

# **Performances of lab-scale anaerobic bioreactors at low temperature using Yukon native microorganisms**

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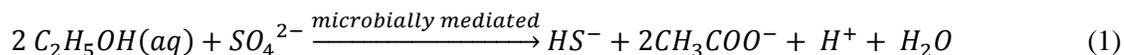
## **Abstract**

Passive biological treatments have been proposed as an efficient and cost effective treatment of metal bearing water discharged from mine sites after closure. However, concerns are typically expressed around biological treatments and their suitability in northern, colder climates, as few examples of passive treatment systems operating under cold conditions have been documented so far. In this study, four laboratory scale anaerobic bioreactors were monitored for over one year for their capacity to reduce metal concentrations at ambient laboratory temperature, then at 6°C and 3°C. As, Cd, Cu, Se and Zn concentrations in the effluent were compared and contrasted with discharge limits in application at the Bellekeno Mine, Yukon Territory. Removal efficiencies in the range of 90 to 100% were observed in the four reactors and for the five metals studied, suggesting that sulfate-reducing bacteria native from the Yukon stayed active under cold conditions. In addition, integration of biochar in the composition of a bioreactor had a positive effect on the treatment efficiencies at the lower temperatures. This finding suggested that addition of a reactive material in the composition of the bioreactor substrate might help reduce the impact of the cold climate on the treatment system by taking advantage of metal adsorption mechanisms, which allows for continued metals removal during changes in temperature.

## **Introduction**

Water management in the mining industry has become a priority focus at all stages of the mine life. Passive water treatment approaches are seen as a solution where long-term treatment of water impacted by old mine workings, waste rocks, tailings facility or other mine components after is required after closure. Past research efforts lead to the development of anaerobic bioreactors targeting the removal of metal contaminants from mine-impacted water using sulfate-reducing bacteria, SRB (USEPA, 2014; MEND, 1996) and numerous passive anaerobic bioreactors have been successfully implemented at large scale (Alexco, 2012; Gusek et al., 2000, Gusek et al., 2011; Kuyucak et al., 2006; Sobolewski, 2010; Dar et al., 2007; Wilmoth, 2002; Germain and Cyr, 2003; Ettner, 2007; Nordwick et al., 2006; Bless et al., 2008;

Wilmoth, 2002). Under anaerobic conditions, sulfate ( $SO_4^{2-}$ ) is reduced into sulfide ( $HS^-/S^{2-}$ ) by sulfate-reducing bacteria using electrons from organic matter (represented as ethanol in the equation 1 adapted from Nagpal et al., 2000). Sulfides then react with metals to precipitate as metal sulfide salts which are generally very insoluble (Eq. 2 where a metal cation is represented as  $Me^{2+}$ , Jong and Parry, 2004; Neculita et al., 2010).



As passive bioreactors are proposed for mine closure in Yukon Territory common concerns includes the performances of biological water treatment in cold climate as low temperatures typically affect microbial growth rate. In order to improve efficiencies, various authors have looked at the addition of organic carbon to the reactor help microorganisms sustain activities in cold temperatures. It has been shown that the addition of readily metabolizable carbon such as methanol, ethanol, lactate, or ethylene glycol, improves the performances of anaerobic reactors (Gould et al., 2012; Alexco, 2012; Sobolewski, 2010; Tsukamoto, 1999). Although several anaerobic bioreactors have displayed high efficiencies in cold climates (Ness et al., 2014), the performances of anaerobic bioreactors still need to be broadly proven and be supported by comprehensive scientific knowledge before it can be classified as best practice in northern environments.

The objectives of this study were to first, assess the potential of Yukon native sulfate-reducing bacteria to remove As, Cd, Cu, Se and Zn from a neutral mine drainage in lab-scale bioreactors and secondly, to measure the impact of cold temperature on the bioreactors performances. Finally, another objective of this study was to evaluate the effect of adding biochar, an adsorbent material, within the bioreactor on metal removal efficiencies at low temperatures.

