Pilot bioreactors commission and operation at Minto Mine

2014 & 2015 Preliminary Results

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EXECUTIVE SUMMARY

Four (4) pilot anaerobic bioreactors were commissioned at the Minto mine site in the summer of 2014 by Amelie Janin, NSERC Industrial Research Chair at Yukon College, and Capstone staff. Installation was completed on August 7th, 2014 and operation started on August 20th, 2014. Bioreactor substrate composition varied among the bioreactors and included mixtures of creek sediments, low-grade or river gravel, wood chips or biochar (a coal made out of wood). During the first year the bioreactors were monitored until September 23rd, 2014, after which they were dismantled and stored for the winter. In the following year, the bioreactors resumed operation on June 2nd and were monitored between June 20th, 2015 and September 26th, 2015.

Results during the four months of operation in the succeeding summer and fall in 2015 suggested that:

- Bioreactors continued to reduce selenium from the influent which had a concentration close to Minto Mine's selenium effluent discharge limit, decreasing to below the discharge limit of 0.003 mg/L as set by Minto's Water Use License;
- Steady copper removal was observed in all bioreactors regardless of temperature drop during the fall months;
- Chipped wood appeared to release acidity in the effluent along with Total Organic Carbon in the month of operation in 2014 but not during operational period in 2015;
- All bioreactors, including two that contained wood chips, met pH requirement of mine discharge limit throughout the 2015 operational period;
- The most effective reduction of sulfate was observed in the bioreactors amended with wood chips;
- Bioreactors helped to reduce nitrate concentrations to below discharge limits of 7.65 ug/L;
- No exceedance of the mine effluent discharge limits were observed for nitrite and ammonia in the bioreactors effluents; and
- Elevated levels of phosphate (not regulated) was observed in the effluent of the biochar amended reactor in the first year but absent in the other bioreactors.

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1. BACKGROUND

Minto Mine (Minto) is an open pit and underground copper mine that has been in commercial production since 2007. The mine is operated by Minto Explorations Ltd., a wholly-owned subsidiary of Capstone Mining Corp. Anaerobic bioreactors have been identified as a potential semi-passive solution for long term water treatment of copper (Cu) and selenium (Se) at the Minto mine.

Building on Capstone Mining Corp's partnership with Yukon College through the Industrial Research Chair program, this project focused on the commissioning and summer operation of four anaerobic bioreactors at the Minto site. The objectives of this project were to start testing and assessing the potential of semi-passive anaerobic bioreactors on-site at a small-scale for Cu and Se treatment. More specifically, the treatment targets were based on effluent standards from Minto's previous water Use Licence QZ96-006 Amendment #8 (WUL); defined as 50 ug/L for Cu and 3 ug/L for Se.

This project was first reported with results from summer/fall 2014 in a report entitled: "Pilot Bioreactors Commission and Operation at Minto Mine – 2014 Preliminary Results", by Amelie Janin, Ryan Herbert and Jennie Gjertsen (April 2015, 38p.). This report presents both additional data obtained in the summer of 2015 as well as the data collected in the late summer/fall of 2014.

1.1. ANAEROBIC BIOREACTORS FOR METAL REMOVAL

(Extracts from "Performances of lab-scale anaerobic bioreactors at low temperature using Yukon native microorganisms" by A. Janin and J. Harrington in the Proceedings submitted for the Mine Water Solution in Extreme Environments, April 2015, Vancouver, BC).

Water management in the mining industry has become a priority focus at all stages of the mine life cycle. Passive water treatment approaches are seen as a potential solution where long-term treatment of mine impacted water are required after closure. Past research efforts led 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 semi-passive anaerobic bioreactors have been successfully implemented on a 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).

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 equation (Eq. 1 adapted from Nagpal et al 2000). Sulfides then react with dissolved 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).

Eq. 1 $$2\ C_2H_5OH(aq) + SO_4{}^{2-} \xrightarrow{microbially\ mediated} HS^- + 2CH_3COO^- + \ H^+ + \ H_2O$$

Eq. 2 $$H_2S + Me^{2+} \ \rightarrow MeS + \ 2H^+$$

1.2. ANAEROBIC BIOREACTORS IN YUKON'S COLD CLIMATE

As semi-passive bioreactors are considered for mine closure in Yukon Territory, common concerns include the performance of biological water treatment in cold climates, as low temperatures typically affect microbial growth rate. In order to improve efficiencies, various studies have evaluated the addition of organic carbon to the reactor to help microorganisms sustain activities at cold temperatures. It has been shown that the addition of readily metabolizable carbon such as methanol, lactate, ethylene glycol, or ethanol, as used in this study, improves the performances of anaerobic reactors (Nielsen et al. 2016, Gould et al 2012, Alexco 2012, Sobolewski 2010, Tsukamoto 1999).

In addition, a new approach for cold-climate bioreactors is to include a reactive substrate within the bioreactor that will provide opportunities for retention of the metal contaminants by chemical sorption (Janin and Harington 2015, 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).

Although several anaerobic bioreactors have displayed high efficiencies in cold climates (Ness et al 2014), further scientific evidences will support the use of this technology in northern environments. This study falls within the Chair's broader objective to generate and gather scientific evidences of the potential of semi-passive and passive treatment systems in Yukon.

2. METHODOLOGY

(Extract from "Pilot Bioreactors Commission and Operation at Minto Mine – 2014 Preliminary Results" by A. Janin, R. Herbert and J. Gjertsen, technical report published in April 2015).

2.1. EXPERIMENTAL DESIGN

The influent water selected for the project was sourced from a mine water collection sump ("water quality station W37") at the mine. Water that is collected at W37 includes mine site impacted runoff water, runoff from the dry stack tailings storage facility and localized surface and groundwater. Water from the W37 sump was pumped weekly and stored into a "feed tank" (1,000 L) where ethanol was added to further support microbial growth in the bioreactors. Figure 1 shows a general view of the bioreactor setup. The water was then pumped into the four individual drums using a peristaltic pump with a multi-head channel setup at 9 ml/min, shown in Figure 2. Water was pumped into the bottom of the bioreactors and flowed in the upward direction. The theoretical hydraulic retention time was set to 15 days, meaning that ~100 L of effluent was being treated each week by every reactor. Although the hydraulic residence time was chosen based on common values in the literature, the retention time will have to be optimized in future studies. Liquid ethanol (99.5%) was added weekly into the feed tank at 1.5 mmol/L of effluent, which was based on the average sulfate concentration of 0.9691 mmol/L (or 82.5 mg/L) measured at W37 sump between June 4th and July 13th in 2014. The ethanol requirements were calculated by taking into account that each sulfate molecule requires 8 electrons to be reduced and ethanol provides 12. Twice the stoichiometric amount was applied in this study (i.e. 1.5 mmol ethanol/L of effluent ethanol molecules required to reduce one sulfate molecule).

Figure 1. Pilot-scale bioreactors at Minto Mine (Picture taken in August 2014).

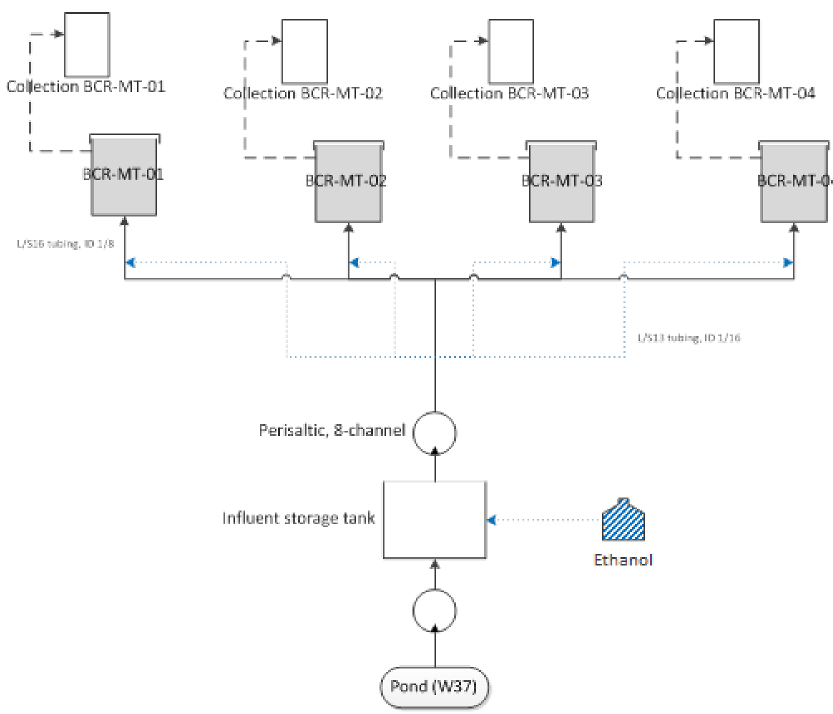

Figure 2. Bioreactors influent feed setup.

