



Bioremediation Research Project

2013 Results



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Recommended citation:

Janin, A., 2014. Bioremediation research project – 2013 results, February 2014, 54p.

Printed in Whitehorse, Yukon, 2014 by Arctic Star Printing Inc., 204 Strickland St.
Photo credit: Yukon Research Centre

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1. EXECUTIVE SUMMARY

Passive water treatment has been identified as one of the areas of focus by the four partners who likely plan to use some sort of biological passive treatment systems at closure (closure plans of Minto, Wolverine, Bellekeno, and likely Eagle Gold) or during operation (Wolverine Mine, possibly Eagle Gold) whether this is wetlands, anaerobic bioreactors, electro-biochemical reactor or a combination of these techniques. Using passive biological treatment at mines in the North can be challenging due to the regulators lacking confidence in this type of technology due to limited Northern operational experience in applying these systems. A common concern relates to the efficiency of biological systems at cold temperature. To address that particular issue, research was conducted through the Chair program, starting in January 2013, to assess and compare different substrates for their potential to support bioreactor efficiencies. Substrates (biochars and wood products) were compared for their ability to remove metals by sorption as well as for organic carbon release in the effluent. Eight lab-scale bioreactors were installed and monitored since then to demonstrate their efficiencies and compare substrates. The first set of 4 bioreactors used creek sediment from Keno Hill District (KHD) and is treating synthetic water containing high concentrations of As, Cd, Cu, Se and Zn to encompass the common metals found at the different partner sites. This set has been in operation for more than 5 months to date. The other set of four used creek sediment from Wolverine creek and is treating true mine effluent from this site with a particular focus on selenium. This set has been in operation for 1 month. Results from the substrate assessment and from the monitoring of the bioreactors are reported here. The four KHD bioreactors all displayed a very high efficiency for As, Cd, Cu, Se and Zn with concentrations in the effluent lower than discharge limits except the wood-amended reactor. Low pH may have been the limiting factor in this bioreactor.

2. PURPOSE

The purpose of this document is to report the results of the Bioremediation Project conducted by the Industrial Research Chair in Mine Life Cycle at Yukon College, between February 2013 to January 2014, to the industrial partners Alexco Resource Group, Capstone Mining Corp, Victoria Gold Corp and Yukon Zinc Corp.

3. BACKGROUND

Beginning in January 2013, the newly established Industrial Research Chair in Mine Life Cycle at Yukon College, Dr. Amélie Janin, assessed the needs for research amongst the industrial partners. A proposition for a research project on bioremediation was submitted and approved by the partners in February 2013. A research plan was finalized in March 2013, which included design and construction of lab-scale bioreactors, review and

assessment of locally available substrates, operation of lab-scale bioreactors including different substrates and research for funding sources.

Passive biological treatments have been proposed as a possible efficient, cost effective treatment of mine water during operation and after closure. The four industrial partners are currently assessing the potential to use passive biological treatment for water treatment at their sites. This research project was built to support the partner's efforts to develop biological treatment systems in Yukon. The primary objectives were to demonstrate the efficiency of sulfate reducing bioreactors for mine water treatment, provide a better understanding of the biochemical processes and assess different low-cost, locally available substrates that could be used efficiently to promote metal sequestration. A secondary objective was to build up capacity at the College and in the community to study bioreactors.

Anaerobic bioreactors using sulfate reducing bacteria for the treatment of mine water have received attention in the last decade. Various examples have been presented in the literature (Alexco Resource US Corp, 2011; Nordin 2010; Doshi 2006; USEPA 2008; Mioska 2012, Dar et al. 2007; Genty 2011; Jong and Parry 2003; McCauley et al. 2009; Neculita et al. 2010). Several biological and chemical mechanisms arise in the reactor and together, lead to a reduction of the effluent's metal concentration. The main process of interest is biological sulfate reduction into hydrogen sulfide. Hydrogen sulfide then precipitates most metals as metal sulfide salts which are highly insoluble (Gazea et al 1996, Blais et al. 2008). Metal precipitation hence takes out the dissolved metals from the effluent to produce a precipitate in the bioreactors. Beside biological reduction, one of the other mechanisms involved is chemical adsorption. Chemical adsorption is usually considered a minor mechanism in bioreactors but compared to biological processes; this chemical reaction is fast and less temperature-dependent. The hypothesis beyond the research described in this report is that, if favored, chemical sorption may improve the efficiency and reliability of an anaerobic bioreactor. Materials like wood and biochar have the ability to promote adsorption mechanisms (Beesley and Marmiroli 2011, Kolodynska et al 2012, Lindsay et al 2011, Argun et al. 2008; Keng et al. 2013; O'Connell et al. 2008). Beside sorption capacity, porous materials such as biochar may support bacterial growth at cold temperatures.

To be active, sulfate reducing-bacteria require energy from organic matter to reduce sulfate into sulfide. The electrons are provided from organic carbon. Two systems of anaerobic bioreactors have been proposed: the compost-free bioreactor (such as the pilot bioreactor operated at Galkeno 900 in the Keno Hill District) for which the organic carbon is fed into the reactor by controlled addition of a liquid substrate (ethanol, sugar, molasses, milk protein, etc.); and the substrate-filled bioreactor (such as the Biopass designed for Wolverine mine) for which the organic carbon comes from the degradation of solid substrate integrated in the composition of the filling materials used in the bioreactor bed.

4. SUBSTRATE ASSESSMENT STUDY

Bioreactors are filled in with solid supports or substrate before operation. Common fillings for operation of bioreactors in southern Canada or the US include gravel, straw, wood chips, alfalfa, sewage sludge or agro-food industry waste, etc. Some of these materials are not readily available in the Yukon, or at higher cost. “Substrate” (or “fillings material”) used in this study were wood products (poplar chips, wood chips and spruce needles) and biochars (wood biochars, from 3 distinct manufacturers). Wood products are cheap and largely available in the Yukon. Biochars can be produced in the Yukon or at mine site. Warren Zakus, from Zakus Farm in Whitehorse, is currently developing the capacity to produce biochar from wood. Before integrating these materials into bioreactors (next section), tests have been conducted to assess their capacity to 1) degrade and release organic matter in water and 2) sequester metals from water by chemical sorption. In addition, preliminary tests were completed with peat, looking only at metal sorption (no carbon release).



Figure 1 Biochars and wood samples tested in laboratory (grinded form)

4.1. Substrate assessment methodology

4.1.1. pH, substrate degradation and carbon release

The purpose of this experiment was to assess the potential of wood products and biochar to degrade and release organic matter when used in a bioreactor. Biochar can probably be considered non-readily biodegradable (labile carbon should have been volatilized during pyrolysis) whereas wood chips may be biodegradable to some extent and should supply some carbon to feed the microbial population. Three biochars from different manufacturers and three wood products (poplar chips, spruce chips, spruce needles) were compared. Forty g of substrate were soaked in 800 mL of DI water (5% Total solids) for 8 weeks in Mason jars. Initial pH in the jar was adjusted to 6. All the tests were completed in duplicate (ie. 2 Mason jar per substrate type). The jars were capped and stored at room temperature in the dark. The conditions of the testing are summarized in Table 6, appendix 1. 50 mL samples were collected from the supernatant after 2, 4, 6, 8 and 16 weeks. pH of the supernatant was measured after each sample collection, the pH of the supernatant was readjusted to 6. The samples were filtered on 0.45µm porosity filters, acidified, stored at 4 degrees and analyzed for TOC at ALS Environmental Laboratory in Whitehorse.



Figure 2 Carbon release batch test

4.1.2. Metal sorption by substrates

The purpose of this set of experiments was to assess the capacity of the different substrates to reduce metal concentration by chemical adsorption. As discussed earlier, adsorption mechanisms are faster than biological mechanisms. To measure the capacity of the three biochars (from Diacarbon, Titan and Zakus Farm) and the three wood products (poplar and spruce chips, spruce needles), 2, 4, 6, 8 and 10g of substrate were mixed with 200 mL of metal-bearing synthetic solution in 500 mL erlenmeyer flasks (1, 2, 3, 4 and 5% Total Solid). The flasks were mixed for 24 hours at room temperature to reach chemical equilibrium. For each substrate, “control” experiments were completed by using 10g of substrate into DI water. Samples of the metal-bearing synthetic effluent were collected prior to mixing and samples of the supernatant were collected after flask mixing. Samples were filtered over 0.45µm, acidified, stored at 4 degrees until metals were analyzed by GFAA.

Kinetic tests were also completed in similar conditions except that samples were collected after mixing for 2, 4, 8 and 24 hours. Samples were analyzed for pH and metal concentration by GFAA using the PinAAcle 900T instrument (Perkin Elmer) in the YRC lab.



Figure 3 Samples collected from metal sorption tests

4.2. Substrate assessment results

4.2.1. pH and carbon release by substrate

pH and DOC values are presented in Table 8, Appendix 2. As expected, biochars tend to be alkaline materials (presence of ash) leading to a high pH at the beginning of the soaking experiment whereas wood products tend to be more acidic. Initial pH in the testing jars was in the range of 8.8 to 9.0 with biochars and 5.0 to 5.7 with wood. pH changed significantly over the 16 weeks of soaking in water. pH of the biochar mixtures decreased steadily from > 8.5 to neutrality (pH 6.8 to 7.2). On the other hand, pH of the wood mixtures quickly dropped down to about 4 then slowly increased toward equilibrium at about pH6 after 16 weeks. The drop in pH observed in the mixture containing wood materials may indicate that phenolic acids contained in the wood structure have been leached into the water. This is also supported by a relatively high DOC content in the supernatant of the wood material mixtures (Fig 4).

The amount of organic carbon released from wood products and biochars are dramatically different. Spruce chips (from the tree trunk) released 445 mg/L of DOC initially, poplar wood chips released 1,540 mg/L and spruce needle release was significantly higher at 2,555 mg/L DOC. On the other hand, DOC release from biochar was much lower with 74, 29 and 7 mg/L DOC release respectively from Titan, Diacarbon and Zakus Farm biochars. Organic carbon released by substrate likely has an impact on the growth of a microbial population as organic carbon is used as a source of energy by many microorganisms. Higher organic carbon may be useful to promote biological mechanisms if the population of bacteria are required to break down long organic carbon chains present in the bioreactor. On the other hand, for bioreactors designed to be more controlled, a boost in organic carbon is not favored as it could boost microbial growth for too short a duration. In this case, wood should be avoided but biochars could be used. Interestingly DOC has been monitored in the supernatant over 16 weeks and it dropped in the poplar (-20%) and spruce needle (-30%) jars, probably by biological degradation, but not in the spruce chips jar.

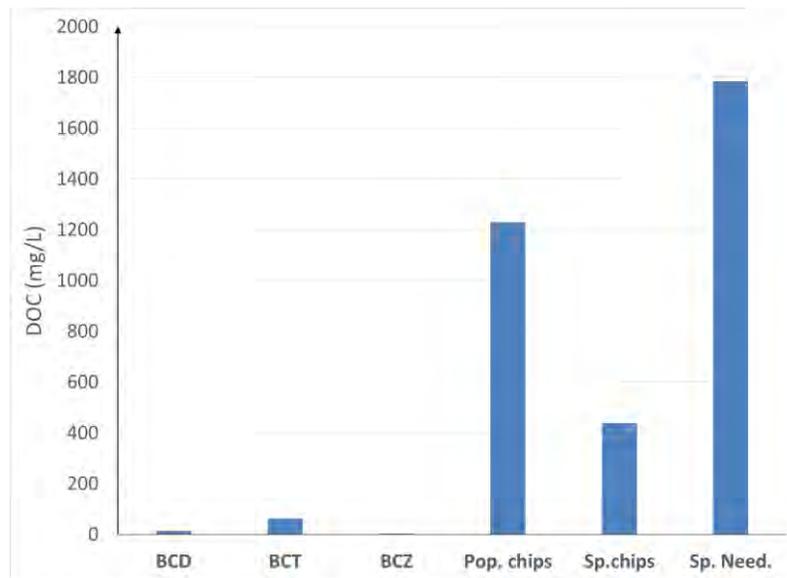


Figure 4 Dissolved Organic Carbon measured in supernatant of wood and biochars mixtures (5% TS) after 16 weeks

4.2.2. Metal sorption by substrate

The metal sorption batch tests were meant to assess the capacity of biochars and wood products to remove metals from water in a very controlled system, over 24 hours, in absence of microorganisms. Synthetic effluent containing metals was mixed with the different substrates studied with variable total solid contents. Many of the results obtained during this study were presented in the conference proceeding prepared for the 2013 Northern Latitudes Mining Reclamation Workshop and 38th Annual Meeting of the Canadian Land Reclamation Association. The conference proceeding is attached to this report in Appendix 9 and the results won't be repeated here. The conclusion of that work was that Cu, Cd and Zn were able to sorb on biochars (>90% of initial metal content) and relatively well on wood products (51-94% of initial metal content). As and Se did not sorb as well as the divalent metals but some adsorption of arsenic was observed on biochars (35-69%) within 24 hours of contact between the metals and substrates.