Metals sorption mechanisms such as adsorption on substrate or on plant materials, are known to co-exist with the microbial mediated sulfate reduction however sorption mechanisms have been considered to play a minor role in the overall metal removal in a bioreactor system (Gusek, 2008; Neculita et al., 2008). A new approach for cold-climate bioreactors is to include reactive substrate within the bioreactor that will provide opportunities for retention of the metal contaminants by chemical sorption (Janin and Harrington, 2013; Conca and Wright, 2006). Chemical sorption is less temperature-dependent than microbial processes, thus it might prove useful under transitions to cold temperatures where microbial activity may be at least temporarily slowed. Biochar, which is defined as a carbon-rich material produced by thermal decomposition of organic material under limited supply of oxygen (Lehmann and Joseph, 2009) has been used in this study.

Biochar is known to have adsorption capacity for transition metals (Regmi et al., 2012; Kolodynska et al., 2012; Tong et al., 2011; Chen et al., 2011; Li et al., 2013).

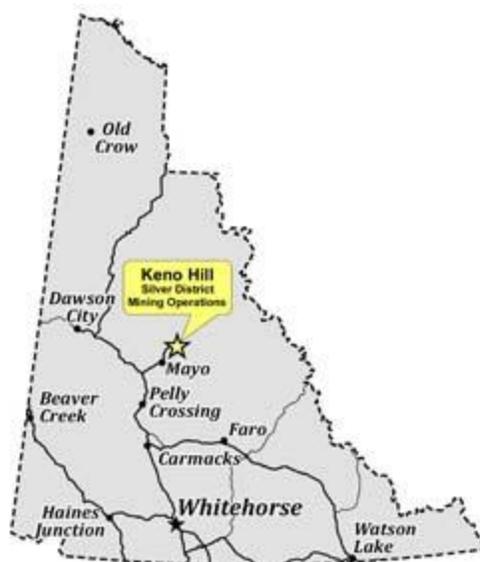
## Material and methods

### Synthetic effluent preparation

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 deionized (DI) water with target concentrations of 5 mg/L for Cu, Fe and Zn, 0.5 mg/L for As, Cd and Se. Actual metal concentrations of the synthetic effluent were measured monthly. To accommodate metal/sulfur ratio required to precipitate metals as sulfide salts ( $FeAsS$ ,  $CdS$ ,  $CuS$ ,  $FeS$ ,  $Se(0)$ ,  $ZnS$ ), 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. In addition to metals, 16 mg/L ethanol was added in the synthetic effluent to provide electrons and support the sulfate-reducing bacteria in the bioreactors.

### Lab-scale bioreactors setup

Sediments were collected from Crystal Creek, in the Keno Hill Mine District, Yukon (Figure 1), in July 2013, in an area with long term exposure to mine drainage. The sediments, high in sandy materials, were sieved onto 2 cm, then used as an inoculum in the composition of the bioreactors to provide Crystal creek-native microorganism in the lab scale bioreactors under study.



**Figure 1: Project location, Keno Hill Silver District, Yukon Territory**

Four lab-scale bioreactors were made from 70 cm high Plexiglass columns with 6.4 cm internal diameter and a bed volume of 2.25 L (Ellis Associates, Edmonton) (Figure 2). The columns were fed with synthetic effluent from the bottom up using a peristaltic pump and Masterflex L/S13 tubing (internal

diameter of 0.8 mm). Target flow rate was 0.1 ml/min at 3.7 rpm with 14 days residence time. Two bioreactors, called “C1” and “C2”, were filled with 100% creek sediment while the bioreactor “C3” was filled with 80%(v/v) creek sediment and 20%(v/v) wood biochar produced by Zaku Farm (YT). The fourth bioreactor which was filled with 20% wood won't be discussed in this study but data is available from the authors.