2.2. BIOREACTORS COMPOSITION

The four bioreactors were composed of different combinations of the following material; fluvial gravel collected from a gravel borrow source on the Minto access road, crushed gravel from low grade run-of-mine waste rock, wood chips (50% (v/v) dry spruce and 50% (v/v) dry poplar) from Yukon College and biochar from Titan Clean Energy Projects based out of Saskatoon, SK. In addition, all the bioreactors contained “creek sediments” which provided microbial inoculum. This inoculum was collected on site, at W32.

BCR-MT-01 was considered a “control” Bioreactor, filled with only fluvial gravel and creek sediment. BCR-MT-02 and 03 both included 37% (v/v) wood chips, but BCR-MT-02 had fluvial gravel while BCR-MT-03 used crushed gravel. Finally, BCR-MT-04 was filled with crushed gravel and wood biochar from Titan Clean Energy Projects. Composition of each bioreactor is provided in Table 1 and presented in Figure 3. The mixes of materials were prepared independently and homogenized by hand in a wheelbarrow, shown in Figure 4.

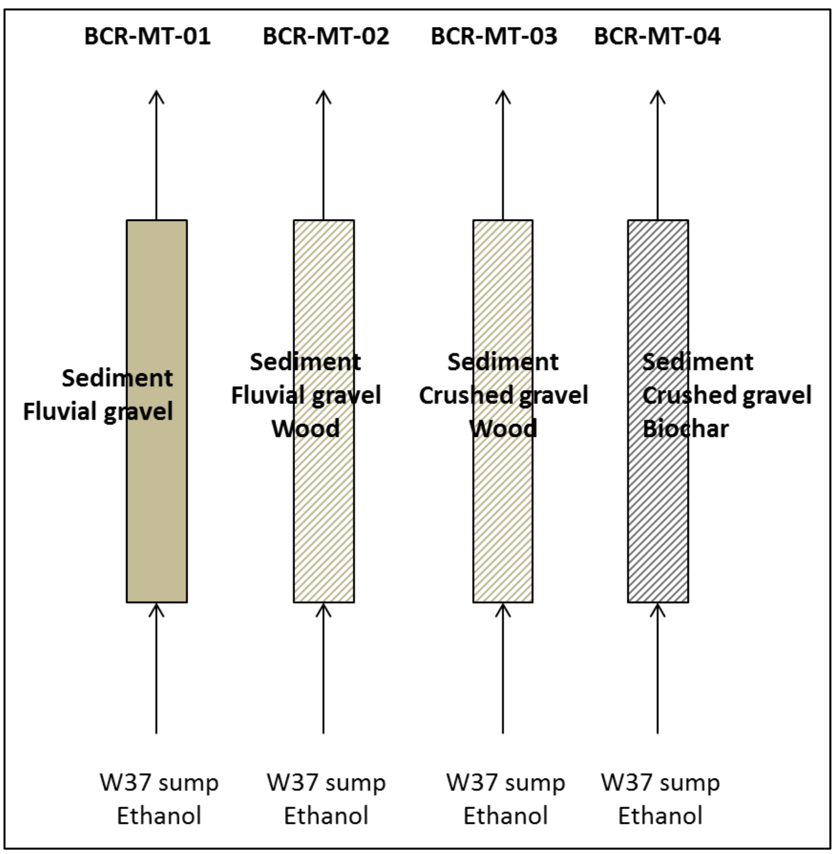

Figure 3. Substrate composition overview.

Table 1. Composition of the pilot bioreactors setup at Minto Mine

	Measured density (kg/L)	Measured weight (kg)	Calculated volume (L)	Proportions by weight (%)	Proportions by volume (%)
BCR-MT-01					
Creek sediment	1.49	44.68	29.99	13.1%	14.2%
Fluvial gravel	1.64	296.2	180.6	86.9%	85.8%
Total		340.9	210.6		
BCR-MT-02					
Creek sediment	1.49	44.82	30.08	18.2%	14.3%
Fluvial gravel	1.64	165.0	100.6	66.9%	47.7%
Wood	0.46	36.80	80.00	14.9%	38.0%
Total		246.6	210.7		
BCR-MT-03					
Creek sediment	1.49	44.70	30.00	20.5%	14.0%
Crushed gravel	1.31	136.5	104.2	62.6%	48.6%
Wood	0.46	36.80	80.00	16.9%	37.3%
Total		218.0	214.2		
BCR-MT-04					
Creek sediment	1.49	44.70	30.00	17.9%	14.7%
Crushed gravel	1.31	175.1	133.7	70.3%	65.6%
Biochar	0.73	29.32	40.16	11.8%	19.7%
Total		249.1	203.8		

Figure 4. Bioreactors substrate mixture preparation.

2.3. BIOREACTORS CONSTRUCTION

Bioreactors were built August 6-7th, 2014 by Amelie Janin and Sabrina Clarke (summer student at Yukon Research Center (YRC)) and by Rick Martin (Minto), and Aaron McGinty, (Selkirk First Nation summer student) at the Minto mine.

The previous report by Janin et al. (2015) presents the step by step construction of the bioreactors and won't be repeated in this report.

2.4. BIOREACTORS MONITORING

Influent and effluent of the reactors were sampled weekly by Minto staff along with measurements of pH, conductivity and temperature. Samples were filtered in the field using 0.45um syringe filters for dissolved metals analysis. Samples that were collected on the first sampling event on September 4th 2014 were sent to Maxaam Analytics lab for analysis, while the subsequent samples collected in September 2014, and between June and September in 2015 were analyzed by YRC for total and dissolved copper and selenium (CuT, CuD, SeT, SeD), and Total Organic Carbon (TOC). Samples for ammonia, nitrite, nitrate and phosphoros (NH_3, NO_2, NO_3 and PO_4) were analyzed at Minto's on-site lab. For samples taken in 2015, sulphate (SO_4) concentrations were measured at the laboratory in YRC.

Water was pumped into the reactors from August 20th to September 23rd in 2014 for a total of 34 days and then the bioreactors were decommissioned for the winter season and stored on site. The operation of the bioreactors was resumed on June 2nd 2015 and continued for 116 days until September 26th, shown in Table 2. Samples were collected at weekly intervals during the operation in 2014 and in 2015.

Table 2. Bioreactors operation timeline

	From	To	Comments
Bioreactor prep	31-Jul-14	1-Aug-14	
Initial fill-up	1-Aug-14	7-Aug-14	Pump at 80 rpm
Incubation	7-Aug-14	20-Aug-14	Pump stopped
Normal operation	20-Aug-14	23-Sep-14	Pump at 19.2 rpm
Winter storage	23-Sep-14	2-Jun-15	Pump Stopped
Normal operation	2-Jun-15	26-Sep-15	Pump at 19.2 rpm
Winter storage	Sep-26-15		Pump stopped

2.5. SAMPLE ANALYSIS

During the periods that the bioreactors were in operation, the collected samples were analyzed for total and dissolved Cu and Se using a Perkin Elmer PinAAcle Graphite Furnace Atomic Absorption (GFAA) analyzer (Perkin Elmer, Waltham, MA) and Flow Injection for Atomic Spectroscopy (FIAS) System (Perkin Elmer, Waltham, MA). For samples taken in 2015, sulfate (SO_4) concentration was analyzed by spectrophotometry using a SmartChem 170® Automated Discrete Analyzer (Westco, Guelph, ON) according to the STM Method D516-90, 02.

The Quantification Limits (QL) used in the GFAA work have been defined as the 10-σ over 10 blanks and were 0.6 ug Cu/L and 0.7 ug Se/L. Due to a change in analytical method, the QL of Se was 0.9 ug /L for samples analyzed in 2015. Calibrations were conducted 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. The QL used in the spectrophotometry work was 4 mg SO_4/L.

Analysis of nutrients were conducted by Minto Exploration staff in the internal lab. Using internal standard operation procedures, the detections limits are 0.01 mg/L for NH_3, 0.1 mg/L for NO_3 and 0.01 mg/L for NO_2.

3. PRELIMINARY RESULTS

3.1. FIELD OBSERVATIONS

During the first year of operation in 2014, the four bioreactors, BCR-MT-01, 02, 03 and 04 were incubated from August 7th to 20th, then operated with flowing water for approximately a month. Field observations are presented in Table 3. Effluents were initially described as having low clarity or high turbidity. This is expected in the early stages of bioreactor operation, when small particles from the substrate materials are flushed out in the first few turn-overs. This includes biochar particles, which colored the effluent from BCR-MT-04 black. In addition, the smell of sulfur observed in effluent from BCR-MT-02 and BCR-MT-03, both containing wood chips, might indicate the presence of hydrogen sulfide. Hydrogen sulfide is also a product of sulfate reduction (Waybrant et al. 1998, Johnson and Hallberg 2005), which may indicate that sulfate-reducing bacteria was successfully established. Based on the volume measured every week, approximately 300 L of water was circulated through each of the 200 L bioreactors during the monitoring period in 2014.