4.2.3. Kinetics of metal sorption

Chemical sorption is a fast reaction. Metal removal rates by sorption are presented in table 9, appendix 3. Copper removal at 2, 4, 8 and 24 hours is presented in the figure 5 as an example. The results show that 1) sorption removal rate is high in batch conditions (no

flow) and 2) sorption happens within the first hours of contact between the substrate and the effluent. Although these results were obtained in a batch experiment, and are not scalable to a continuous flow system, it means that if favored, the sorption mechanisms happen faster than biological reactions. Hence, metals could potentially be retained in a reactor by sorption before undergoing precipitation with the sulfides produced by the biological mechanisms. That might be of interest in freshet conditions where bioreactors are stressed by high flow rates at freshet (lower residence time in bioreactor) and by cold temperatures (lower biological activities).

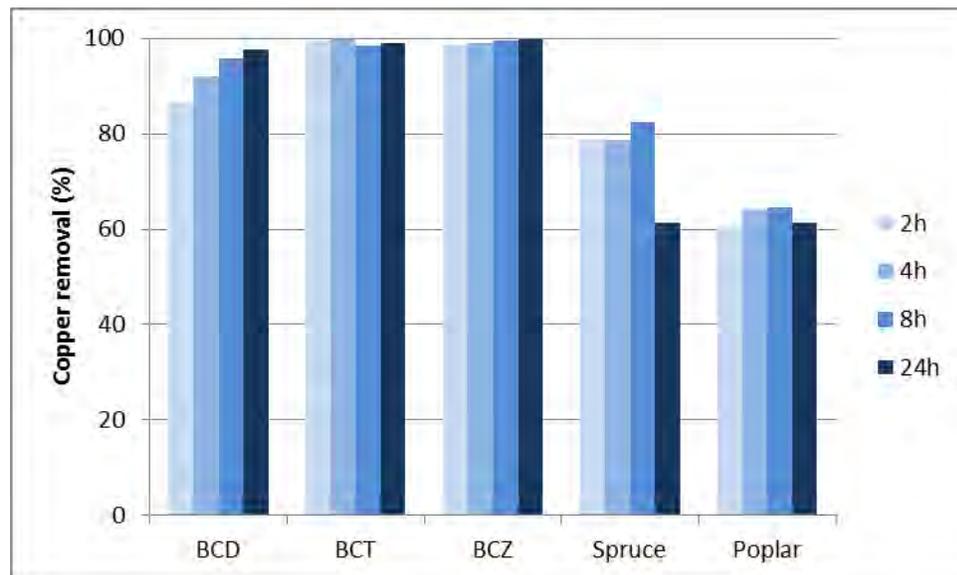


Figure 5 Removal rate of copper by sorption at 2, 4, 8 and 24 hours of contact between substrates and effluent (initial Cu concentration 2.105 mg/L; BCD: biochar from Diacarbon; BCT: biochar from Titan; BCZ: biochar from Zaku Farm)

5. LAB-SCALE BIOREACTOR PERFORMANCES

5.1. Condition of operation of lab-scale bioreactors

5.1.1. Lab scale bioreactors design

Height Plexiglass columns with end caps were built by Ellis Associates (Edmonton). The columns are 70 cm high, four columns had an internal diameter of 6.4 cm (Fig 6) and the other four an internal diameter of 14 cm (Fig 7). The columns are fed with effluent from the

bottom up using a peristaltic pump and Masterflex L/S13 tubing (internal diameter of 0.8 mm). Flow rate was set at 0.1 ml/min of synthetic effluent in the column bioreactors. According to the volume of the reactor and the flow rate, the residence time was set at 14 days for the 6.4 cm ID columns and 69 days for the 14 cm ID columns. The hydraulic residence time, accounting for the duration it takes the effluent to travel through the reactor, was not calculated.

The 8 reactors have been filled with different materials and are fed with different effluents (Table 1). The bioreactors called C1, C2, C3 and C4 were prepared using inoculum from the Keno Hill District (sediments from Crystal Creek, high gravel content), incubated then continuously fed with synthetic effluents. The bioreactors called C5, C6, C7 and C8 were prepared using inoculum from Wolverine Mine (sediment from Wolverine Creek, low gravel content, sludge-like consistence), incubated then fed continuously with dewatering effluent from the Wolverine Mine.

The effluent coming out of each reactor was collected in 2L jars over 1 week, every week for measurements of pH, Total and Dissolved Metals and sulfate.

Table 1 Condition of operation of the 8 lab-scale bioreactors (filling material and effluent)

	C1	C2	C3	C4	C5	C6	C7	C8
Condition model	Keno Hill District + As, Se				Wolverine Mine			
Filling materials (% of total volume)								
Creek sediment	100	100	80	80	40	40	40	40
Gravel					30	30	20	20
Sand					30	30	15	20
Biochar			20					20
Wood				20			20	
Limestone							5	
Reactor feed								
Effluent	Synt. Eff.	Synt. Eff.	Synt. Eff.	Synt. Eff.	Dewater.	Dewater.	Dewater.	Dewater.
Ethanol addition	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes



Figure 6 Bioreactors C1 to C4 (Keno Hill District conditions, 6.4 cm ID)



Figure 7 Bioreactors C5 to C8 (Wolverine conditions (Biopass), 14 cm ID)

5.1.2. Effluent

1.5.1.2 Bioreactors C1 to C4

To feed the first set of four bioreactors, synthetic effluents were prepared by dissolving metal salts (As_2O_5 , CdSO_4 , CuSO_4 , FeSO_4 , SeO_2 , ZnSO_4 , Na_2SO_4) in DI water. The target concentrations were 5 mg/L for Cu, Fe and Zn, 0.5 mg/L for As, Cd and Se. The exact metal concentrations of each synthetic effluent produced are presented in Table 10, Appendix 4. According to the metal/sulfur ratio required to precipitate metals in the bioreactor in their sulfide form (FeAsS , CdS , CuS , FeS , Se(0) , ZnS), the sulfate concentration required was 49 mg/L, provided as Na_2SO_4 . The pH of the synthetic effluent was adjusted to 6 using NaOH.

Ethanol was added in the synthetic effluent before use, at 16 mg/L, to act as a substrate to provide electrons to the sulfate-reducing bacteria to reduce sulfate into hydrogen sulfide (8 electrons required per sulfate molecule).

2.5.1.2 Bioreactors C5 to C8

Bioreactors C5 to C8 were fed with effluent coming from the Wolverine site. Effluent is collected from “Sump 2”, a pond collecting the underground dewatering water. This effluent is used to be as close as possible to the effluent that will be possibly treated by the Biopass system at closure of the Wolverine mine. The Biopass will be positioned to intercept ground water which may have infiltrated through the underground workings.

5.1.3. Bioreactors operation timelines

The first set of bioreactors (C1 to C4) were prepared on August 1st 2013 then filled with synthetic water, containing sulfate and metals. The pump was then stopped to let the reactors incubate for 13 days. After incubation, on August 23rd 2013, the columns were fed continuously with synthetic effluent flowing at 0.1 ml/min. Residence time in this set of bioreactors was 14 days.

The second set of bioreactors (C5 to C8) were prepared on December 19th 2013 and filled with a synthetic solution of sodium sulfate at 1g/L to provide the sulfate required for incubation of the sulfate reducing bacteria. The pump was stopped on December 23rd 2013 to let the reactors incubate. On January 21st, dewatering effluent started to flow through the reactors. Residence time is expected to be 69 days.

Details on the timelines of operation are presented in table 11, Appendix 5.

5.1.4. Flow rates (bioreactor C1 to C4)

The volume of effluent collected is measured. The volume of influent is assumed equal to the volume of effluent. This value is used for calculation of the flow rates of the influents for each bioreactor.

$$\text{Influent flow rate (assumed)} = \frac{\text{Volume of collected effluent}}{\text{Duration of effluent collection}}$$

However, flow rates vary during testing (Table 13, Appendix 6), as it depends on the hydraulic conductivity of the reactors. Clogging of the tubes has been observed, mainly at the outlet of the reactors where biofilms can develop and can slow and stop the flow. In this case, the volume of effluent, V_{eff} , is 0, no samples are collected and no metal concentrations data are available. At that time, tubes were unclogged, and the flow restarted.

Target flow rate was 0.1 ml/min and actual average flow rate are 0.09 ml/min, 0.10 ml/min respectively for the bioreactors C1 and C3 and 0.12 ml/min in both C2 and C4 bioreactors. Variation in the flow rate impacted the hydraulic residence time, calculated as follow:

$$\text{Hydraulic residence time} = \frac{\text{Volume of reactor}}{\text{Average flow rate}}$$

Variations in residence time were observed: range 13 to 18 days. C1 had the longest residence time of 18 days while C2 (Duplicate of C1) and C4 (wood) have the shortest time of 13 days.

5.2. Bioreactors (C1 to C4) performances

The concentrations of Total As, Total Cd, Total Cu, Total Se, Total Zn and SO₄ in the effluent samples collected weekly are presented in Appendix 7, tables 14, 15, 16 and 17.

5.2.1. pH evolution

pH of the four reactors were generally going down, from early September to late December, with pH of the reactors C1 and C2 generally displaying the higher pH (Table 2). pH has a significant impact on metal chemistry. As a general rule, lower pH indicates that a larger portion of the metals are in a dissolved state, which is not favored for metal removal treatments. C1 and C2 had a pH close to 8 decreasing to about 7 after 154 days of operation. C4, which displayed a lower pH started at 6.4 and decreased to 5.4. A decrease in pH of the wood-amended reactor likely comes from the release of organic acid from the wood into the water. No buffer materials were used in any of the bioreactors and no pH adjustments were completed. **The results indicate that when using wood in a bioreactor at**

a mine site, adjustment of the pH would be required. This could be done by addition limestone or other alkaline materials in the reactor filling.

Table 2 pH values in weekly samples of C1-C4 reactors effluents

Sampling date	Duration (day)	pH			
		C1	C2	C3	C4
9/6/2013	14	7.89	8.41	N/A	6.43
9/20/2013	28	7.28	8.27	7.58	6.29
9/27/2013	35	7.77	7.99	7.07	6.17
10/4/2013	42	N/A	7.28	6.9	6.67
10/11/2013	49	8.53	8.24	7.47	7.09
10/18/2013	56	7.79	7.44	7.13	6.47
10/25/2013	63	7.03	7.16	6.33	5.85
11/1/2013	70	7.09	7.31	7.12	6.6
11/7/2013	77	7.02	6.96	6.87	5.79
11/14/2013	84	6.99	6.58	6.28	5.32
11/21/2013	90	6.7	6.74	6.53	5.76
11/28/2013	98	7.01	7.03	6.73	5.81
12/4/2013	105	7.12	6.72	6.48	5.94
12/12/2013	112	7.09	6.85	6.39	5.87
12/19/2013	119	6.42	6.84	6.59	6.11
1/23/2014	154	7.96	6.61	7.59	5.45

5.2.2. Arsenic removal

The mean concentration of the reactor influents was about 340 ppb, and it was dropped down below the As discharge limit of 100 ppb at the Bellekeno by the four bioreactors (Fig 8). The biochar-amended bioreactor was less effective at reducing arsenic concentration in the effluent but it reduced As concentration to 48 ppb on average over five months of monitoring. The other reactors, C1 and C2 (the duplicates) and C3, the wood amended column were more efficient, with As concentrations lower than 40 ppb in most cases. The spike seen in the recent sampling event, at the beginning of January, may be due to the discontinuity of the flow, when the pump has been stopped over the Christmas period.

