extraction with NH_4-acetate) and the residual fraction. The sum of all the fractions was made and compared with a second sample treated by *aqua regia* in order to control the mass balance. It turned out that the mass balance was relatively good knowing that the aquifer was not homogeneously contaminated. There was a large difference (increase of metal deposition) between T0 and T2 as during those two years continuously metals were precipitated on the aquifer. The metal concentrations were highest at the inlet (C1) of the column as there the oxygen was first consumed and redox potential decreased. Leachable, exchangeable and carbonate fractions are decreased compared to the original situation. This indicates that all the metals move into a more tightly bound precipitate. Nearly all the metals are found in the reducible (Fe-Mn oxide) and mostly in the oxidizable fraction (organic). In fact the metal sulfide precipitates are expected in the oxidizable fraction.

Table 4 presents a comparison of sequential extraction for three columns operated under natural attenuation condition or with molasses or lactate (+N/P) as electron donor. The leachable and exchangeable fractions are stable in the NA column and are reduced in the molasses and lactate columns. However the reduction is always higher in the lactate column compared to the molasses column. The same is true for the carbonate fractions, but the differences are smaller. It is also observed that the Zn is precipitated in the reducible fraction and the highest amount can be recovered from the oxidizable fraction (ZnS). However in the case of Ni, it can be recovered in the reducible and mostly in the oxidizable fraction in the lactate amended column. Only very small amounts of Ni could be recovered from the aquifer of the molasses amended column. Here a large difference is observed between the use of molasses and lactate as electron donors. Lactate always tend to precipitate the metals in a more stable form and this especially true for Ni. Ni removal and stable precipitation is rather low in the case of molasses amended columns and optimal conditions could be obtained with lactate. Zn and Cd, both group IIb elements, showed the same behavior as well as Ni and Co, the latter being transition metals and belonging to group VIII.

In some further studies on sequential extraction were performed under aerobic or anaerobic conditions. These two methods performed on a same aquifer sample showed one big difference. The aquifer material sequentially extracted under aerobic conditions gave a rather large metal carbonate fraction in some cases. The anaerobic treated aquifer did not show a carbonate fraction. This difference still needs further research and elaboration.

In a final study some aquifer material was studied by Scanning Electron Microscopy. First a separation method was developed in order to enrich the fine precipitates. In the enriched samples sand particles could be observed with a metallic layer on it. After EDAX evaluation Zn and S were detected at equimolar concentrations indicating the presence of ZnS. As these tests were performed on aquifer samples from batch experiments the precipitates were rather small and below the detection level for X-ray diffraction analysis. The following study will focus on the metal precipitates of the higher mentioned columns (after a two year operation).

Table 4. Sequential extraction for Zn and Ni from aquifer derived material from columns operated with molasses or lactate as electron donor compared to natural attenuation

	Zn (mg/kg dm)			Ni (mg/kg dm)		
	NA	Molasses	Lactate N/P	NA	Molasses	Lactate N/P
Leachable						
T0	227	227	227	81	81	81
C1	267	71	16	93	77	103
C2	306	80	1	111	75	35
C3	292	77	1	108	75	12
C4	293	42	2	107	76	10
Exchangeable						
T0	809	809	809	22	22	22
C1	763	262	45	23	23	81
C2	890	308	0	31	37	46
C3	828	281	0	62	46	19
C4	720	155	0	21	39	17
Carbonate						
T0	93	93	93	22	22	22
C1	119	51	83	23	23	81
C2	124	38	9	31	37	46
C3	191	41	6	62	46	19
C4	75	20	2	21	39	17
Fe-Mn oxide						
T0	144	144	144	5	5	5
C1	189	689	445	189	11	126
C2	199	625	430	7	11	95
C3	198	572	381	7	12	39
C4	265	423	228	26	16	32
Organic						
T0	69	69	69	3	3	3
C1	69	3153	2652	4	6	3425
C2	83	2783	1594	5	7	1706
C3	91	1536	684	5	10	794
C4	99	2647	1217	8	44	530

In all the former experiments the electron donor was added in the batch with aquifer and groundwater or was pumped continuously with the groundwater through the columns. A new series of tests was performed by pumping just groundwater over columns, filled with aquifer. Some centimeters above the inlet an injection of molasses or HRC$^{®}$ was done at single stages. After the first injection with HRC$^{®}$ the redox turns negative and stays negative in contradiction to the molasses column where after a while the redox increased again. In both columns the pH decreases first and increases afterwards to a pH of more than one unit higher than the original groundwater pH. Sulfate decreased much better in the HRC$^{®}$ column compared to the molasses column. Also the formation of black iron sulfides could much better be observed in the HRC$^{®}$ column. Zn removal went faster with HRC$^{®}$. Indeed 10 days after injection Zn was removed by 100%. In the case of the molasses column 53 days after the second carbon source injection Zn removal starts to decrease.