In 2015, the operation of reactors BCR-MT-01, 02, 03 and 04 was approximately four months from June 2nd to September 26th after a pause of water flow during the winter. Field observations are shown in Table 4. Black coloring of effluent observed in the previous year from the reactor containing biochar BCR-MT-04 was relieved and the bioreactor showed the best clarity for the first two weeks of operation. Overall, bioreactors with wood chips, especially BCR-MT-03 demonstrated effluent characterized with dark color and strong odor, whereas effluent from control and biochar reactors had lighter color and no significant odor. This can be an indication that there were more active microbial reactions in bioreactors with wood chips than control or bioreactors with biochar. Total effluent volume over the 116-day-operation in 2015 was 809 L from BCR-MT-01, 716, 602, and 510 L from BCR-MT-02, BCR-MT-03, and BCR-MT-04 respectively. With the volume of the barrel being 200L, the ratio of the volume of water to the volume of the bioreactor were 4.0, 3.6, 3.0, and 2.6. This data includes estimates where effluent volume was missing.

Table 3. Field observations at sampling events in 2014

Date	Bioreactor	Effluent volume (L)	Clarity	Effluent temp-erature (°C)	Time of day	Comments
4-Sep-14	BCR-MT-01	120	<25 cm	4.7	2:25 PM	Light brown.
	BCR-MT-02	120	<25 cm	8.5	2:30 PM	Light brown.
	BCR-MT-03	120	<25 cm	6	2:35 PM	Light brown.
	BCR-MT-04	120	<25 cm	9	2:40 PM	Light brown.
13-Sep-14	BCR-MT-01	100	60 cm	7.9	9:45 AM	
	BCR-MT-02	102	12 cm	8.2	9:50 AM	Smelly
	BCR-MT-03	104	20 cm	7.9	9:55 AM	Smelly
	BCR-MT-04	100	20 cm	8.2	10:00 AM	
18-Sep-14	BCR-MT-01	55	55 to 30L	4.3	10:30 AM	Light sheen, no odor
	BCR-MT-02	55	55 to 50L	5.5	10:25 AM	Full sheen on surface, strong odor
	BCR-MT-03	55	55 to 40L	5.1	10:05 AM	Light sheen on surface, strong odor
	BCR-MT-04	55	55 to 50L	5.7	10:15 AM	Light sheen on surface, less odor than MT03
23-Sep-14	BCR-MT-01	37	37 to 0L	2.7	2:20 PM	Full visibility
	BCR-MT-02	37	37 to 0L	7.1	2:10 PM	Odor of sulphur
	BCR-MT-03	37	37 to 33L	3.8	2:00 PM	Sheen on surface, frothy on surface, no odor, required lot of filters
	BCR-MT-04	37	37 to 20L	7	1:45 PM	Water color black, no odor

Note: Clarity was observed in the field either as the depth (in cm) until which someone could not see through or as the volume graduations (in L) one could read from the surface. Observations can be compared from one bioreactor to another but not from one week to another.

Table 4. Field observations at sampling events in 2015

Date	Bioreactor	Effluent volume (L)	Clarity	Effluent temperature (°C)	Time of day	Comments
20-Jun-15	BCR-MT-01	120	5 cm	21.8	11:20 AM	Light brown
	BCR-MT-02	120	4 cm		11:30 AM	Medium brown, light film on surface, light smell
	BCR-MT-03	60	2 cm		11:40 AM	Dark water, black, light smell, light film
	BCR-MT-04	15	15 cm		11:50 AM	Light brown
27-Jun-15	BCR-MT-01	30	full	15.6	2:50 PM	Full visibility, very light tint
	BCR-MT-02	28	3 cm	17	3:00 PM	Dark
	BCR-MT-03	62	3 cm	14.8	3:10 PM	Dark
	BCR-MT-04	20	full	16.1	3:20 PM	Light tint
5-Jul-15	BCR-MT-01	56*	n/a	22	5:25 PM	Clear
	BCR-MT-02	49*	n/a	20.9	5:00 PM	Moderate dark
	BCR-MT-03	44*	n/a	18.4	5:40 PM	Darkest
	BCR-MT-04	39*	n/a	19.7	4:50 PM	Light tinge, light film on surface
12-Jul-15	BCR-MT-01	58	n/a	13.4	8:55 AM	Light sheen, no odor
	BCR-MT-02	43*	n/a	13.4	9:15 AM	Dark
	BCR-MT-03	59	n/a	12.9	9:05 AM	Dark, strong smell
	BCR-MT-04	18	n/a	12.6	8:45 AM	Semi-clear
19-Jul-15	BCR-MT-01	54	n/a	13.1	4:10 PM	
	BCR-MT-02	53	n/a	13.4	4:20 PM	
	BCR-MT-03	50	n/a	13.2	4:30 PM	
	BCR-MT-04	8	n/a	11.7	4:40 PM	
26-Jul-15	BCR-MT-01	65	n/a	20.6	3:45 PM	
	BCR-MT-02	68	n/a	20.8	3:50 PM	
	BCR-MT-03	55	n/a	19.1	3:55 PM	Darker than the other tanks
	BCR-MT-04	45	n/a	22.6	4:00 PM	
	BCR-MT-02	41	n/a	13.5	1:35 PM	Medium-dark

Table 5. Field observations at sampling events in 2015 (Continued)

Date	Bioreactor	Effluent volume (L)	Clarity	Effluent temperature (°C)	Time of day	Comments
31-Jul-15	BCR-MT-01	37	n/a	13.3	1:25 PM	Medium brown
	BCR-MT-03	31	n/a	11.9	1:45 PM	Dark brown
	BCR-MT-04	40	n/a	14	1:55 PM	Dark brown
11-Aug-15	BCR-MT-01	88	n/a	14.7	2:30 PM	Semi-clear
	BCR-MT-02	91	n/a	13.1	2:40 PM	Light brown
	BCR-MT-03	58	n/a	13	2:50 PM	Brown
	BCR-MT-04	41	n/a	13.9	3:00 PM	Light brown
22-Aug-15	BCR-MT-01	95	n/a	10.1	2:15 PM	
	BCR-MT-02	85	n/a	10.8	2:15 PM	
	BCR-MT-03	58	n/a		2:15 PM	
	BCR-MT-04	90	n/a	10.7	2:15 PM	
27-Aug-15	BCR-MT-01	31	n/a	10.8	1:40 PM	
	BCR-MT-02	18	n/a	10.4	1:45 PM	
	BCR-MT-03	14	n/a	10.5	1:50 PM	
	BCR-MT-04	45	n/a	10.6	1:55 PM	
6-Sep-15	BCR-MT-01	70	n/a	5.7	8:00 AM	
	BCR-MT-02	49	n/a	4.2	8:10 AM	
	BCR-MT-03	38	n/a	5.7	8:30 AM	
	BCR-MT-04	60	n/a	4.6	8:40 AM	
17-Sep-15	BCR-MT-01	50	n/a	6.6	1:30 PM	
	BCR-MT-02	38	n/a	7.8	1:40 PM	
	BCR-MT-03	33	n/a	7.5	1:50 PM	
	BCR-MT-04	38	n/a	8.5	2:00 PM	

Table 6. Field observations at sampling events in 2015 (Continued)

Date	Bioreactor	Effluent volume (L)	Clarity	Effluent temp-erature (°C)	Time of day	Comments
26-Sep-15	BCR-MT-01	55	n/a	2.5	10:10 AM	
	BCR-MT-02	33	n/a	3.1	10:20 AM	
	BCR-MT-03	40	n/a	3.9	10:20 AM	
	BCR-MT-04	51	n/a	4.5	10:30 AM	

* Missing value. Figure was extrapolated and accounted as equal to the average flow rate x duration.

3.2. FLOW – HYDRAULICS

Flow rates presented in Table 5 and Table 6 are calculated using equation 3 and are based on the observed volume on each of the outlet tanks. It should be noted that the volume markings on the outlet tank were made manually and may not be accurate and in addition, the outlet tanks had a 120 L capacity. Therefore, the tanks had likely overflowed before the first sampling event of each year on September 4th 2014 as well as June 20th 2015, and the volume reading of 120 L was likely an underestimate.