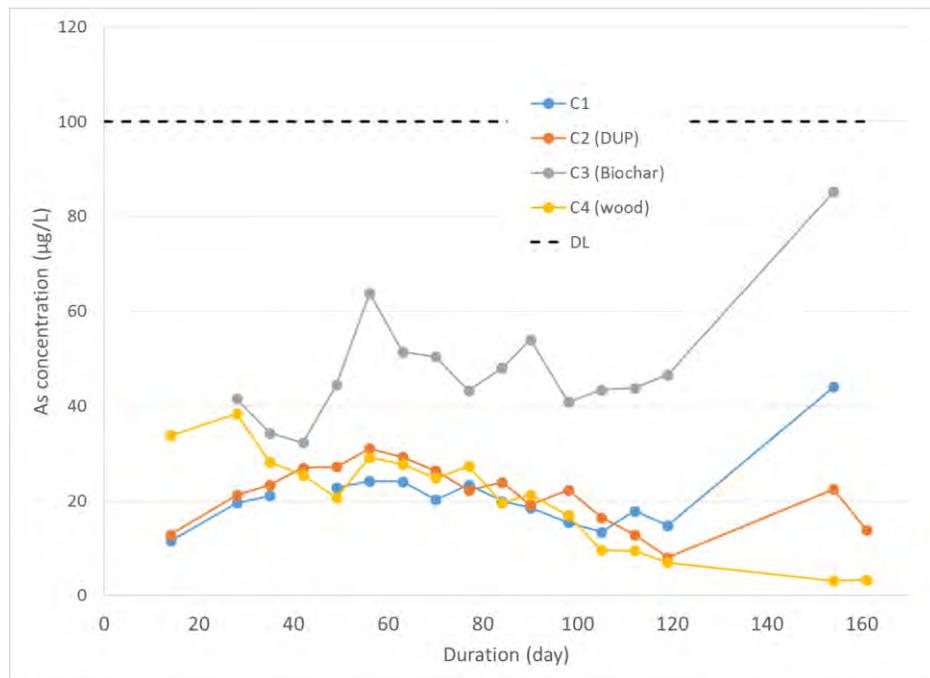


Figure 8 Arsenic concentration in effluents from the C1 to C4 bioreactors (weekly monitoring; DL: Discharge Limit for Bellekeno; $[As]_0 = 342$ ppb in average)

5.2.3. Cadmium removal

The discharge limit at the Bellekeno Mine is 10 ppb for cadmium. The synthetic effluent used to feed the reactors had 513 ppb in average. The effluent of the reactor C3 (biochar) never exceeded the discharge limit of 10 ppb for cadmium, while C1 and C2 exceeded only once, after 14 days of operation, which is still be considered within the incubation period. **From day 28 to 90, the effluents from the reactors C1-C3 were all compliant, below 10 ppb Cd on a weekly basis (Fig 9).** On the other hand, Cd in the effluent of the bioreactor C4 (wood) were non-compliant and well above the discharge limit. Amendment with wood was detrimental to Cadmium removal, possibly due to the lower pH.

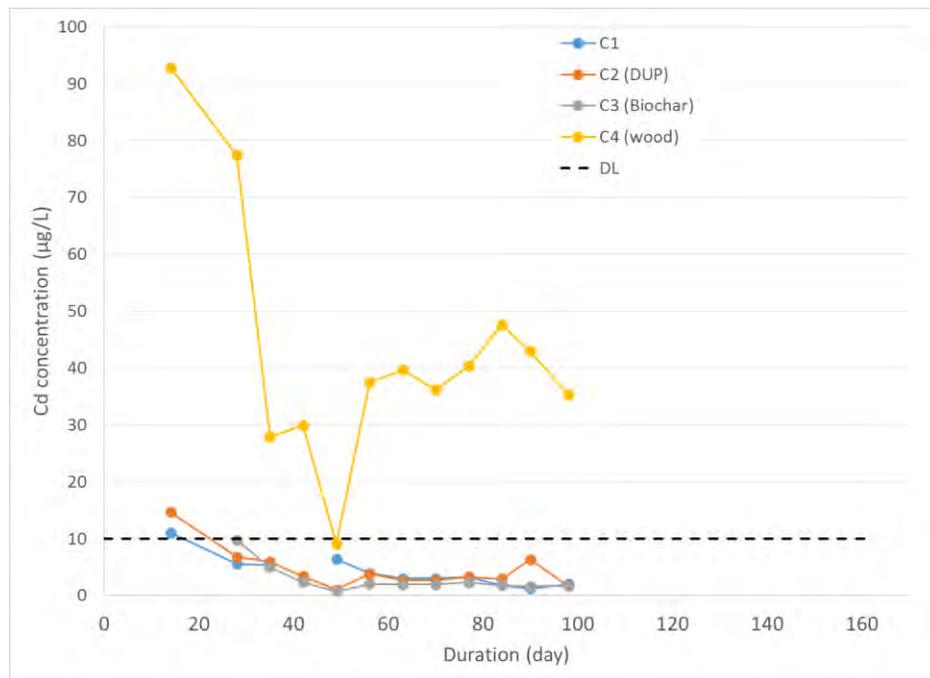


Figure 9 Cadmium concentration in effluents from the C1 to C4 bioreactors (Weekly monitoring; DL: Discharge Limit for Bellekeno; $[Cd]_0 = 513$ ppb in average)

5.2.4. Copper removal

Copper discharge limit was set at 100 ppb at the Bellekeno site and all the effluent from all four columns were compliant. **Cu concentration in the synthetic effluent was 4,601 ppb and it was reduced to less than 100 ppb in all reactors (Fig 10).** C1, C2 and C3 exhibited similar Cu concentration, between 1.9 and 21.1 ppb, whereas the reactor C4 (wood) produced effluent containing higher Cu concentration, between 12 and 64 ppb.

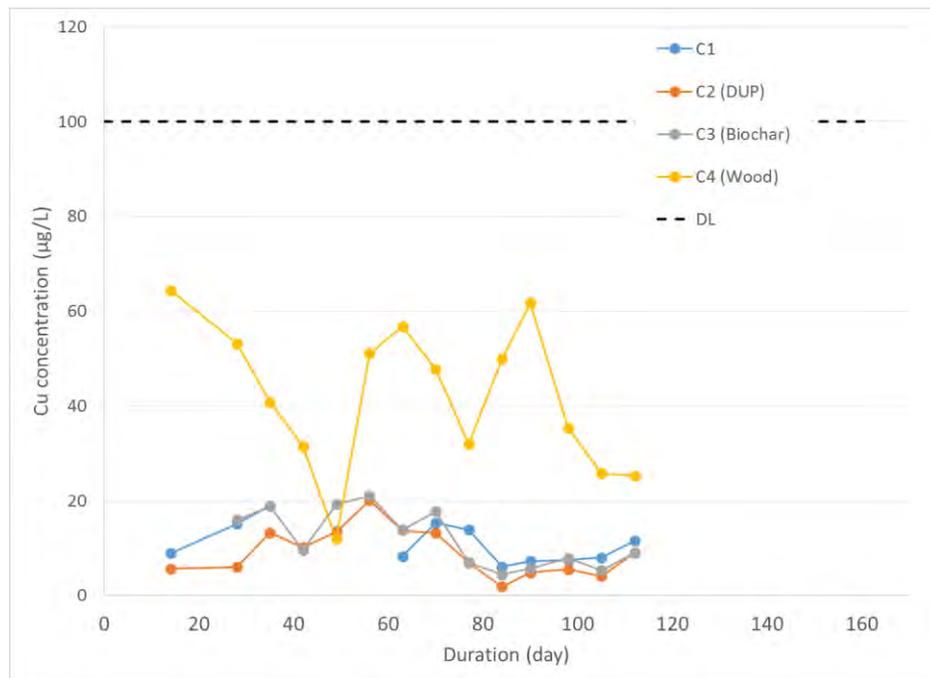


Figure 10 Copper concentration in effluents from the C1 to C4 bioreactors (Weekly monitoring; DL: Discharge Limit for Bellekeno; $[Cu]_0 = 4601$ ppb in average)

5.2.5. Selenium removal

Selenium removal by the four bioreactors was impressive. **Influent contained 466 ppb of selenium and this was reduced to less than 3.1 ppb over 5 months of monitoring.** A majority of the samples analyzed were below quantification limit of 1.5 ppb (reported as 1.5 ppb value in the figure 11 below).

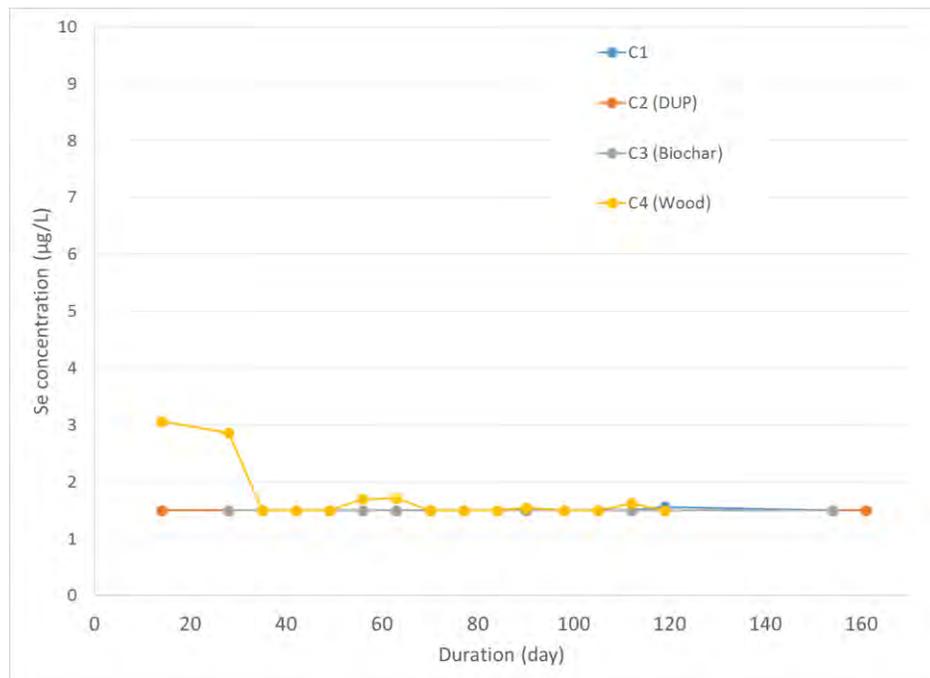


Figure 11 Selenium concentration in effluents from the C1 to C4 bioreactors (Weekly monitoring; quantification limit was 1.5 ppb, value below this limit were reported as 1.5; no discharge Limit for Bellekeno; $[Se]_0 = 466$ ppb in average)

5.2.6. Zinc removal

Similar to the observation made for cadmium, the removal of zinc was satisfactory for reactors C1, C2 (dup) and C3 (biochar) but not efficient enough in C4 (wood). **Effluent from C1-C3 were below the discharge limit of 500 ppb Zn, however the wood-amended reactor displayed Zn concentrations exceeding compliance level.** Zn concentration in the influent was 5440 ppb on average. Again, this lower efficiency for Zn removal in the wood-amended column might be due to the release of organic acids from the wood, which decreases the pH and may be deleterious to microorganism growth.