**Figure 2: Bench-scale bioreactors**

### **Bioreactors operation and monitoring**

The lab-scale bioreactors were prepared on August 1<sup>st</sup> 2013 then filled with synthetic effluent. The pump was then stopped to let the reactors incubate for 13 days. After incubation, on August 23<sup>rd</sup> 2013, the columns were fed continuously with synthetic effluent flowing at 0.1 ml/min at room temperature between September 2013 and April 2014 (259 days). Later, in May 2014, the reactors were transferred into a fridge at 6°C for 2 months (57 days), then at 3°C for 55 days). The effluents from the bioreactors were gathered in 2L collection jars over which were monitored weekly for pH and volume. Samples for total metal were collected weekly from the collection jar, preserved with HNO<sub>3</sub> then stored at 4°C until analysis.

### **Analytical Techniques**

Total As, Cd, Cu, Se and Zn metal concentrations were measured using Perkin Elmer PinAAcle Atomic Absorption (AA) analyzer (Perkin Elmer, Waltham, MA). As, Se, Cd and Cu were analyzed by Graphite Furnace (GFAA) and Zn by Flame (FAA). The Quantification Limits (QL) used in this work have been defined for each element 10- $\sigma$  as 0.8 ug As/L, 0.05 ug Cd/L, 0.6 ug Cu/L, 0.7 ug Se/L and 0.4 ug Zn/L. Calibrations were completed on a daily basis using single element standard (SCP Science, Baie D'Urfé, QC), and blanks and mixed verification standards (Perkin Elmer, Waltham, MA) were analyzed every 15

samples. Standards were made from commercial standards purchased from SCP Science. Solution pH was measured using Oakton PCD650 meter (Vernon Hills, IL) with a double junction pH electrode.

## Results and discussion

### Synthetic effluent characteristics

Metal concentration in the influent varied along the duration of the experiment with the averages and standard deviation presented in Table 1. As, Cd and Se average concentrations in the solutions entering the bioreactors were respectively 342 ug As/L, 521 ug Cd/L and 466 ug Se/L while Cu and Zn concentrations were 4,654 ug Cu/L and 5,525 ug Zn/L. Variability between 17 and 24% was observed for the metal concentrations in the synthetic effluent over 371 days. Variations likely came from metals precipitation and co-precipitation in the influent container while the solution was pH 6. Yearly variations between 15 and 25% in metal concentrations are not uncommon in drainage monitored at mine sites and are due to seasonality and variations in water flows and temperatures.

**Table 1: Metal concentrations in synthetic effluent feeding in the bioreactors (monthly monitoring over 371 days)**

Metals	Concentration (ug/L)		
	Average $\pm$ SD	Min	Max
As	342 $\pm$ 81	204	450
Cd	521 $\pm$ 93	446	766
Cu	4,654 $\pm$ 1,022	1,755	5,543
Se	466 $\pm$ 117	154	621
Zn	5,525 $\pm$ 960	4,592	7,517

### Effluent flows

The total volume of effluent collected from the outlet of the four lab-scale bioreactors were used to assess and compare the hydraulic characteristics of each four reactors (Table 2). Variation was observed amongst the bioreactors, with average flow rates between 0.080 to 0.089 ml/min over the duration of the experiment. Consequently, the total number of bed volumes treated through the reactors varied from 19 to 21 (ie. number of reactor volumes that has passed through the reactor). Similarly, the hydraulic residence time which is calculated by dividing the volume of the reactor by the flow rate at which it is operated, varied from 17 days for the reactors C2 and C3 to 19 days for the reactor C1. It should be noted that although the two reactors C1 and C2 are referred to as a duplicate, C2 received about 10% higher flow than C1. Variation in flow rate depends on the hydraulic conductivity of the substrate and its porosity but the major challenge

observed during this lab-scale study was clogging of the inlet tubing which are required to be small (0.8 mm ID) to deliver small flow rates (0.09 ml/min) and therefore susceptible to be obstructed by suspended particles. Such a challenge is usually not an issue at larger scale, where inlet tubing are much larger.