Eq. 3 $$Effluent\ flow\ rate = \frac{Volume\ of\ collected\ effluent}{Duration\ of\ effluent\ collection}$$

Importantly, flow measurements indicate that the bioreactors received equivalent flow rates and were operated in the same conditions, which allows for easier comparison between the four bioreactors. In 2014, the target flow rate was 9 ml/min and the measured rates were 7.4 ml/min on average. In 2015, the pump speed was set as the same as previous year (9 ml/min), however, the actual flow rate was almost half the target of 9 ml/min at 4.9, 4.3, 3.8, and 3.4 ml/min on average for BCR-MT-01, 02, 03 and 04, respectively. Lower flow rate might indicate lower permeability and hydraulic conductivity due to physical changes inside the bioreactors. The long dry wintering and moving back and forth of the bioreactor in the fall and spring might have affected the structure of the materials inside the bioreactors.

Table 7. Bioreactors flow characteristics from Aug-20-2014 to Sept 21-2014

Date	Bioreactor	Volume outlet (L)	Duration (days)	Flow (mL/min)	Comments
20-Aug-14	N/A	N/A	0	N/A	
4-Sep-14	BCR-MT-01	120	15	5.6	Likely overflowed, underestimated
4-Sep-14	BCR-MT-02	120	15	5.6	Likely overflowed, underestimated
4-Sep-14	BCR-MT-03	120	15	5.6	Likely overflowed, underestimated
4-Sep-14	BCR-MT-04	120	15	5.6	Likely overflowed, underestimated
13-Sep-14	BCR-MT-01	100	24	7.7	
13-Sep-14	BCR-MT-02	102	24	7.9	
13-Sep-14	BCR-MT-03	104	24	8.0	
13-Sep-14	BCR-MT-04	100	24	7.7	
18-Sep-14	BCR-MT-01	55	29	7.6	
18-Sep-14	BCR-MT-02	55	29	7.6	
18-Sep-14	BCR-MT-03	55	29	7.6	
18-Sep-14	BCR-MT-04	55	29	7.6	
21-Sep-14	BCR-MT-01	37	32	8.6	
21-Sep-14	BCR-MT-02	37	32	8.6	
21-Sep-14	BCR-MT-03	37	32	8.6	
21-Sep-14	BCR-MT-04	37	32	8.6	

Table 8. Bioreactors flow characteristics from Jun-20-2014 to Sept 26-2015

Date	Bioreactor	Volume outlet (L)	Duration (Days)	Flow (mL/min)	Comments
2-Jun-15	N/A	0	286	N/A	
20-Jun-15	BCR-MT-01	120	304		Likely overflowed, underestimated
20-Jun-15	BCR-MT-02	120	304		Likely overflowed, underestimated
20-Jun-15	BCR-MT-03	60	304		
20-Jun-15	BCR-MT-04	15	304		
27-Jun-15	BCR-MT-01	30	311	3.0	
27-Jun-15	BCR-MT-02	28	311	2.8	
27-Jun-15	BCR-MT-03	62	311	6.2	
27-Jun-15	BCR-MT-04	20	311	2.0	
5-Jul-15	BCR-MT-01	56*	319	4.9**	
5-Jul-15	BCR-MT-02	49*	319	4.3**	
5-Jul-15	BCR-MT-03	44*	319	3.8**	
5-Jul-15	BCR-MT-04	39*	319	3.4**	
12-Jul-15	BCR-MT-01	58	326	5.8	
12-Jul-15	BCR-MT-02	43*	326	4.3**	
12-Jul-15	BCR-MT-03	59	326	5.9	
12-Jul-15	BCR-MT-04	18	326	1.8	
19-Jul-15	BCR-MT-01	54	333	5.4	
19-Jul-15	BCR-MT-02	53	333	5.3	
19-Jul-15	BCR-MT-03	50	333	5.0	
19-Jul-15	BCR-MT-04	8	333	0.8	
26-Jul-15	BCR-MT-01	65	340	6.4	
26-Jul-15	BCR-MT-02	68	340	6.7	
26-Jul-15	BCR-MT-03	55	340	5.5	

Table 9. Bioreactors flow characteristics from Jun-20-2014 to Sept 26-2015 (Continued)

Date	Bioreactor	Volume outlet (L)	Duration (Days)	Flow (mL/min)	Comments
26-Jul-15	BCR-MT-04	45	340	4.5	
31-Jul-15	BCR-MT-01	37	345	5.1	
31-Jul-15	BCR-MT-03	31	345	4.3	
31-Jul-15	BCR-MT-04	40	345	5.6	
11-Aug-15	BCR-MT-01	88	356	5.6	
11-Aug-15	BCR-MT-02	91	356	5.7	
11-Aug-15	BCR-MT-03	58	356	3.7	
11-Aug-15	BCR-MT-04	41	356	2.6	
22-Aug-15	BCR-MT-01	95	367	6.0	
22-Aug-15	BCR-MT-02	85	367	5.4	
22-Aug-15	BCR-MT-03	58	367	3.7	
22-Aug-15	BCR-MT-04	90	367	5.7	
27-Aug-15	BCR-MT-01	31	372	4.3	
27-Aug-15	BCR-MT-02	18	372	2.5	
27-Aug-15	BCR-MT-03	14	372	1.9	
27-Aug-15	BCR-MT-04	45	372	6.3	
6-Sep-15	BCR-MT-01	70	382	4.9	
6-Sep-15	BCR-MT-02	49	382	3.4	
6-Sep-15	BCR-MT-03	38	382	2.6	
6-Sep-15	BCR-MT-04	60	382	4.2	
17-Sep-15	BCR-MT-01	50	393	3.2	
17-Sep-15	BCR-MT-02	38	393	2.4	
17-Sep-15	BCR-MT-03	33	393	2.1	
17-Sep-15	BCR-MT-04	38	393	2.4	
26-Sep-15	BCR-MT-01	55	402	4.2	

Table 10. Bioreactors flow characteristics from Jun-20-2014 to Sept 26-2015 (Continued)

Date	Bioreactor	Volume outlet (L)	Duration (Days)	Flow (mL/min)	Comments
26-Sep-15	BCR-MT-02	33	402	2.5	
26-Sep-15	BCR-MT-03	40	402	3.1	
26-Sep-15	BCR-MT-04	51	402	3.9	

***** Missing value. Figure was extrapolated and accounted as equal to the average flow rate in 2015 times duration.

** Average flow rate due to missing value of effluent volume.

3.3. INFLUENT WATER QUALITY

Water was pumped from the W37 pond into a 1000 L feed tank. Samples of the feed water were collected weekly and analyzed for total metals and dissolved metals as well as nutrients. The monitored parameters in the influent are presented in Table 7. In addition, on September 4th 2014, these samples were sent to a Maxxam Analytics lab (Maxxam) and were analyzed for a full suite of dissolved and total metals as well as nutrients. Data from this analysis is presented in Table 8. Effluent discharge limits for metals set by Minto's Water Use Licence are included for comparison.

Selenium is the only metal which exceeded the prescribed limit of 3.0 ug/L in the Water Use Licence. Total and dissolved selenium were measured at 6.9 ug/L and 6.4 ug/L respectively on September 4th 2014. In the following sampling events, total selenium was measured at 5.3, 5.4 and 5.8 ug/L on September 13th, 18th and 23rd 2014, respectively. In 2015, Selenium concentration in the influent was slightly lower than discharge limit at 2.8 ug/L for total Se and 2.5 ug/L for dissolved Se at the beginning of the monitoring period on June 20th. It stayed around the discharge limit until mid-August and rose dramatically after mid-August up to 11.0 ug/L and 11.2 ug/L for total and dissolved selenium concentration at the last sampling on September 26th 2015. As it was seen in the previous year, the majority of selenium was in dissolved form regardless of temperature or total selenium concentration (Appendix 1).

Copper in the feed water pumped at W37 did not exceed the WUL limit of 50 ug/L during both periods of operation. In 2014, total and dissolved Cu were measured at 48.9 ug/L and 16.9 ug/L respectively on Sept 4th 2014. In the following sampling events, Total Cu was measured at 42.5, 43.7 and 43.2 ug/L on September 13th, 18th and 23rd 2014, respectively. In 2015, the concentration of copper also remained below the discharge limit during weekly sampling but rose after September 6th up to 49.9 ug/L for total and dissolved copper concentration respectively (Appendix 1). Unlike selenium, a significant portion of copper was present in the particulate form (>0.45 um particle size) with on average, 18% of Cu in the particulate form.