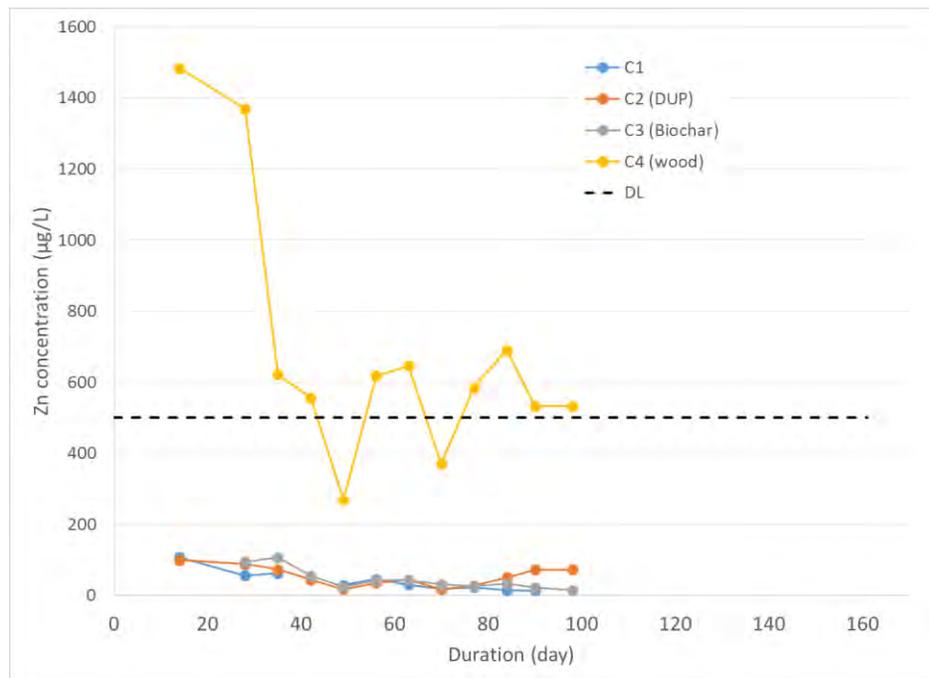


Figure 12 Zinc concentration in effluents from the C1 to C4 bioreactors (Weekly monitoring; DL: Discharge Limit for Bellekeno; $[Zn]_0 = 5440$ ppb in average)

5.2.7. Sulfate reduction rates

Sulfate concentrations in effluents were measured weekly from Nov 11th 2013 (Table 3 and 4). The synthetic effluent feeding the four reactors contains 49 mg/L of sulfate. Reactors C1 and C2 consumed sulfate and produced effluent containing less than 5 mg/L (quantification limit). The sulfate reduction rate was > 90%, indicating that the bioreactors were functioning properly and that sulfate was likely reduced to hydrogen sulfide, which in turn favors precipitation of metal sulfide salts such as CdS, CuS and ZnS.

SO₄ concentration in effluent of C3 was between 9.1 and 14.1 mg/L with reduction rates between 71 and 81 %. Finally, Reactor C4 exhibited a lower efficiency of sulfate reduction, more variable, with rates between 34 and 80%. Sulfate reduction may not be as favored in the biochar and the wood-amended reactors (C3 and C4), which may indicate that less sulfide is produced. This may explain the difference observed in Cd, Cu and Zn concentration in the C4 effluent.

Overall, the reduction in sulfate concentrations was efficient in all four reactors with an average removal > 71%, with C1 and C2 consuming more than 90% of the sulfate feeding the reactors. However, it should be noted that creek sediment made up 100% of the filling used in C1 and C2 and 80% of the filling used in C3 and C4. The amount of creek sediment

used in the composition of the reactors might impact the amount of organic matter present initially which can be used by the microorganisms in the sulfate reduction reaction.

Table 3 Sulfate concentrations in effluents from reactors C1 to C4 (Limit of quantification is 5 mg/L; N/A: not available)

Sampling date (dd-mm-yy)	Duration (day)	SO ₄ concentrations (mg/)			
		C1	C2	C3	C4
14-11-2013	84	<5	<5	14	12.1
21-11-2013	90	<5	<5	12.6	11.7
28-11-2013	98	<5	<5	11.7	9.6
04-12-2013	105	<5	<5	14.1	6.7
12-12-2013	112	<5	<5	10.8	<5
19-Dec-13	119	<5	<5	9.1	32.3
23-Jan-14	154	N/A	11.8	N/A	17
30-Jan-14	161	N/A	<5	N/A	N/A

Table 4 Sulfate reduction rates in reactors C1 to C4 (rate assumed to be above 90% when effluent concentration is below 5 mg/L; N/A: not available)

Sampling date (dd-mm-yy)	Duration (day)	SO ₄ reduction rate (%)			
		C1	C2	C3	C4
14-11-2013	84	>90	>90	71	75
21-11-2013	90	>90	>90	74	76
28-11-2013	98	>90	>90	76	80
04-12-2013	105	>90	>90	71	80
12-12-2013	112	>90	>90	78	>90
19-Dec-13	119	>90	>90	81	34
23-Jan-14	154	N/A	76	N/A	65
30-Jan-14	161	N/A	>90	N/A	N/A

5.3. Bioreactors efficiencies

5.3.1. Calculation of the removal efficiencies

The reduction of As, Cd, Cu, Se and Zn concentrations by the four bioreactors are significant: commonly 97 to 99.9% reduction weekly. However, the flow rates and volume flowing through each reactor are different. In order to compare the four reactors adequately, the metal removal efficiencies were calculated using the weekly metal concentrations (in mg/L) in influent and effluents and the volume of effluent collected weekly. Amount (in mmol) of metals coming into and out of the reactors are calculated on a weekly basis and then summed to get the “total amounts”. The total amount of metal coming into the reactors was compared with the total amount coming out from October 20th to the last samples analyzed. This allows for calculation of the metal removal efficiency. Note: the calculations are made from October 20th 2013, after the first bed volume has passed (after the water present in the reactors during incubation has been entirely replaced).

5.3.2. Impact of the residence time: duplicate comparison

Comparing the quantity of metals coming in (table 10, appendix 4) and out (Table 14, 15, 16 and 17, appendix 7) of the four reactors and taking into account the variations in flow rates (table 13, appendix 6), the amount of metals sequestered by each reactor per liter of effluent treated was calculated.

Reactor C1 and C2 are two duplicates, filled with the same materials and fed with the same synthetic effluents. The reproducibility, in terms of metal concentrations in the effluent of the duplicate bioreactors C1 and C2 was satisfactory. In terms of quantity of metal sequestered, ie the amount of metal retained in the vicinity of the reactor (ex. precipitated, sorbed, encapsulated), was greater in C1 than in C2, especially for the divalent metals Cd, Cu and Zn (Fig 13). However, the flow rates of these two reactors were slightly different and led to an 18 days residence time in C1 versus 13 days in C2. Effluent took 5 more days to flow through reactor C1 compared to C2, reactor C1 had 27% more time to complete the chemical and biological treatment. **Residence time is known to be a key factor for bioreactor design and efficiency and its impact is indicated in these results.**

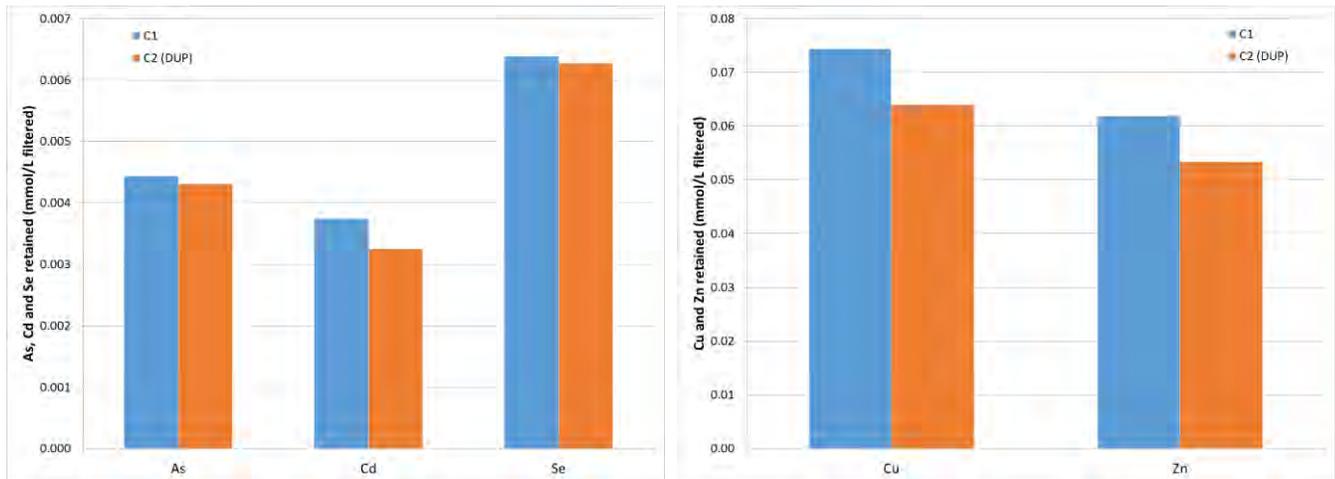


Figure 13 Relative metal contents retained in bioreactor C1 and C2 (Fig a) As, Cd and Se; Fig g) Cu and Zn)

5.3.3. As, Cd, Cu, Se and Zn removal efficiencies

The metal removal efficiencies presented in table 5 are calculated over durations of monitoring between 77 and 127 days. **Removal efficiencies were high in all four reactors and varied from 88% to 99.9% for the five metals.** Cadmium, copper and selenium removal efficiencies are all above 99%. Zinc removal efficiency is high (>98%) except in the fourth reactor, amended with wood (88%). Arsenic removal efficiencies was 94% in all reactors except the biochar-amended reactor (87%).

Table 5 Metal removal efficiencies of bioreactors C1 to C4 with amount of metals coming in and out of the reactors for designated period of time (C1: gravel and sediment, C2: gravel and sediment duplicate, C3: Gravel sediment and biochar, C4: gravel, sediment and wood)

	Period	Duration	Bioreactors			
	mm-dd-yy to mm-dd-yr	(day)	C1	C2 (DUP)	C3 (Biochar)	C4 (Wood)
As _{in} (mmol)	09-20-13 to 01-23-14	127	0.0615	0.0838	0.0703	0.0830
As _{out} (mmol)			0.0035	0.0054	0.0093	0.0049
As removal efficiency (%)			94.4	93.6	86.8	94.1
Cd _{in} (mmol)	09-20-13 to 11-28-13	77	0.0494	0.0597	0.0534	0.0627
Cd _{out} (mmol)			0.0003	0.0004	0.0003	0.0044
Cd removal efficiency (%)			99.4	99.3	99.5	93.0
Cu _{in} (mmol)	09-20-13 to 12-12-13	90	0.9772	1.1651	1.0564	1.2011
Cu _{out} (mmol)			0.0026	0.0022	0.0025	0.0099
Cu removal efficiency (%)			99.7	99.8	99.8	99.2
Se _{in} (mmol)	09-20-13 to 01-23-14	127	0.0838	0.1141	0.0968	0.1113
Se _{out} (mmol)			0.0001	0.0001	0.0001	0.0003
Se removal efficiency (%)			99.9	99.9	99.9	99.7
Zn _{in} (mmol)	09-20-13 to 11-28-13	77	0.8215	0.9805	0.8713	1.0342
Zn _{out} (mmol)			0.0110	0.0096	0.0075	0.1192
Zn removal efficiency (%)			98.7	99.0	99.1	88.5

5.3.4. Bioreactor performances comparison

Reactors C1 and C2 contain 100% of creek sediment, made out of gravel, sand and organic matter. Reactors C3 and C4 contain 80% (v/v) of creek sediment, which prevent a clear and direct comparison between C1, C2 and C3, C4. This restriction has been adjusted in the design of the experiment going on with C5-C8 (Biopass) reactors. However, It is clear that all four reactors were highly efficient for metal removal.