**Table 2: Flow characteristics for C1, C2 and C3 bioreactors over 371 days of operation (weekly monitoring)**

<b>Bioreactor</b>	<b>C1</b>	<b>C2 (Dup)</b>	<b>C3 (Biochar)</b>
Total volume treated (L)	42.99	47.67	44.95
Nb bed volumes	19.1	21.2	20.0
Average flow rate (mL/min)	0.080	0.088	0.089
Min (mL/min)	0	0	0
Max (mL/min)	0.16	0.16	0.15
Hydraulic residence time	19 days	17 days	17 days

### **Metal removal efficiencies**

Weekly monitoring of As, Cd, Cu, Se, Zn and effluent volume allowed for calculation of the total load (in mmol) which entered each bioreactors along with the total load of metals which were not retained by the bioreactors. This was used to calculate the total efficiencies for the three bioreactors studied under variable temperatures for over a year of operation (Table 3). Cadmium, copper, selenium and zinc were steadily removed by the control reactors, C1 and C2 (creek sediment duplicates) and C3 (biochar) with metal removal efficiencies >98.7% in the conditions studied. Arsenic removal rates were slightly lower than the other metals studied, however the removal efficiencies were still measured between 89% and 95.4%. Although metal removal efficiencies are largely dependent on the initial concentrations, initial water chemistry and the addition of ethanol, the observed efficiencies were well aligned with typical removal efficiencies observed at pilot or full-scale bioreactors as reported in a literature review by Ness et al., 2014.

**Table 3: Metal loads coming in and out of the C1 to C4 lab-scale bioreactors and removal efficiencies over 371 days (C1: gravel and sediment, C2: gravel and sediment duplicate, C3: gravel, sediment and biochar)**

	<b>C1</b>	<b>C2 (DUP)</b>	<b>C3 (Biochar)</b>
As <sub>in</sub> (mmol)	0.1883	0.2024	0.1862
As <sub>out</sub> (mmol)	0.0086	0.0107	0.0205
<b>As removal efficiency (%)</b>	<b>95.4</b>	<b>94.7</b>	<b>89.0</b>
Cd <sub>in</sub> (mmol)	0.1667	0.1829	0.1696
Cd <sub>out</sub> (mmol)	0.0010	0.0018	0.0007
<b>Cd removal efficiency (%)</b>	<b>99.4</b>	<b>99.0</b>	<b>99.6</b>
Cu <sub>in</sub> (mmol)	3.7991	4.1114	3.8973
Cu <sub>out</sub> (mmol)	0.0072	0.0054	0.0058
<b>Cu removal efficiency (%)</b>	<b>99.8</b>	<b>99.9</b>	<b>99.9</b>
Se <sub>in</sub> (mmol)	0.3078	0.3357	0.3153
Se <sub>out</sub> (mmol)	0.0005	0.0005	0.0004
<b>Se removal efficiency (%)</b>	<b>99.8</b>	<b>99.9</b>	<b>99.9</b>
Zn <sub>in</sub> (mmol)	3.0201	3.3560	3.0952
Zn <sub>out</sub> (mmol)	0.0273	0.0438	0.0228
<b>Zn removal efficiency (%)</b>	<b>99.1</b>	<b>98.7</b>	<b>99.3</b>