Table 7. Measured water quality parameters in bioreactor influent (BCR-Tank)

Sampling date	Duration (day)	pH	Se-T (ug/L)	Se-D (ug/L)	Cu-T (ug/L)	Cu-D (ug/L)	TOC (mg/L)	SO4 (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. (uS/cm)	Temp. (°C)
4-Sep-14	15	8.0	6.9	6.4	48.9	16.9	43.0	N/A	0.04	3.4	<0.01/0.02	N/A	498	5.1
13-Sep-14	24	8.2	5.3	6.1	42.5	42.3	42.6	N/A	<0.01	N/A	N/A	N/A	594	8.1
18-Sep-14	29	9.1	5.4	5.7	43.7	34.2	49.1	N/A	<0.01	3.1	<0.01	N/A	934	6.8
23-Sep-14	34	9.0	5.8	4.9	43.2	33.4	39.8	N/A	0.09	4.3	0.04	N/A	940	7.8
20-Jun-15	304	N/A	2.8	2.5	39.8	35.1	25.5	63.4	N/A	6.3	<0.01	N/A	N/A	N/A
27-Jun-15	311	N/A	3.1	3.2	34.2	29.0	18.4	58.0	N/A	6.1	<0.01	N/A	N/A	N/A
5-Jul-15	319	N/A	3.0	3.1	48.7	28.0	21.1	68.1	N/A	2.5	0.029	N/A	N/A	N/A
12-Jul-15	326	N/A	3.0	2.7	31.9	27.4	26.3	65.7	N/A	3	0.071	N/A	N/A	N/A
19-Jul-15	333	N/A	3.0	3.0	32.1	30.8	24.9	63.0	N/A	10.3	0.023	N/A	N/A	N/A
26-Jul-15	340	8.5	3.2	3.0	33.5	32.4	21.8	89.3	N/A	7.4	<0.01	N/A	813	20.5
31-Jul-15	345	8.2	3.3	3.7	34.2	26.4	24.3	132.8	N/A	7.9	<0.01	N/A	696	12.8
11-Aug-15	356	8.4	3.1	2.8	30.1	22.2	23.5	108.6	N/A	5.5	0.011	N/A	782	14.1
22-Aug-15	367	8.2	3.8	3.8	26.9	23.4	21.8	96.8	N/A	5.8	<0.01	N/A	836	10.4
27-Aug-15	372	8.2	5.0	5.1	31.1	27.5	23.1	71.6	N/A	13.3	0.014	N/A	654	11.3
6-Sep-15	382	8.4	5.0	5.0	30.9	27.6	27.2	86.6	N/A	N/A	N/A	N/A	905	5.7
17-Sep-15	393	8.4	9.7	9.5	37.2	33.3	29.5	99.4	N/A	27.2	<0.01	N/A	942	7.2
26-Sep-15	402	8.6	11.0	11.2	49.9	45.6	25.3	110.4	N/A	3.8	0.029	N/A	1111	3.1

Table 8. Total and Dissolved metals measured by Maxxam Analytics in the "BCR-Tank" influent samples collected on Sept 4th 2014

Parameter	Unit	Total	Dissolved	WUL Discharge limit (Total)
Hardness ($CaCO_3$)	mg/L	477	464	
Sulfate	mg/L	N/A	128	
Organic Carbon (C)	mg/L	43	N/A	
Aluminum (Al)	µg/L	23.9	11.8	
Antimony (Sb)	µg/L	<0.50	<0.50	
Arsenic (As)	µg/L	0.38	0.4	
Barium (Ba)	µg/L	126	134	
Beryllium (Be)	µg/L	<0.10	<0.10	
Bismuth (Bi)	µg/L	<1.0	<1.0	
Boron (B)	µg/L	<50	<50	
Cadmium (Cd)	µg/L	0.041	0.044	0.15
Calcium (Ca)	mg/L	129	127	
Chromium (Cr)	µg/L	<1.0	<1.0	
Cobalt (Co)	µg/L	<0.50	<0.50	
Copper (Cu)	µg/L	48.9	46.9	50
Iron (Fe)	µg/L	119	50	
Lead (Pb)	µg/L	<0.20	<0.20	
Lithium (Li)	µg/L	<5.0	<5.0	
Magnesium (Mg)	mg/L	37.8	35.6	
Manganese (Mn)	µg/L	214	204	
Mercury (Hg)	µg/L	<0.010	<0.010	
Molybdenum (Mo)	µg/L	8.2	8.1	
Nickel (Ni)	µg/L	1	1.1	
Phosphorus (P)	µg/L	26	23	

Table 8. Total and Dissolved metals measured by Maxxam Analytics in the "BCR-Tank" influent samples collected on Sept 4th 2014 (Continued)

Parameter	Unit	Total	Dissolved	WUL Discharge limit (Total)
Potassium (K)	mg/L	4.81	4.89	
Selenium (Se)	µg/L	6.89	6.4	3
Silicon (Si)	µg/L	8040	7970	
Silver (Ag)	µg/L	<0.020	<0.020	
Sodium (Na)	mg/L	22.2	21.7	
Strontium (Sr)	µg/L	1310	1340	
Sulphur (S)	mg/L	50	51.9	
Thallium (Tl)	µg/L	<0.050	<0.050	
Tin (Sn)	µg/L	<5.0	<5.0	
Titanium (Ti)	µg/L	<5.0	<5.0	
Uranium (U)	µg/L	3.16	3.14	
Vanadium (V)	µg/L	<5.0	<5.0	
Zinc (Zn)	µg/L	57.8	55.8	150
Zirconium (Zr)	µg/L	<0.50	<0.50	

3.4. SELENIUM REMOVAL

Selenium was one of the two targeted metals for treatment. The objective was to reduce it to less than the WUL effluent discharge limit of 3 ug/L.

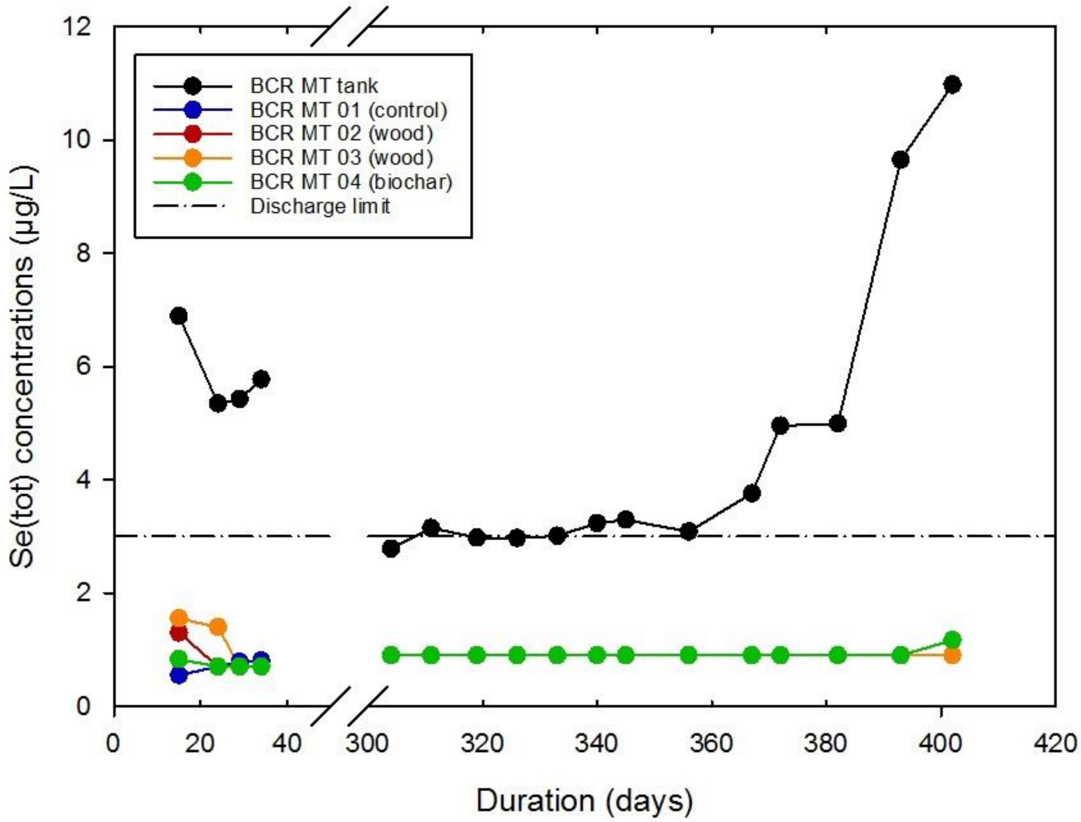

Figure presents the selenium concentration before and after the treatment by each of the four bioreactors. Reduction of the selenium concentration was observed persistently in the four reactors in the one month of operation in 2014 as well as the four months in 2015, with values below discharge limits (3 ug/L). Results were often below the detection limit when analyzed at the YRC lab (Note: Due to changes in analysis method at the YRC lab, detection limit was 0.7 ug/l for samples analyzed in 2014 and 0.9 ug/l in 2015). Both total and dissolved selenium were measured and the concentrations are presented in Tables 1-4, Appendix 1. It was observed that more than 90% of the total selenium was present in a dissolved form (excluding outliers). The reduction of selenium follows a similar trend to results observed from bench-scale bioreactors at the Yukon College, treating either a synthetic mine drainage (Janin 2014a) or dewatering water from the Wolverine mine (Janin 2014b). Prior to decommissioning the bioreactors on September 23rd 2014 after a month of operation, selenium removal efficiencies between 86% and 88% were measured. At the end of operation in 2015 (September 26th 2015), all bioreactors were performing with a selenium removal rates between 89% and 92%.