With a lower amount of sediment, the biochar-amended reactor led to the same removal efficiency for Cd, Cu, Se and Zn removal, with concentrations in the effluents very similar to those observed in the non-amended reactors. On the other hand, arsenic was less well removed in the biochar-amended reactor than in the other. This difference may be due to the lower amount of organic matter available to the microorganism or to some unknown

mechanisms. Iron was not monitored so far, but this could have provide some indications on the removal mechanisms of As. Besides metal removal, it has been observed that sulfate consumption was lower in the biochar-amended reactor than in the non-amended ones. Sulfate is used to precipitate Cd, Cu and Zn as sulfide. With lower consumption of sulfate, the biochar-amended reactor still produced the same efficiency for removal of these metals. According to this set of experiment (substrate study and bioreactors), the results give evidence that mechanisms other than biological sulfate reduction are going on in the reactor, likely chemical metal sorption. Hence these results indicate that metal sorption has the potential to co-exist with biological sulfate reduction reaction and to be efficient at metal retention. Although it will never be the major process for metal removal, it may help improve the reliability of the reactor by adding Cd, Cu, Se and Zn retention capacity. Biochar did not prove to improve As removal in the condition tested in this study.

Wood-amended reactor, C4, produced effluent with higher Cd, Cu and Zn concentrations than the reactors C1, C2 and C3. Again, this reactor has 80% (v/v) creek sediment but the lower efficiency may be related to the lower pH of the effluent (close or less than 6). Solubility of metal sulfide salts increases when pH is lower (Fig 14). Lower pH may come from the release of organic acid from the wood, as shown in the substrate study. Wood also released high DOC in water. This DOC may be toxic to the microorganism and lead to less reliable sulfate reduction rate (34-90%). Fresh spruce was used in the composition of the reactor C4. A spruce tree was cut down, the trunk grinded and the chips integrated in the reactor within 1 month. A suggestion to improve the efficiency of a wood-amended bioreactor would be to use older or weathered wood chips, or poplar instead of spruce as it contains less phenolic acid and certainly to incorporate alkaline materials. The new wood-amended bioreactors (Biopass set) setup in the laboratory were amended with lime from a gardening store to assess if that will help raise the efficiency for divalent metal removal. On the other hand, the efficiency of removal of As and Se was very good, even better for As than the three other reactors.

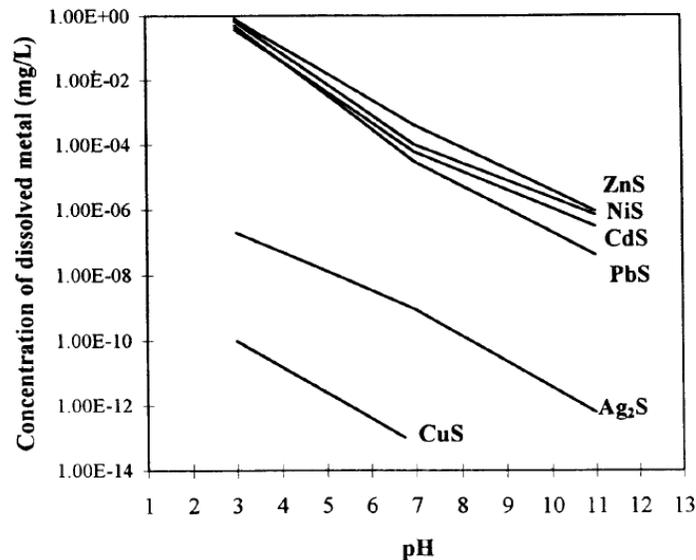


Figure 14 Solubility of metal sulfides (from Gazea et al. 1996)

6. PROPOSED PROJECT DEVELOPMENT

6.1. Variation of operational conditions

A proposition for the next development of this project was detailed in the “Bioremediation Research Project Proposal” (Feb 2014) was sent to the partners. This includes:

- Operating the reactors C1 to C4 in temperature stress conditions
- Setup of a new set of bioreactors using Minto produced water and inoculum
- Assessing the capacity of reactors to remove antimony (Sb spike in reactor influent)

6.2. Field study

The lab-scale bioreactors studied so far has proved to be highly efficient at decreasing metal concentration from metal-bearing effluents. To build on the results obtained, the next steps of this research should include field study, with larger bioreactors. Such bioreactors could be made out of 45 gallons plastic tanks or out of a water truck tank (6,000 gallons, Fig 15, Appendix 8). Field bioreactors could be installed on site, close to a drainage

or pond, and fed in parallel. The bioreactors could be filled with different materials of interest, as were the bioreactors studied in the laboratory.

One of the challenges to pursue this route is the high cost of the equipment. To overcome that issue, the Chair applied for funding from NSERC for their “Applied Research Tools and Instruments” grant (application available to the partners upon demand). If successful, this grant would provide about \$70,000 toward equipment and staff to build a transportable pilot bioreactor (design presented in Appendix 8, Fig 16). The results of this application will be known by mid-march 2014. If successful, the Chair will engage discussion with the partners to define the design and to plan for installation at mine sites.

7. CONCLUSIONS AND LESSONS LEARNED

In this study, the anaerobic bioreactors were operated in very favorable conditions: warm temperature (room), ethanol feed and high percentage of inoculum composed of organic matter. In this setting, the reactors displayed a very high efficiency, with >98% removal of Cd, Cu, Se and Zn in the reactors C1-C3 and As removal was >94% in C1, C2 and C4. After incubation, the effluents of the reactors C1-C3 all complied with the discharge limit in use at the Bellekeno Mine. Although the metal removal efficiencies may be lowered a little bit in a full scale bioreactor due to some limitations, this set of results indicates that bioremediation technology certainly has the potential to treat mine waters with higher efficiencies than commonly used technologies such as lime precipitation.

Lessons were learned from this study:

- 1) If wood is to be used as a bioreactor filling, pH should be adjusted and the toxicity of the wood to the microorganisms should be assessed.
- 2) Metal sorption seems to be a non-negligible mechanism. It could be very beneficial and should be investigated more.
- 3) Clogging: the size of the outlet pipes to be used for a bioreactor should be large to lower the risk of clogging by biofilms and accessible to allow for scrubbing.

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APPENDIX 1 – EXPERIMENTAL CONDITIONS FOR SUBSTRATE ASSESSMENT STUDY

Table 6 Experimental conditions for assessment of organic carbon release from substrate

Parameter	Experimental conditions
Type	Batch (no flow)
Duration	16 weeks
Total solids	5 % of biochar in DI water (in duplicate), 3 biochars from Diacarbon, Titan and Zaku Farm 5% wood chips in DI water (in duplicate), poplar and spruce chips, spruce needles
pH	Controlled at 6 with H ₂ SO ₄ at the start of the experiment

Table 7 Experimental conditions for assessment of metal sorption by substrate

Parameter	Experimental conditions
Type	Batch (no flow)
Duration	24 hours; 2, 4, 8, 24 hours for kinetic tests
Total solids	1.0, 2.0, 3.0, 4.0 and 5.0% of biochar or wood products in synthetic drainage water
Control	5% biochar or wood chips in DI water
pH	Controlled at 6 with H ₂ SO ₄

APPENDIX 2 - PH AND CARBON RELEASE FROM SUBSTRATES

Table 8 Dissolved Organic Carbon released from biochars and wood chips in water and pH generated (BCD: Biochar from Diacarbon Energy Inc; BCT: biochar from Titan; BCZ: biochar from Zaku Farm)

Parameter	Duration (wk)	BCD	BCT	BCZ	Pop. chips	Sp.chips	Sp. Need.
DOC	2	29.2	73.7	6.9	1540	445	2555
	4	23.8	72.6	6.9	1405	417	2310
	6	24.6	71.4	5.1	1385	411	2195
	8	19.1	65.5	6.2	1380	415	2190
	16	15.2	61.5	4.4	1229	438	1785
pH	0	8.77	8.77	8.84	5.68	5.06	5.01
	2	7.72	7.19	7.86	3.93	4.61	4.20
	4	7.24	6.51	7.62	4.48	5.22	5.35
	6	7.13	6.41	7.63	5.54	5.50	5.60
	8	7.01	6.55	7.80	5.61	5.86	6.01
	16	7.01	6.61	7.74	6.30	6.57	6.62

APPENDIX 3 - SORPTION REMOVAL RATES

Table 9 Removal rates for As, Cd, Cu and Se by sorption at 2, 4, 8 and 24 hours (initial contents: 394 ppb As, 322 ppb Cd, 2105 ppb Cu and 387 ppb Se; BCD: biochar from Diacarbon; BCT: biochar from Titan; BCZ: biochar from Zaku Farm)

Substrate	Metal	Duration (hours)			
		2	4	8	24
BCD	As (%)	72.7	76.9	78.3	76.1
	Cd (%)	91.1	94.9	97.6	97.9
	Cu (%)	86.6	92.0	95.8	97.7
	Se (%)	31.6	26.7	26.3	19.6
	Zn (%)	TBD	TBD	TBD	TBD
BCT	As (%)	78.7	73.6	68.9	60.0
	Cd (%)	87.8	96.7	99.4	99.4
	Cu (%)	99.4	99.7	98.5	99.1
	Se (%)	36.2	24.8	25.5	17.3
	Zn (%)	TBD	TBD	TBD	TBD
BCZ	As (%)	87.7	86.1	81.1	73.7
	Cd (%)	94.6	98.9	99.5	99.6
	Cu (%)	98.6	98.9	99.4	99.8
	Se (%)	47.5	44.9	38.8	34.4
	Zn (%)	TBD	TBD	TBD	TBD
Spruce	As (%)	43.2	29.2	20.8	13.7
	Cd (%)	93.0	93.8	95.0	96.6
	Cu (%)	79.1	78.8	82.5	61.2
	Se (%)	26.5	13.5	9.4	5.1
	Zn (%)	TBD	TBD	TBD	TBD
Poplar	As (%)	40.7	22.5	1.5	10.0
	Cd (%)	88.9	89.4	89.6	86.4
	Cu (%)	60.4	64.0	64.6	61.4
	Se (%)	28.2	7.7	20.9	32.9
	Zn (%)	TBD	TBD	TBD	TBD

APPENDIX 4 - SYNTHETIC EFFLUENT DATA

Table 10 Synthetic effluent use and metal concentrations (HM: Hiromi Moriya, TC: Timothy Castillon, N/A: Not Available, TBA: To be analyzed)

Date prepared	Period of use		Operator	Metal concentrations ($\mu\text{g/L}$)				
	From	To		As	Cd	Cu	Se	Zn
06-Aug-13	06-Aug-13	05-Sep-13	HM	441.7	473	5543	556.1	7456
06-Sep-13	06-Sep-13	26-Sep-13	HM	397.9	475.9	4820	485.2	5543
27-Sep-13	27-Sep-13	10-Oct-13	HM	267.5	457.5	3647	353.6	4592
11-Oct-13	11-Oct-13	22-Oct-13	HM	450.4	504.7	5199	488.5	5145
22-Oct-13	23-Oct-13	05-Nov-13	HM	290.8	532.7	5137	497.3	4714
7-Nov-13	6-Nov-13	17-Nov-13	TC	295	632	4973	534.7	5192
19-Nov-13	18-Nov-13	29-Nov-13	TC	N/A	N/A	N/A	N/A	N/A
29-Nov-13	30-Nov-13	06-Dec-13	TC	N/A	N/A	N/A	N/A	N/A
06-Dec-13	07-Dec-13	20-Dec-13	TC	390.1	TBA	5144	621.0	TBA
13-Jan-14	13-Jan-14	24-Jan-14	TC	10.2*	TBA	1755	154.1	TBA
24-Jan-14	25-Jan-14	On-going	TC	204.4	TBA	5193	499.7	TBA

* As was suspected to have precipitated

APPENDIX 5 - BIOREACTORS OPERATION TIMELINES

Table 11 Operation timelines and incubation periods for Bioreactors C1 to C4 and C5 to C8

Period	Operation
Bioreactors C1, C2, C3, C4 (Keno Hill District conditions)	
08-01-13	Columns preparation
08-02-13 to 08-09-13	Columns filling in with synthetic solution
08-10-13 to 08-22-13	Incubation (no flow)
08-23-13	Start continuous feed with synthetic effluent
08-23-13 to 09-06-13	Period required to allow first bed volume to pass through reactors
Bioreactors C5, C6, C7, C8 (Wolverine conditions)	
12-19-13	Columns preparation
11-19-13 to 23-12-13	Columns filling in with synthetic solution
24-12-13 to 01-20-13	Incubation (no flow)
01-21-13	Start continuous feed with synthetic effluent
01-21-13 to 03-31-13	Period required to allow first bed volume to pass through reactors

Table 12 Experimental conditions for assessment of metal sorption by substrate

Parameter	Experimental conditions
Type	Batch (no flow)
Duration	24 hours; 2, 4, 8, 24 hours for kinetic tests
Total solids	1.0, 2.0, 3.0, 4.0 and 5.0% of biochar or wood products in synthetic drainage water
Control	5% biochar or wood chips in DI water
pH	Controlled at 6 with H ₂ SO ₄

APPENDIX 6 - BIOREACTORS FLOW RATES

Table 13 Volume of effluent collected and calculated flow rates for bioreactors C1 to C5 from Oct 20th 2013 to Jan 23rd 2014 (note, the pump has been stopped from Dec 20th 2013 to Jan 12th 2014).