Removal efficiency for transition metals such as Cd, Cu, Zn are typically in the 90-100% range, and while As and Se removal by anaerobic bioreactors have been less studied, some examples report removal of >95% Se (Blumenstein and Gusek, 2008; Rutkowski et al., 2010) and 50 to 96% removal of As (Nordwick et al., 2006; Germain and Cyr, 2003; Alexco, 2012). Finally, the efficiencies of the biochar-amended reactor, C3, were very similar to the efficiencies observed in the non-amended, control reactors C1 and C2 except for As performances, which was slightly lower in C3 reactor. However, when compared with the discharge limits in effect at the Bellekeno mine in the Keno Hill District described by the Quartz Mining Licence QZ07-078, the biochar-amended bioreactor performed better than the other two reactors, with more infrequent exceedance of the discharge limits (Table 4). The discharge limits of 0.1 mg/L As, 0.01 mg/L Cd, 0.1 mg/L Cu and 0.5 mg/L Zn, as defined by the Water Use Licence QZ09-092 (Bellekeno mine, Keno Hill District, YT) constituted the water treatment objectives against which the results obtained in this study were compared. Exceedances of limits have been observed 8 times out of 140 data for C1, 21 times out of 148 data for C2 and 6 times out of 151 data. This might indicate that amendment with biochar helped keep effluent below discharge limits as it relates to As, Cd, Cu, and Zn. In a larger scale reactor, it is expected that the compliance efficiency would be higher.

**Table 4: Number of metal measurements complying with discharge limits at the Bellekeno mine according to license QZ07-078 (weekly sampling for As, Cd, Cu, and Zn from lab-scale reactors monitored over 371 days of operation at room temperature, 6°C and 3°C)**

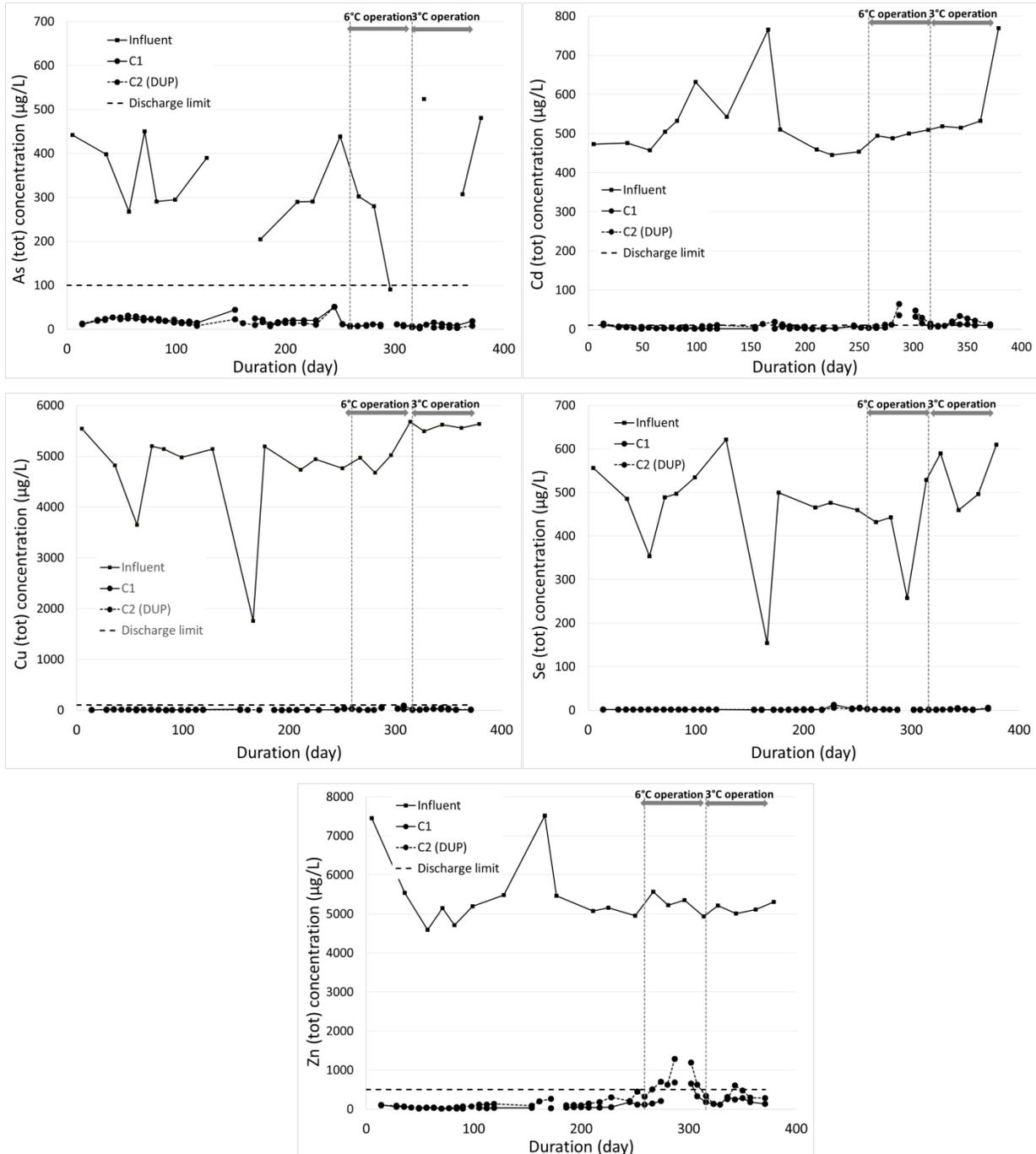
	Discharge limits*	Number compliance/Non compliance		
	(mg/L)	C1	C2 (DUP)	C3 (Biochar)
As	0.1	35/35	37/37	36/37
Cd	0.01	29/35	24/37	33/38
Cu	0.1	35/35	36/37	38/38
Zn	0.5	33/35	30/37	38/38
n		140	148	151
Non-compliance events		8 (6%)	21 (14%)	6 (4%)