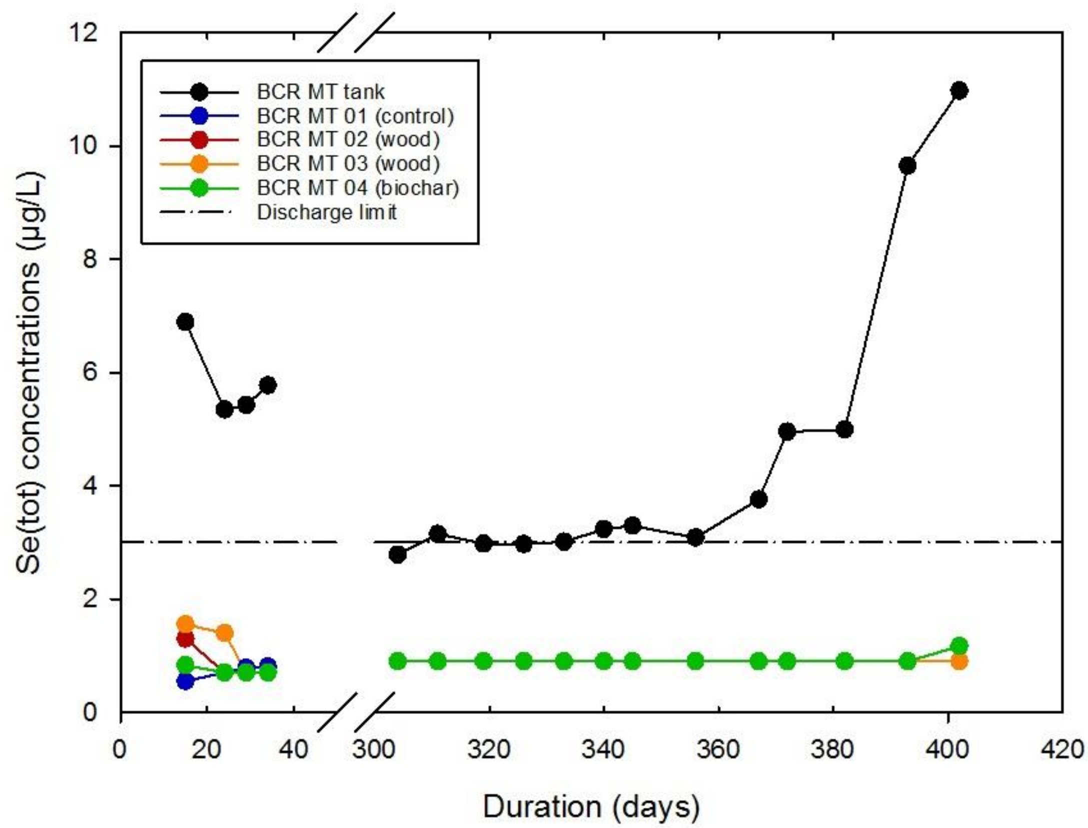

Figure 5. Selenium concentration in influent and effluent of the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 and September 26th 2015 (Discharge Limit of 0.003 mg/L).

3.5. COPPER REMOVAL

Copper was the other targeted metal for treatment by the bioreactors.

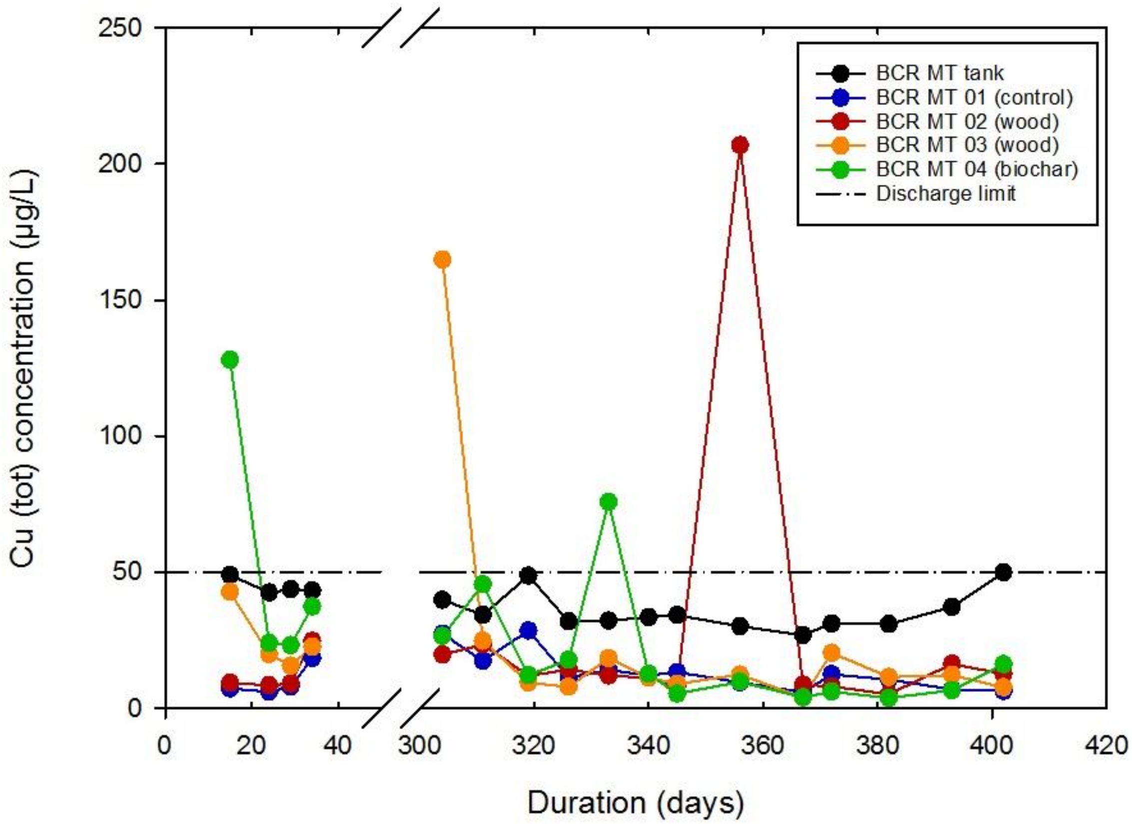

Figure presents the total copper concentrations before and after treatment. The first sample collected on September 4th, 2014 at the outlet of the biochar amended reactor (BCR-MT-04) was measured at 128 ug/L fot total copper and 90 ug/L for dissolved copper by Maxxam, above the WUL effluent discharge limit of 50 ug/L. It is unclear if this high copper concentration if this should be considered an outlier or if it was a result of flushing the water that stood inside the bioreactor during incubation. The following measurement which taken on September 13th when the 220 L passed through the 200L bioreactor was much lower at 23.9 ug/L and consistent with copper concentrations measured in the outlet afterward.

Cu concentrations in the influent were close to the discharge limit of 50 ug/L and a decrease in Cu concentration was observed after treatment by all four bioreactors in the one month of operation in late August and September 2014. On September 23rd, 2014 at the 30 day mark, Cu removal efficiencies were measured at 57% for the control reactor, BCR-MT-01; 43 and 48% respectively for the wood amended reactors, BCR-MT-02 and BCR-MT-03; and 13% for the biochar amended reactor, BCR-MT-04. Hence, copper removal efficiencies have improved over a 1-month period when air temperatures were decreasing. These results were encouraging promising and were expected to improve the following summer with better establishment of the community of microorganisms and warmer summer temperatures in future operating periods.