Bioreactors	C1		C2		C3		C4	
	V _{eff}	Flow rate						
Sampling date	(L)	(mL/min)	(L)	(mL/min)	(L)	(mL/min)	(L)	(mL/min)
20-09-2013	1.80	0.18	0.96	0.10	0.49	0.05	0.56	0.06
27-09-2013	0.57	0.06	1.10	0.11	1.16	0.12	1.04	0.10
04-10-2013	0.00	0.00	1.17	0.12	1.24	0.12	1.24	0.12
11-10-2013	0.48	0.05	1.14	0.11	1.20	0.12	1.13	0.11
18-10-2013	1.20	0.12	1.10	0.11	0.75	0.07	1.95	0.19
25-10-2013	1.23	0.12	1.23	0.12	1.31	0.13	1.21	0.12
01-11-2013	1.30	0.11	1.32	0.11	0.97	0.08	1.24	0.11
07-11-2013	0.60	0.07	1.06	0.12	1.14	0.13	1.03	0.12
14-11-2013	1.17	0.12	1.25	0.12	1.00	0.10	1.09	0.11
21-11-2013	1.13	0.11	1.16	0.11	0.92	0.09	1.19	0.12
28-11-2013	1.04	0.10	1.29	0.13	1.25	0.12	1.80	0.18
04-12-2013	0.87	0.10	1.27	0.15	1.25	0.14	1.15	0.13
12-12-2013	1.08	0.09	1.20	0.10	1.23	0.11	1.04	0.09
19-12-2013	0.61	0.06	1.23	0.12	1.42	0.14	1.21	0.12
23-01-2014	0.03	0.00	1.71	0.12	0.09	0.01	0.95	0.07
Total (L)	13.10		18.18		15.41		17.82	
Min (mL/min)		0.00		0.10		0.01		0.06
Max (mL/min)		0.18		0.15		0.14		0.19
Average (mL/min)		0.09		0.12		0.10		0.12
Adjusted residence time		18 days		13 days		15 days		13 days

APPENDIX 7 - BIOREACTOR EFFLUENTS DATA

Bioreactor C1 (gravel and sediment)

Table 14 Metals and sulfate concentration in effluent of reactor C1 (gravel and sediment, N/A: Not Available, TBA: To be analyzed)

Total metal concentrations in C1 effluent (µg/L)						SO ₄ (mg/L)
Sampling date	As	Cd	Cu	Se	Zn	SO ₄
QL	1.5	0.05	0.6	1.5	0.4	5
06-09-2013	11.6	11.1	8.9	<1.5	107.6	N/A
20-09-2013	19.6	5.6	15.2	<1.5	56.8	N/A
27-09-2013	21.1	5.3	18.9	<1.5	63.1	N/A
04-10-2013	N/A	N/A	N/A	N/A	N/A	N/A
11-10-2013	22.8	6.4	13.7	<1.5	29.6	N/A
18-10-2013	24.1	4.0	35.0*	<1.5	45.8	N/A
25-10-2013	24.1	3.0	8.3	<1.5	30.4	N/A
01-11-2013	20.3	3.0	15.4	<1.5	18.7	N/A
07-11-2013	23.4	3.2	13.9	<1.5	22.9	N/A
14-11-2013	19.9	1.8	6.1	<1.5	15.0	<5
21-11-2013	18.5	1.2	7.3	<1.5	13.8	<5
28-11-2013	15.4	2.0	7.5	<1.5	385.9	<5
04-12-2013	13.4	TBA	8.0	<1.5	TBA	<5
12-12-2013	17.9	TBA	11.6	0.809	TBA	<5
19-Dec-13	14.8	TBA	TBA	1.56	TBA	TBA
23-Jan-14	44.04	TBA	TBA	1.11	TBA	TBA
30-Jan-14	N/A	N/A	N/A	N/A	N/A	TBA

* Outlier

Bioreactor C2 (gravel and sediment, duplicate)

Table 15 Metals and sulfate concentration in effluent of reactor C2 (Duplicate: gravel and sediment, N/A: Not Available, TBA: To be analyzed)

Sampling date	Total metal concentrations in C2 effluent ($\mu\text{g/L}$)					SO ₄ (mg/L)	
	As	Cd	Cu	Se	Zn	SO ₄	
QL	1.5	0.05	0.6	1.5	0.4		5
06-09-2013	12.8	14.7	5.7	<1.5	99.8	N/A	
20-09-2013	21.2	6.8	6.0	<1.5	88.3	N/A	
27-09-2013	23.4	6.0	13.3	<1.5	73.9	N/A	
04-10-2013	27.0	3.4	10.2	<1.5	44.6	N/A	
11-10-2013	27.2	1.0	13.4	<1.5	17.5	N/A	
18-10-2013	31.0	3.8	20.1	<1.5	35.7	N/A	
25-10-2013	29.2	2.6	13.7	<1.5	44.3	N/A	
01-11-2013	26.4	2.7	13.2	<1.5	17.3	N/A	
07-11-2013	22.2	3.3	6.9	<1.5	28.2	N/A	
14-11-2013	23.9	3.0	1.9	<1.5	50.8	<5	
21-11-2013	19.1	6.4	4.8	<1.5	72.9	<5	
28-11-2013	22.3	1.7	5.5	<1.5	72.5	<5	
04-12-2013	16.5	TBA	4.1	<1.5	TBA	<5	
12-12-2013	12.9	TBA	9.1	<1.5	TBA	<5	
19-Dec-13	8.0	TBA	TBA	<1.5	TBA	TBA	
23-Jan-14	22.4	TBA	TBA	<1.5	TBA	TBA	
30-Jan-14	13.9	TBA	TBA	<1.5	TBA	TBA	

Bioreactor C3 (gravel and sediment and biochar)

Table 16 Metals and sulfate concentration in effluent of reactor C3 (Biochar, gravel and sediment, N/A: Not Available, TBA: To be analyzed)

Sampling date	Total metal concentrations in C3 effluent ($\mu\text{g/L}$)					SO ₄ (mg/L)	
	As	Cd	Cu	Se	Zn	SO ₄	
QL	1.5	0.05	0.6	1.5	0.4		5
06-09-2013	N/A	N/A	N/A	N/A	N/A	N/A	
20-09-2013	41.5	9.8	16.0	<1.5	95.5	N/A	
27-09-2013	34.3	5.0	18.8	<1.5	107.3	N/A	
04-10-2013	32.3	2.3	9.6	<1.5	55.5	N/A	
11-10-2013	44.4	0.8	19.3	<1.5	24.4	N/A	
18-10-2013	63.8	2.0	21.1	<1.5	42.8	N/A	
25-10-2013	51.4	2.0	13.9	<1.5	43.4	N/A	
01-11-2013	50.4	2.0	17.8	<1.5	31.2	N/A	
07-11-2013	43.2	2.3	7.0	<1.5	25.7	N/A	
14-11-2013	48.0	1.8	4.5	<1.5	33.2	14	
21-11-2013	54.0	1.6	5.8	<1.5	22.1	12.6	
28-11-2013	40.9	1.7	7.9	<1.5	14.7	11.7	
04-12-2013	43.4	TBA	5.4	<1.5	TBA	14.1	
12-12-2013	43.8	TBA	9.0	<1.5	TBA	10.8	
19-Dec-13	46.6	TBA	TBA	<1.5	TBA	TBA	
23-Jan-14	85.2	TBA	TBA	<1.5	TBA	TBA	
30-Jan-14	N/A	N/A	N/A	N/A	N/A	N/A	

Bioreactor C4 (gravel, sediment and wood)

Table 17 Metals and sulfate concentration in effluent of reactor C4 (Wood, gravel and sediment, N/A: Not Available, TBA: To be analyzed)

Sampling date	Total metal concentrations in C4 effluent ($\mu\text{g/L}$)					SO ₄ (mg/L)	
	As	Cd	Cu	Se	Zn	SO ₄	
QL	1.5	0.05	0.6	1.5	0.4		5
06-09-2013	33.8	92.8	64.4	3.1	1483.0	N/A	
20-09-2013	38.3	77.5	53.2	2.9	1369.0	N/A	
27-09-2013	28.1	27.9	40.8	<1.5	621.4	N/A	
04-10-2013	25.4	30.0	31.5	<1.5	556.3	N/A	
11-10-2013	20.7	9.1	12.0	<1.5	270.2	N/A	
18-10-2013	29.1	37.5	51.1	1.7	618.5	N/A	
25-10-2013	27.7	39.7	56.8	1.7	645.9	N/A	
01-11-2013	24.8	36.2	47.8	<1.5	371.0	N/A	
07-11-2013	27.3	40.4	32.0	<1.5	584.2	N/A	
14-11-2013	19.5	47.6	49.9	<1.5	690.7	12.1	
21-11-2013	21.3	43.0	61.7	1.5	532.8	11.7	
28-11-2013	16.9	35.3	35.3	<1.5	532.5	9.6	
04-12-2013	9.6	TBA	25.8	<1.5	TBA	6.7	
12-12-2013	9.5	TBA	25.3	1.6	TBA	<5	
19-Dec-13	7.0	TBA	TBA	<1.5	TBA	TBA	
23-Jan-14	3.1	TBA	TBA	TBA	TBA	TBA	
30-Jan-14	3.3	TBA	TBA	TBA	TBA	TBA	

APPENDIX 8 - POTENTIAL DESIGN OF FIELD BIOREACTORS



Figure 15 Example of 45 gallons tank and a 5,000 gallon tank (Images from <http://forums.pondboss.com/> and from <http://www.marketbook.ca/>)

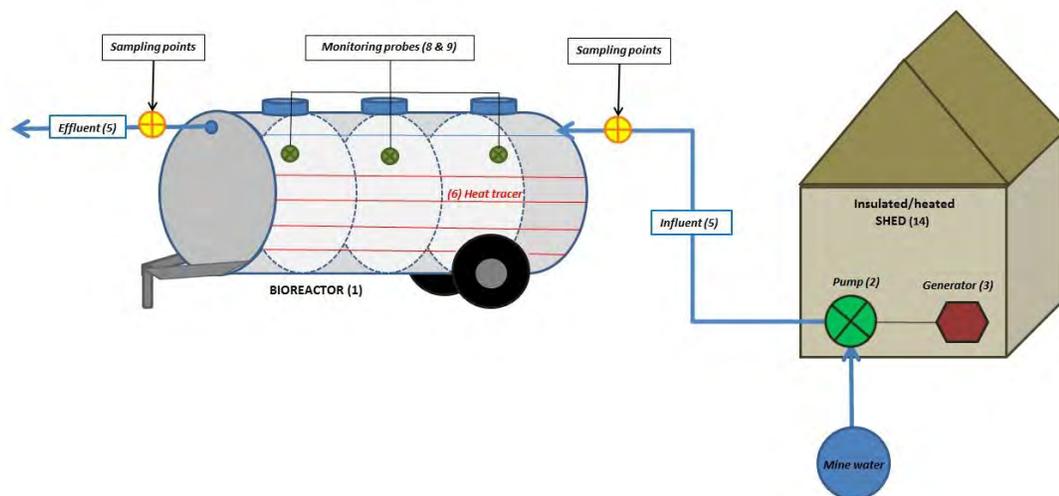


Figure 16 Transportable pilot bioreactor design proposed in ARTI application

As per figure 16, the materials required to build the transportable pilot bioreactor includes: (1) 5000 to 6000 gallons water tank, (2) peristaltic pump Verderflex, (3) diesel generator, (4) heater to protect the shed from freezing; (5) insulated, heat traced pipes for tank influent and effluent, (6) heat tracers to protect the tank from freezing, (7) tank insulation,

(8) multimeter and probes for pH, conductivity and ORP (with extra probes), (9) thermometer, (10) trailer rental to transport the shed on-site, (11) excavator rental with trailer to fill in the bioreactor with (12) biochar (20% by weight) and (13) gravel, (14) insulated wooden shed, (15) Typar membrane for protection of the bioreactor insulation.