The glucose concentration 10 cm above the injection point decreased in 6 days from 12mg/l to 3mg/l. Afterwards it stabilized between 3 an 6mg/l. At the exit of the column the glucose concentration increased in 14 days after injection from 5mg/l to 10mg/l. two weeks later it decreased again to 5mg/l. 11 days after the second injection the volatile fatty acids contained mostly acetate. However 14 days after injection this acetate could not be measured anymore. After the second injection lactate was measured 20cm above the injection point. Initially the concentration was very high (3911mg/l). But this concentration decreased slowly.

CONCLUSIONS

This paper focused on the further development of the ISBP-process combined with other techniques and with special attention to the longevity and sustainability of the process. Parameters as pH, redox potential, carbon source and sulfate concentration seem to play a very important role in the induction of the sulfate reduction process. On the other hand the selection of the electron donor seemed to be important for keeping the process going on. Presumably combinations of electron donors must be selected in order to induce and to keep the process. This was for instance suggested by Agathos et. al. (2005) for the dehalogenation process. They suggested to induce the process with lactate as it induced a broad bunch of dehalogenating bacteria. Later on the process could be kept going on by adding the much cheaper methanol. Some experiments also showed that the presence of trace elements can become necessary in order to make the process sustainable. This was shown by some first results that indicated that the addition of a nitrogen and phosphorous source to lactate kept the process much more stable. Perhaps on the long run other metals or elements can become limiting too. However on the other hand, the mineral aquifer can probably be a source of slow release of micro-elements.

An other point that needs strong evaluation is the comparison between the regular injection of electron donor and the slow release based processes. Some first tests, going on at the moment indicate no difference between continuous injection and slow release systems. Although the regular injection of an electron donor has certainly some drawbacks as it is laborious. Even when it is fully automatically operated it needs more maintenance. A very regular injection of a carbon source in an injection or monitoring well leads to risks related to bacterial growth on the housing of the injection well and finally can lead to biofouling and blocking of the filters. On the other hand slow release compounds are more expensive but need only to be injected one or two times a year and can be injected by a push system (e.g. by a Geoprobe system) without risks of clogging or biofouling. At the moment a comparative study is started and results will be available in the beginning of 2006.

Another intriguing point is certainly the different behavior of Cd-Zn at one end and Ni-Co at the other end. It seems that in the experiments performed until now the Cd- and Zn-precipitates are much more stronger and stable compared to the Ni- and Co-precipitates. Further investigations are underway in order to find out if this unstable behavior is rather temporarily than continuously. Indeed some studies indicate that metal sulfide formation processes tend to transform from an amorphous state to a more crystalline state making the precipitate indeed much more stable. This study is planned and already started and will give a final answer on the sustainability of the ISBP-process. It will be necessary to convince authorities about the final usefulness of this risk reduction process.

Tests, performed with single injections of molasses and HRC®, showed that metals could be precipitated in both cases. However the frequency of necessary injections to keep the system under operation was much higher for molasses that in the case with HRC®.

As many sites are contaminated by several metals (anions and cations or radionuclides) by activities as non-ferrous metals processing, surface treatment and mining activity (including also coal mining in some cases) pump & treat can not always deliver an economically acceptable solution. Therefore the development of *in situ* technology is becoming very important. The results and problems encountered up to now point out that in many cases the combination of different rather passive systems (e.g. also in combination with wetlands) will be necessary. The use of the *in situ* bioremediation process all or not combined with other passive systems as wetlands, PRBs, etc. will allow an economically acceptable management of risks related to metals and acid spreading into the environment from large contaminated sites (e.g. mining sites). Pump and treat technology can be useful in source removal whereas the passive treatment systems will be more applicable for the diffuse pollution of largely affected sites.

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