The impact of cold temperatures on biological water treatments in the Yukon has raised concerns in the past and a few studies were done to assess the impact of cold temperatures at laboratory scale. In a previous study conducted at YRC, copper removal was affected when decreasing the temperature from 20 degrees Celsius to 6 and 3 degrees Celsius but only for a short period of time. After 30 days at lower temperatures, removal efficiencies returned to the same copper removal rate of 99% observed at higher temperatures. This suggested that the microorganisms acclimatized to the cold temperatures (Janin and Harrington 2014). In another study by Gould et al (2012), the removal efficiency for zinc was measured at approximately 99% at 20 degrees Celsius before the bioreactors were transferred in a cold chamber at 4 degrees celcius. At this temperature, the efficiency decreased but went back up to 95% after 170 days. In this study, the monitoring has occurred during the 2015 spring-summer-fall season when the temperature gradually decreased from between 10 and 20 degrees Celsius in the summer to below 10 degrees Celsius in September. Over this period of time, the copper removal capability has improved, meaning that the removal of copper was not adversely affected by cold temperatures. At the last sampling event on September 26th, 2015 the removal rates of 87, 75, 85, and 68% at the last sampling event for BCR-MT-01, BCR-MT-02, BCR-MT-03, and BCR-MT-04 respectively.

In 2015, one sample out of the 13 samples collected for the bioreactors BCR-MT-01, 02 and 04 exhibited total copper concentrations above the discharge limit of copper and above the Cu concentration in the influent: 164.9 ug/L on June 20th 2015 in BCR-MT-03, 75.78 ug/L on July 19th 2015 in BCR-MT-04 and 206.9 ug/L on August 11th 2015 in BCR-MT-02. Dissolved copper concentrations from these three samples were below 20 ug/L (Appendix 1). The high total concentrations observed may be due to precipitated copper particles exiting the bioreactors or in the sampling jar and may simply be outliers.

The partitioning of copper between particulate and dissolved form evolved over the 2 years of monitoring (Table 9). Effluent from the bioreactors containing wood chips had higher concentrations of Cu in particulate form than the effluents from the control bioreactor BCR-MT-01 (no wood) and the biochar-amended bioreactor BCR-MT-04. For both BCR-MT-01 and -04 bioreactors, copper was present mostly in dissolved form with 33% and 26% of the total copper in particulate form in 2014 respectively and 29% and 35% in 2015. On the other hand 85% and 84% of the total copper measured in the effluents from the bioreactors BCR-MT-02 and BCR-

MT-03 was present in particulate form respectively in 2014, and 51.6% and 59.4% respectively in 2015.

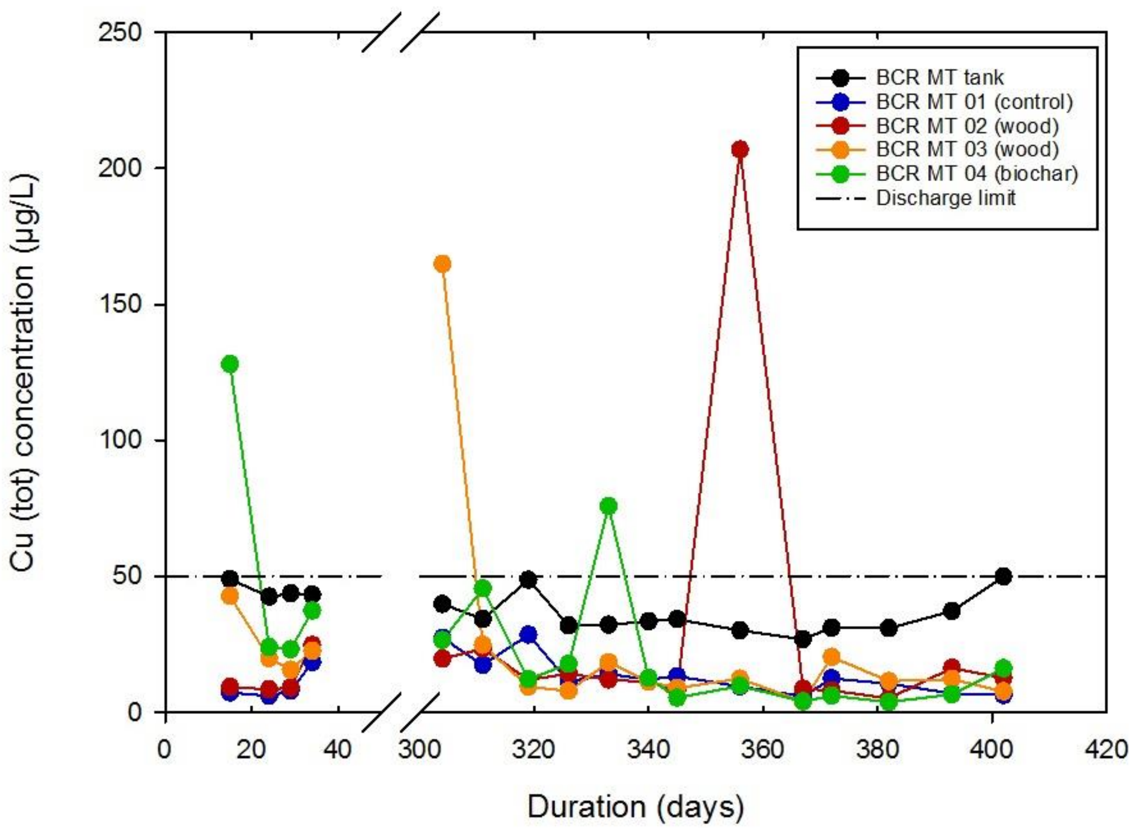

Figure 2. Copper concentrations in influent and effluent of the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 and September 26th 2015 (Discharge Limit of 0.050 mg/L).

Table 9. Minimum, Maximum and average Cu concentration and average % particulate in year 1 and 2

Year	Total Cu (µg/L)			Dissolved Cu (µg/L)			Average particulate
	min.	max.	ave.	min.	max.	ave.	(%)
Year 1							
BCR MT tank	42.5	48.9	44.6	16.9	42.3	31.7	27.6
BCR MT 01	6.1	18.4	9.9	4.1	7.7	5.3	33.3
BCR MT 02	8.4	24.7	12.9	0.8	2.1	1.6	85.0
BCR MT 03	15.7	42.7	25.2	1.2	11.0	4.7	83.6
BCR MT 04	23.2	128.0	53.1	21.8	90.4	47.5	26.3*
Year 2							
BCR MT tank	26.9	49.9	35.4	22.2	45.6	29.9	15.0
BCR MT 01	5.8	28.4	13.5	3.3	18.2	9.1	28.6
BCR MT 02	5.1	206.9	27.5	2.2	18.5	7.0	51.6
BCR MT 03	3.9	164.9	24.1	0.9	19.0	5.8	59.4
BCR MT 04	3.7	75.8	18.6	2.9	18.8	8.9	34.8

* Average calculated with one outlier removed

3.6. PH

The pH was measured in the field for the influent and effluent of each bioreactor. Influent pH was measured between 8.0 and 9.1 while the WUL discharge limits are 6.5 to 9. pH in the effluent varied from one bioreactor to the other and ranged between 5.8 and 9.4. pH values out of the pH range required for discharge were seen only in the one month of operation in the first year. In 2014, pH of the influent and effluent were very similar for BCR-MT-01 (control) and BCR-MT-04 (biochar) indicating that the treatment did not affect the pH. Furthermore, when the pH of the influent exceeded the discharge limit of pH 9.0 on Sept 18^{th} and Sept 23^{rd} 2014, the effluent from these two bioreactors also exceeded the discharge limit. The following year the influent stayed within the discharge limit, as did the effluents from BCR-MT-01 and BCR-MT-04 although the pH of these effluents were slightly more alkaline than the influents during the second year of operation. The highest pH marked was 8.7 for BCR-MT-01 and 9.0 for BCR-MT-04 in 2015.

The wood amended bioreactors, BCR-MT-02 and BCR-MT-03, lowered the pH by 2 to 3 pH units in the first month of operation for both bioreactors. This acidification may be due to the release of organic acids by the wood chips. Softwood contain various organic acids such as uronic acid, glucuronic acid, dehydroabietic acid, abietic acid, pimaric acid, and isopimaric acid, etc. (Pettersen 1977, Wang et al 1995).
In this study, exceedance of the lower range of the pH limit was observed. During monitoring in the 2015 operation period, pH of effluent from both bioreactors containing wood chips were neutral to alkali and the values were between 6.8 and 8.1 which satisfied the discharge limit. It is thought that the reduced impact to pH in 2015 may be because the organic acids from the wood chips has been flushed out of the bioreactors in 2014. In addition, the generation of alkalinity is expected with improved SRB activities (Waybrant et al. 1998; Mayes et al. 2011) in the second year of operation of the bioreactors. It suggests that to use wood chips in an anaerobic bacterial bioreactor, longer incubation time would be required to avoid the undesired effect of low pH.