APPENDIX 9 - CONFERENCE PROCEEDINGS

PASSIVE TREATMENT OF MINE DRAINAGE WATERS: THE USE OF BIOCHARS AND WOOD PRODUCTS TO ENHANCE METAL REMOVAL EFFICIENCY

Reference

Janin A. and J. Harrington, “Passive treatment of mine drainage waters: The Use of biochars and wood products to enhance metal removal efficiency”, Proceedings of the 2013 Northern Latitudes Mining Reclamation Workshop and 38th Annual Meeting of the Canadian Land Reclamation Association. Overcoming Northern Challenges. Whitehorse, YT, September 9 – 12, 2013, p. 90-99.

PASSIVE TREATMENT OF MINE DRAINAGE WATERS: THE USE OF BIOCHARS AND WOOD PRODUCTS TO ENHANCE METAL REMOVAL EFFICIENCY

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1. ABSTRACT

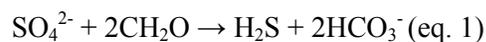
Passive biological treatments have been proposed as a possible efficient and cost effective treatment method for metal bearing water discharged from mine sites after closure. Several biofilters are under study in Yukon and have produced variable, but promising results up to now. However, concerns are typically expressed around biological treatments and their suitability in northern, colder climates. Biofilters allow for metal removal using a variety of chemical, physical and biological mechanisms. If biological processes are affected by a cold climate to some extent, chemical processes are typically not affected by the temperature the same way and can be reliable in cold waters. This study focused on metal sorption and metal removal by chemical mechanisms and assessed the sorption capacity of biochar and wood products which could be later introduced in bioreactors to help with metal removal from mine-impacted cold waters.

Biochars allowed for more than 90% removal of Cd, Cu and Zn from a metal-bearing effluent along with 35 to 69% removal of arsenic. Wood products displayed good removal capacity as well, in the range of 51 to 94% for Cd, Cu and Zn. However, arsenic and selenium removal by wood products was limited; Se also showed minimal sorption on biochars and was in one case released during sorption testing. Metal leaching from the materials was observed to some extent, including Cu and Zn from poplar and spruce products. Amongst spruce products, the chips from the trunk proved to be slightly more efficient than the needles. Overall, biochars and wood products showed potential for use in water treatment for metal sequestration in combination with other mechanisms such as sulfide precipitation in sulfate-reducing bioreactors. Such materials could be collected or produced on remote mine sites and could help with mine remediation.

Key Words: Water Treatment, Metal Removal, Metal Sorption, Adsorption, Bioreactors, Bioremediation.

2. INTRODUCTION

Water management in the mining industry has become a priority focus in our world, which has been increasingly concerned with sustainable development. To lower their footprint on the environment, mines are constantly working toward limitation of contaminant discharge to the environment. Hard rock mines have to closely control the concentrations of metals according to federal and provincial/territorial regulations, which leads to the treatment of mine-affected waters, including run-off waters, drainage from tailings or waste rocks pads, process waters, etc. Water treatment is required during operation of the mines as well as after closure for the long term. Current water treatment technology development is focused on long-term passive treatments that require low operation and maintenance. Various passive treatments are available, including chemical adsorption and bioremediation (Johnson and Hallberg 2005). Along with metals, mine waters commonly contain high sulfate content that results from the breakdown of sulfide minerals (Akcil and Koldas 2006; Kalin et al. 2006). There is an increased interest in the use of Sulfate-Reducing Bacteria (SRB) to help with metal removal from mine waters (Dar et al. 2007; Genty 2011; Jong and Parry 2003; McCauley et al. 2009; Neculita et al. 2010; USEPA 2002). Under anaerobic conditions, SRB reduce sulfate (SO_4^{2-}) into sulfide (S^{2-}) using electrons from organic matter. Sulfides, in turn, precipitate metals. The solubility of metal sulfides being generally very limited, it consequently lowers the concentration of metals in the effluent and provides a stable metal precipitate (Blais et al. 2008). To accomplish sulfide production, SRB catalyze the oxidation of organic carbon from the surrounding organic matter (eq. 1), where CH_2O represents organic carbon (Waybrant et al. 1998):



Anaerobic bioreactors are being studied in the mining industry for effluent treatment. In these systems, the effluent passes through a biofilter (e.g., in trenches) filled with permeable solid support (gravel, sand) and substrate (organic matter). The substrate used to support SRB growth can be variable. Neculita and Zagury (2008) showed that cellulosic materials like wood waste have a beneficial effect on SRB treatment efficiency. Wood products (leaf mulch, wood chips, sawdust, wood compost, peat moss) mixed with sewage sludge or manure can be an excellent substrate for SRB and have been shown to achieve reduction of metals concentration in mine effluent (Neculita et al. 2010; Waybrant et al. 1998). Besides efficiency in the short-term, the ideal mixture of substrate should also last in the long term, i.e., not be too biodegradable and deplete before the end of the life time of the bioreactor, or to extend the useful life of the bioreactor before it would require replacement or refreshment of the media. Drury (2006) used a mathematical model and showed that organic matter with an older apparent age, less biodegradable, can sustain bioreactor efficiency for longer duration. Additionally, the residual organic products may improve the stability of the metal sulfide precipitate once the treatment system is closed.

In northern climates, when the temperature is low, concerns have been expressed about the efficiency of SRB to sustain a sufficient level of biological activity during winter time to maintain treatment efficiency (Nordin 2010). The objective of this study is to assess if a range of substrates can also help with metal removal using chemical mechanisms, which are generally not as temperature-dependent as biological mechanisms. Besides providing feed to SRB, solid substrates can also act as a metal adsorbent. Cellulosic materials like sawdust and wood chips are known for their metal adsorption capacity (Argun et al. 2008; Keng et al. 2013; O'Connell et al. 2008) due to reactive groups within the substrate. Wood is an abundant resource in remote mine sites in northern Canada and other northern climates, and wood chips could easily be included in bioreactors to help metal removal by providing biodegradable organic matter and metals site adsorption. This study looked at the adsorption capacity of Spruce (trunk and needles) and Poplar (trunk) chips, as both species are very common in Yukon mine sites. In addition, biochars made from wood products were also studied. Biochar is defined as a carbon-rich material produced by thermal decomposition of organic material under limited supply of oxygen at relatively low temperature (<700°C) (Lehmann and Joseph 2009). On-going projects look at the construction of mobile pyrolysis ovens (personal communications with K. Stewart, Yukon Research Centre, M. Garcia-Perez, University of Washington). Hence biochars could be produced in remote locations, providing that the mine site has access roads. Due to the thermal decomposition, the remaining biochar is recalcitrant and is likely to persist in bioreactors on the long-term. Biochars are also capable of adsorbing metals on their surfaces and several biochars proved to have good potential for metal removal from effluent, although metal adsorption capacities can be very variable. Metal sorption by biochars depends largely on biochar characteristics, including feedstock, pyrolysis temperature, oxygen content, etc. Table 1 presents the adsorption capacity measured by various authors.

Table 18 Review of adsorption capacity of biochars from literature

Metal	Adsorption capacity (mg/g)	Adsorption pH	Biochar feedstock	References
Cd	1.5	5	Alamo switch grass	Regmi et al. 2012
Cu	4	5		
Cd	16.6	6		
Cu	6.3	5	Pig manure	Kolodynska et al. 2012
Pb	19.8	6		
Zn	4.2	5		
Pb	4.1	5	pinewood residues	Liu and Zhang 2009
Pb	2.4	5	rice husk residues	
Cr (VI)	3.0	2	Oak wood	Mohan et al. 2011
Cr (VI)	4.6	2	Oak bark	
Cu	0.04	5	peanut straw	Tong et al. 2011
Cu	0.09	5	canola straw	
Cu	12.5	5	corn straw	
Zn	11	5	corn straw	Chen et al. 2011
Cu	6.8	5	Hardwood	
Zn	4.5	5	Hardwood	
Cu	48.5	6	Salt-marsh plant	Li et al. 2013

Three different biochars, poplar wood chips, spruce wood chips and spruce branch mulch were studied as metal adsorbents in this study.

3. MATERIAL AND METHODS

4. Adsorbents Sampling and Preparation

Poplar and spruce trees were cut down in the Whitehorse region, Yukon Territory, Canada. Branches were removed from the tree before the trunks were ground into chips using a log chipper (Bandit M65 XP, USA). Spruce branches were ground separately into mulch using the same equipment. Wood chips and mulch were used fresh, with less than a week of drying.

Three biochars were collected from different manufacturers. Biochar made from mixed spruce, pine, and fir was produced by Diacarbon Energy Inc. (Burnaby, BC, Canada) and named “BCD”. Biochar made of spruce, pine, fir, willow and poplar was produced by Zakus Farms (“BCZ”). The biochar collected from Titan (Saskatoon, SK, Canada) was made from Willow and fish bone meal (“BCT”).

Adsorbents pH Measurements

Suspensions were made using 1:10 (w/w) ratio of biochar or wood with DI water. The pH of the suspension was measured at $t=0$ ($\text{pH}_{t=0}$) and after a week ($\text{pH}_{1 \text{ week}}$) at room temperature using an pH meter (Oakton pH5+, Vernon Hills, IL, USA) equipped with Ag/AgCl combination reference electrodes. pH calibration was done using certified pH 4, pH 7 and pH 10 standards (Fisher, catalogue number SB101-500, SB107-500 and B115-500).

Batch Adsorption Studies

Synthetic drainage effluent was produced using sulfate metal salts (As_2O_5 , $\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, SeO_2 , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{NaSO}_4 \cdot 10\text{H}_2\text{O}$, all ACS reagents) dissolved in DI water at pH 6. Then 2, 4, 6, 8 and 10 g of adsorbent materials were mixed with 200 ml of synthetic drainage effluent in a 500ml baffled Erlen Meyer and shaken for 24 hours at room temperature to allow for metal sorption equilibrium. To assess metal leaching from the materials, 10g of adsorbent was mixed with DI in the same conditions. Supernatant was then filtered through 0.45 μm porosity glass fiber filters (Cole Parmer, catalogue number RW03-04700) and stored for further analysis.