### Effect of cold temperatures

Low temperatures are known to affect metabolic activities and bacterial growth but compliance of the effluent with the metal discharge regulations is obviously required all-year round. In order to allow development of bioreactors in cold climate locations, these biological systems have to be tailored to sustain efficiencies over a wide range of temperature, and where temperature variation will necessarily be part of the design constraints. A major improvement proposed was to supplement bioreactors with liquid carbon sources that will help microorganism to sustain a level of metabolic activities in cold temperatures (Gould et al., 2012; Alexco, 2012; Sobolewski, 2010) and this was applied to this study, with addition of 16 mg/L ethanol to the bioreactor influent.

The following figure presents As, Cd, Cu, Se and Zn concentrations in the influent and effluents of the control bioreactors C1 and C2 over the duration of the testing at room temperature, 6°C and 3°C. As discussed above, overall removal efficiencies were significant, even after the temperature was drastically lowered from room temperature to 6°C. This aligns well with the observations from Sagemann et al. (1998) and Robador et al. (2009) that cold-adapted mesophilic bacteria from natural cold arctic environments are capable to perform sulfate-reduction over a large range of temperatures, -3°C to 40°C. However, the change in temperature affected Cd and Zn removal to some extent, with non-compliance event observed in the 50 days following the transition of the bioreactor into the colder temperature. After 2 months at 6°, the Cd and Zn concentrations in the effluents did return back to compliance as observed before the temperature change, which might indicate that the microorganisms had acclimatized to the lower temperature, which correlates well with results from Londry (2013). Still, effluent concentrations were often measured above discharge limits for Cd and sporadically measured above discharge limits for Zn at 6°C and 3°C. Some impact has been observed on Cu with a maximum measured at 0.089 mg/L but never exceeding the 0.1 mg/L discharge

limit. The impact of temperature was less noticeable on As and Se than Cd, Cu and Zn. The effluent did continue to comply with As discharge limits of 100 ug/L (no discharge limits for Se).