Analytical Techniques

Effluent pH was measured using Oakton meter (Oakton pH5+, Vernon Hills, IL, USA with Ag/AgCl combination reference electrodes). Total Solids contents were measured according to APHA method 2540B. Biochar and wood products were partially digested using ACS grade nitric acid and hydrogen peroxide (method USEPA 3050b) to allow for determination of metal contents. Metal concentrations were measured using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), Vista-AX CCO, by Varian (Palo Alto, CA, USA). Quality controls were performed with certified multiple element standards from SCP Science (Lasalle, QC, Canada) to ensure conformity of the measurement apparatus. Limit of Quantification (LQ) was calculated as 10 times the standard deviation measured obtained after measurement of 10 blanks.

5. RESULTS AND DISCUSSION

6. Material Characteristics

The material studied includes three biochars (BCD, BCZ and BCT) and three wood products (poplar chips, spruce chips and spruce needles). Table 2 presents the results of characterization. As expected, biochars displayed alkaline pH, initially between 9 and 10 and reducing down to close to 8.5 after a week. On the other hand, the wood products were acidic, with the poplar chips producing the most acidic conditions after a week, at pH 3.84. Arsenic and selenium contents in all materials were low, under the quantification limits of 0.9 mg/kg for As and 2.1 mg/kg for Se, except the biochar made of willow and bone meal (BCT) at 6.3 ± 0.7 mg/L Se. Overall, the BCT material, made of willow and bone meal, displayed higher concentrations of metals, including significant amount of Cd, Zn, Fe and Na. In general, higher metal contents were measured in biochars than in wood products.

Biochars were made by pyrolysis, which involved volume reduction and subsequent concentration of the metals in the residual product.

Table 19 Measured adsorbent characteristics (1:10 water suspension was used for pH measurements; HNO₃/H₂O₂ digestion was used for metal contents analysis)

Material	Total solid	pH₀	pH_{1week}	As	Cd	Cu	Fe	Na	Se	Zn
	%			mg/kg						
				g	g	g	g		g	g
BCT	81.0	9.84	8.27	<0.9	11.0	3.3	3755.8	15814.5	6.3	164.2
BCD	81.1	9.91	8.51	<0.9	0.8	26	1362.3	1281.0	<2.1	55.8
BCZ	96.5	9.23	8.47	<0.9	0.2	15.2	939.1	123.0	<2.1	60.0
Pop. chips	93.7	6.06	3.84	<0.9	0.2	30.8	32.6	53.3	<2.1	30.0
Sp. chips	93.6	5.94	5.5	<0.9	2.0	67.9	54.5	111.4	<2.1	27.0
Sp. needles	89.9	5.28	5.11	<0.9	2.4	22.9	110.1	124.8	<2.1	51.1

7. Metal Leaching

Wood products and biochars contained heavy metals to some extent. Mixing of the materials with DI water for 24 hours was completed to assess the potential for metal leaching. The results are presented in Fig 1 along with the concentration measured in the synthetic drainage water for comparison. Wood products leached out more metals than biochar products, even if metals contents were generally lower (Table 2). The metals contained in pyrolysis products may be tightly bound and less available for leaching. Poplar chips and spruce needle leached significant amount of copper and zinc in the first 24 hours of being submerged in water, with concentrations 0.13 and 0.12 mg Cu/L and 0.13 and 0.17 mg Zn/L respectively using 5% S/L ratio. No selenium leaching was observed (< LQ of 0.021 mg/L) and arsenic leaching was observed only for BCT (0.012 mg As/L, otherwise < LQ of 0.0093 mg/L). Hence, the use of natural materials such as wood, in bioreactors should be managed carefully, with special attention to metal leaching potential in the initial operation period.

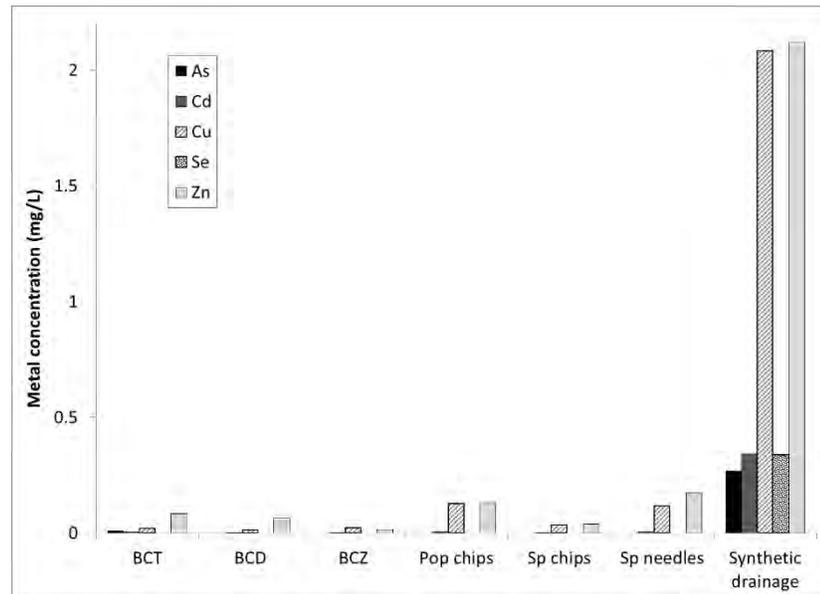


Figure 17 As, Cd, Cu, Se and Zn concentration observed after leaching from biochars BCT, BCD, BCZ, poplar chips, spruce chips and spruce needles and in the prepared synthetic drainage water (Solid to liquid ratio of 5% in DI water, 24 hours equilibrium, LQ are 0.009 mg/L for As).

Metal adsorption

The primary objective of this study was to assess the sorption capacity of materials which can be collected or produced on-site in many northern and especially Yukon mines, to help with metal sequestration. Three biochars and three wood products were mixed with pH 6 synthetic drainage water containing metals commonly found in mine impacted waters, namely As, Cd, Cu, Se and Zn. Figure 2 presents the relative concentration (C/C_0) of the different metals remaining in the effluent after exposure to each of the adsorbents for 24 hours. Biochars results (BCT, BCD and BCZ) are displayed in Fig 2a, b and c. The profiles are very similar for Cd, Cu and Zn, with more than 90% removal on average using 1% to 5% S/L ratio. Arsenic removal was lower, with 54, 69 and 35% respectively using BCT, BCD and BCZ. The selenium profiles were somewhat surprising. The three biochars studied were not able to remove more than 30% of selenium and the Biochar BCZ actually released selenium to a significant extent. Although 10 g of BCZ leached less than 2 μg of Se in DI water, when mixed with synthetic drainage the same amount of biochar released more than 80 μg of Se. Selenium initially bound to the biochars may have been displaced through an exchange process during sorption of other metals with higher affinities like Fe, Na, Zn, Cu, Cd on the biochar. The mechanisms of selenium release should be further studied.

Divalent metal removal by wood products was reasonably effective. Although not as efficient as biochar, poplar, spruce chips and spruce needles were able to remove Cd up to 87, 94 and 84% respectively, Cu up to 76, 81 and 66% respectively and Zn up to 83, 88 and

51% respectively. The spruce chips obtained from trunks displayed higher ability than the needles for metal removal. This difference may be due to the different structure, porosity, and surface chemistry of the needles versus the trunk chips. Amongst the three wood products, Spruce chips gave the best results.

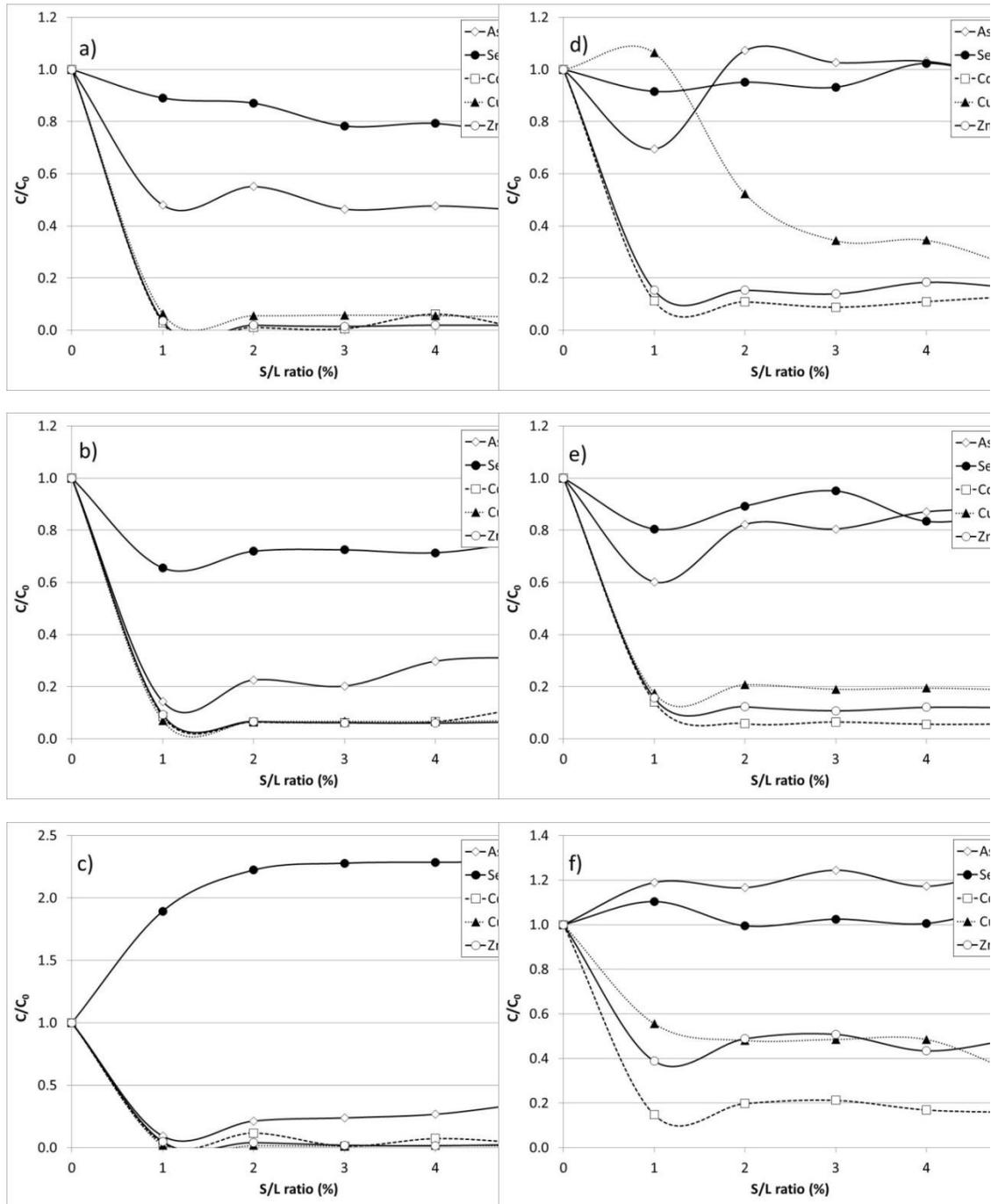


Figure 18 Relative concentrations (C/C_0) of As, Se, Cd, Cu and Zn after 24 hours equilibrium with variable solid to liquid ratio using a) BCT, b) BCD, c) BCZ, d) poplar

chips, e) spruce chips and f) spruce needles (initial concentrations of 0.27 mg As/L, 0.34 mg Cd/L, 2.08 mg Cu/L, 0.34 mg Se/L and 2.12 mg Zn/L).

8. CONCLUSION

Natural wood products and biochars were studied for metal removal from a synthetic drainage effluent. Biochars exhibited alkaline properties whereas wood products generated acidity however, both materials displayed good capacity for divalent metal removal like Cd, Cu and Zn although biochars were slightly more efficient than wood products. As and Se were less amenable to adsorptive removal than divalent metals but biochars helped to remove arsenic to some extent. Overall, this study gave evidence of the potential of wood products and biochars for water treatment and metal sequestration, providing that metal leaching from the material itself is controlled. Further study should investigate the effect of using such materials on sulfate-reducing bacteria growth and bioreactor efficiency, and these tests are planned using some of the same media as was tested in this study.

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