**Figure 1: Arsenic (a), cadmium (b), copper (c), selenium (d) and zinc (e) concentrations in influents and effluents of two duplicate bench-scale bioreactors C1 and C2 (Discharge limits are 100 ug As/L, 10 ug Cd/L, 100 ug Cu/L and 500 ug Zn/L (Quartz mining license QZ07-078)**

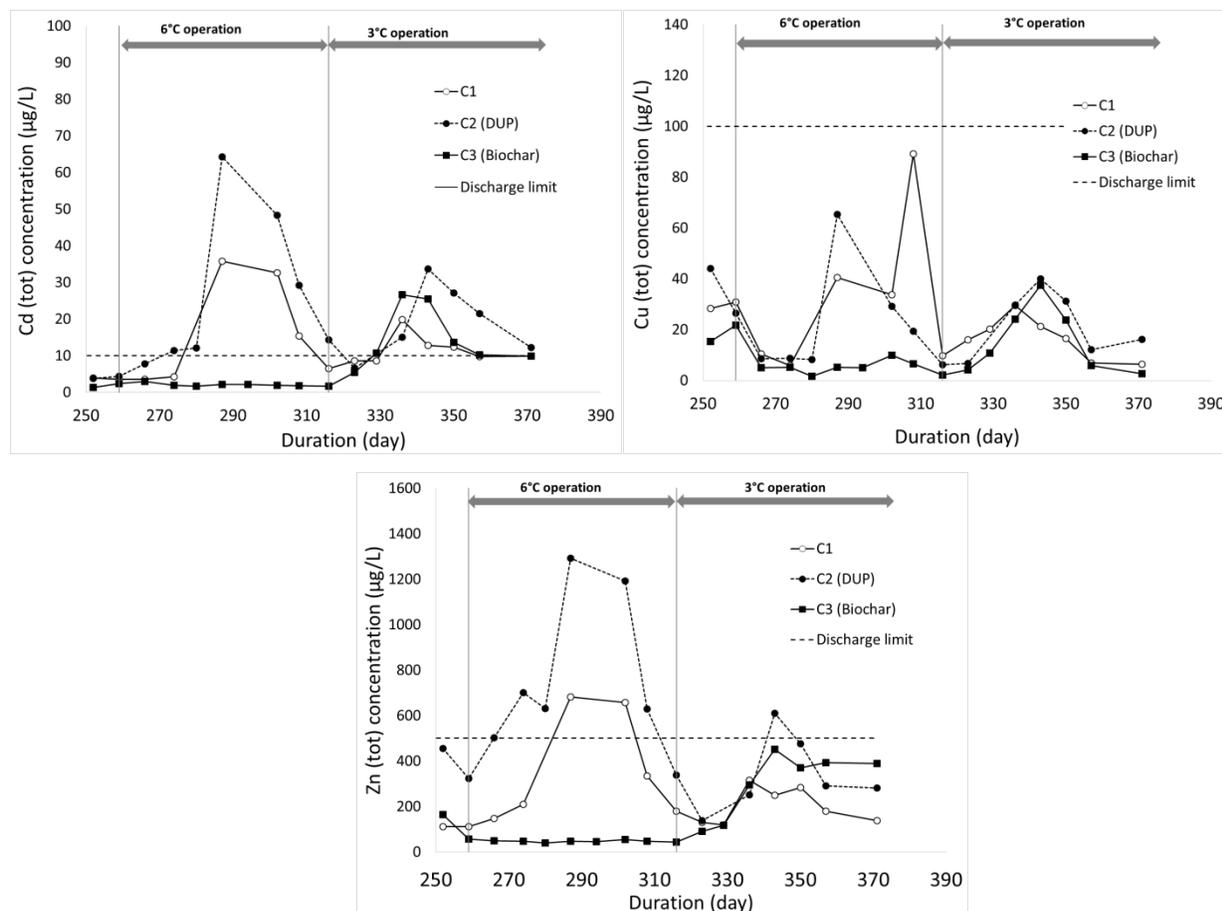
### **Bioreactor amendment with reactive substrate**

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 might improve reliability in cold waters. Metal sorption is known to be one of the mechanisms to remove metals in a bioreactor although it is usually considered a minor mechanism in temperate climate. One hypothesis on why it may be a minor mechanism is that the precipitation of metal sulfides provides a lower solubility sink than the solubility control from the sorption mechanism. In this study, the C3 bioreactor, composed of 20% (v/v) biochar and 80% creek sediment was contrasted with the control C1 and C2 bioreactors which contained 100% of the same creek sediment (Figure 4). As discussed earlier, Cd, Cu and Zn removal were affected by the cold temperature, leading to an increase in effluent concentrations with some exceedance of the site-specific discharge limits.

A common profile was observed for the three metals in the two duplicate reactors with an increase in metal concentrations starting when the temperature was dropped to 6° (259 days), peaking at about 40 days later. The metal effluent concentrations then began to decrease, possibly due to the acclimatization of the sulfate reducing bacteria and subsequent active sulfide production and metal sulfide precipitation. At day 316, when the temperature was decreased from 6°C down to 3°C, another smaller and temporary peak in metal concentrations was observed. The concentration then went down again about 40 days later. Interestingly, when biochar was added in the bioreactor substrate (C3), the first peak in concentration at 6°C was truncated while the second peak at 3°C is comparable as the control reactors for the three metals. The biochar is apparently supporting metal removal in the bioreactor, possibly by promoting sorption during the phase where microbial sulfide precipitation was slowed. The addition of this sorptive substrate might be an appropriate tool to add to bioreactor designs for operation in cold climate to enhance stability and reliability of metal removal by bioreactors especially for less variability during seasonal temperature transitions.

Adsorption capacity of the wood biochar was highlighted in a previous study by Janin and Harrington (2013) and measured as 0.034 mg Cd/g biochar, 0.212 mg Cu/g biochar and 0.209 mg Zn/g biochar. Sorption is a finite and reversible mechanism. In this study, 20% (v/v) biochar was included in the composition of the bioreactor. Using these values and the mass of biochar used in the bioreactors C3, the maximum, theoretical, amount that biochar could sorb was 0.020 mmol Cd, 0.280 mmol Cu and 0.268 mmol Zn. After one-year, these values have been largely exceeded, providing evidence that metal retention within this reactor is not primarily due to sorption but most likely co-existing with microbial mediated mechanism. However, these results indicate that these sorption processes might help offset the decreased biological activity during transition to colder temperatures such as would be experienced in winter time and thereby enhance the reliability of the treatment, as highlighted by the fewer exceedance of the discharge limits in this study. The integration of biochar or another sorbent material could prove useful to better adapt

an anaerobic bioreactor for application in northern, cold, environments. Many natural sorbent materials are known to help metal removal from mine water by sorption (Westholm et al., 2014), offering a variety of materials that could be used to tailor passive water treatment to northern mines.



**Figure 2: Cd, Cu and Zn concentration in effluents from C1, C2 and C3 bioreactors between day 250 and 400 (Influent concentrations between 454 and 533 µg/L Cd, 4,676-5,677 mg/L Cu and 4,939-5,570 mg/L Zn) when bioreactors were operated at 6°C (Days 259-316) and 3°C (days 316-371)**

## Conclusion

Lab-scale bioreactors successfully removed cadmium, copper, selenium and zinc from synthetic mine water with removal efficiencies above 98% along with more than 89% arsenic. Operation of the lab-scale bioreactors at 6°C then 3°C did affect the performances of the bioreactor to some extent and did lead to some out-of-compliance events however, it was shown that the bacteria used in this study and native from northern environment were adapted to the low temperature and the bioreactor were still capable to perform metal removal at high efficiencies when liquid carbon substrate was provided to the bioreactors. The integration of biochar as part of the substrate was shown to have lessened the impact of the cold temperature,

more reliably keeping the metal concentrations in the bioreactors effluent below the discharge limits. Further expansion of this study into field work with pilot-scale bioreactors would help confirm or infirm the hypothesis by which using biochar as an amendment helps with compliance. In addition, the impact of the freeze/thaw cycle on the microbial activities should be assessed if a bioreactor is to be designed in an area where reactor freezing is expected.

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