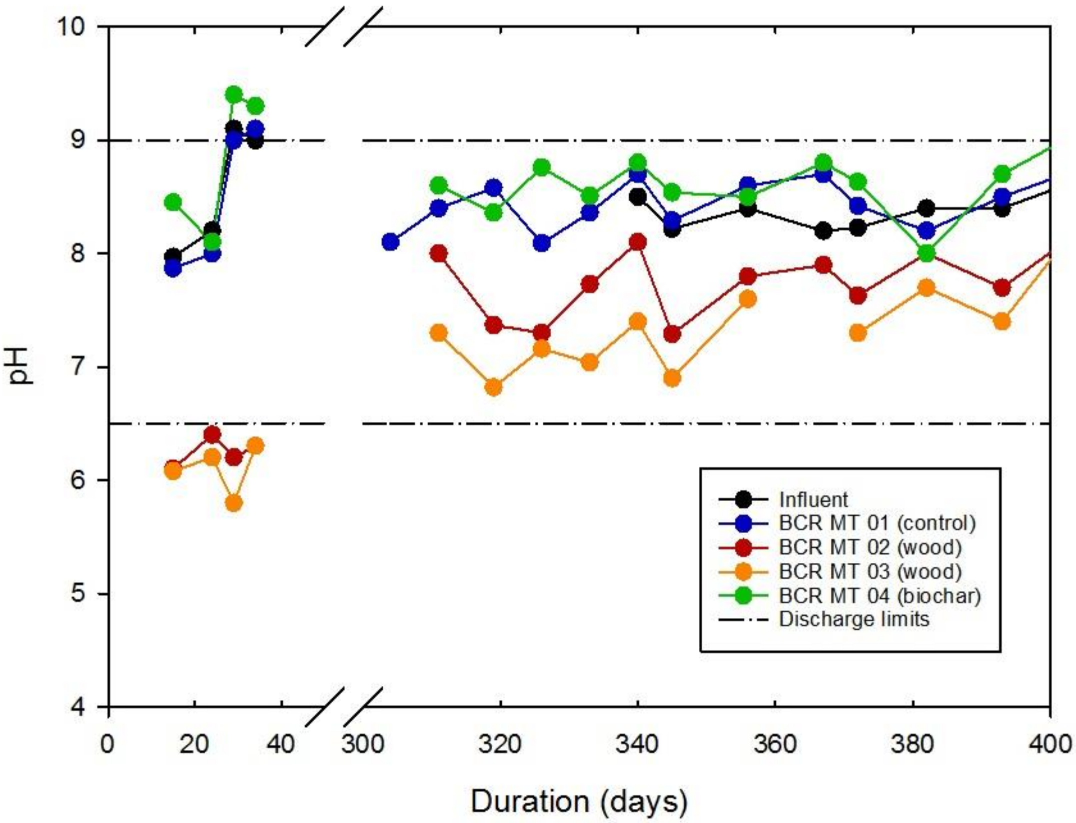

Figure 7. pH in influent and effluent of the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 to September 26th 2015 (Discharge Limit Range: 6.5 to 9).

3.7. NUTRIENTS

Ammonia (NH_3), nitrate (NO_3) and nitrite (NO_2) are also regulated by Minto's previous WUL for effluent discharge with limits of 0.89 mg NH_3/L, 7.65 mg NO_3/L and 0.15 mg NO_2/L. These limits were not exceeded in the bioreactors influent, nor were they exceeded in the effluents from each of the bioreactors during the month of operation in 2014 (Table 10 to 14). However, during the operational period in 2015, nitrate in the influent occasionally exceeded its limit of 7.65 mg/L (Figure 8). Observed trends include the reduction of nitrate concentrations by the anaerobic bioreactors, with 3.1 to 4.3 mg NO_3/L in the influent and <0.2 mg NO_3/L in the effluent in the last sampling event on September 23rd, 2014. Nitrate, an oxidized form of nitrite, was likely consumed by the reducing conditions which were established in the bioreactors and favouring denitrification (Del Pozo and Viel 2003). Although the nitrate concentration in influent went up to as high as 27.2 mg NO_3/L, the maximum value of effluent was 4.6 mg NO_3/L from BCR-MT-03 on July 19th 2015 and all reactors measured <0.1 mg NO_3/L on effluent at the last sampling on September 26th 2015.

Nitrite concentration never exceeded the limit of 0.15 mg NO_2/L in 2014 and 2015 and were under detection limits in all samples in 2015 except on July 5th and on July 12th 2015. On these dates, nitrite concentrations increased to a maximum of 0.056 mg/L (Table 10-14).

Finally, ammonia was measured only in 2014 and did not exceed the limit of 0.89 mg NH_3/L. It was not measured in 2015.

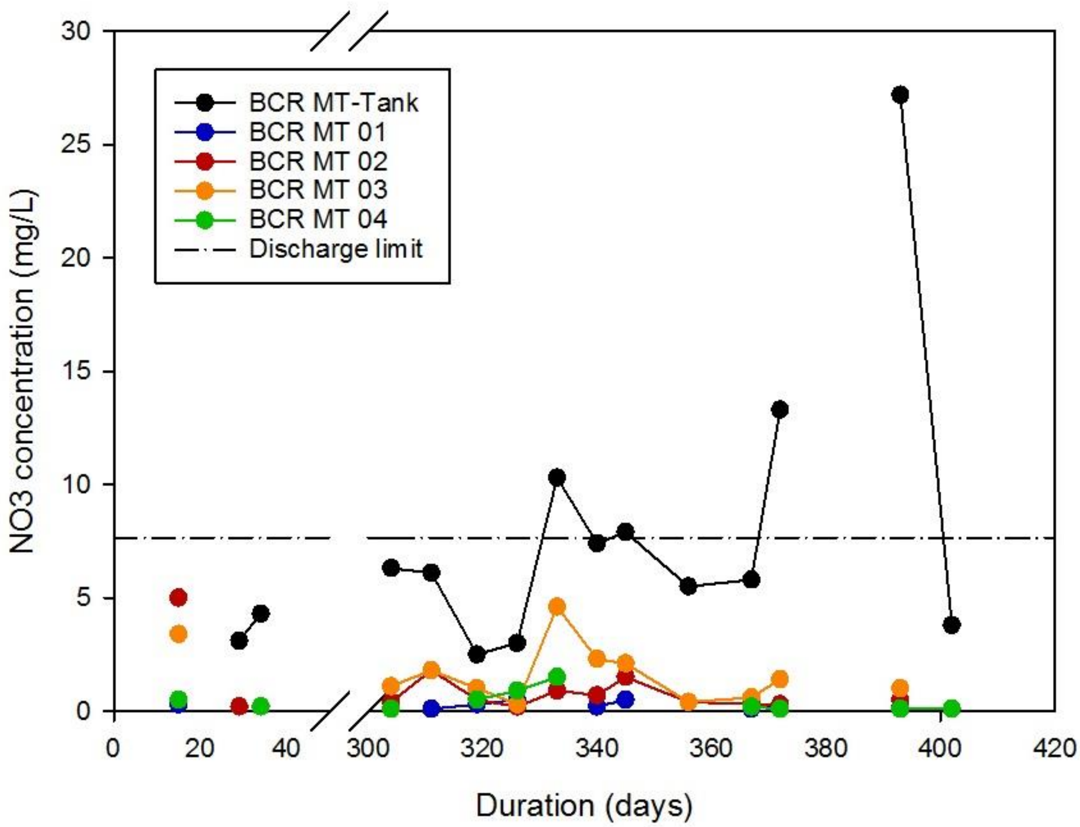

Figure 8. NO_3 concentrations in influent and effluent of the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 and September 26th 2015 (Discharge Limit of 7.65).

Phosphate (PO_4) is not regulated and was only measured in effluents during the first year. PO_4 concentrations were significantly higher in the effluent from the biochar amended bioreactor, BCR-MT-04, than from the other three reactors. This is not unexpected as the willow and bone meal biochar used in this study was known to contain elevated phosphate and the black color of the effluent reported by the field observations may indicate that some of the biochar had been flushed out of the reactor. PO_4 was not measured in 2015, however, its release is not desired and should be monitored during future operating periods.

Table 10. NH_3, NO_3, NO_2, PO_4 and conductivity data in "BCR-MT Tank" influent

Sampling date	**Duration** (day)	**NH3** (mg/L)	**NO3** (mg/L)	**NO2** (mg/L)	**PO4** (mg/L)	**Cond.** uS/cm
4-Sep-14	15	0.04	3.4	<0.01/0.02	N/A	498.1
13-Sep-14	24	<0.01	N/A	N/A	N/A	594
18-Sep-14	29	<0.01	3.1	<0.01	N/A	934
23-Sep-14	34	0.09	4.3	0.04	N/A	940
20-Jun-15	304	N/A	6.3	<0.01	N/A	N/A
27-Jun-15	311	N/A	6.1	<0.01	N/A	N/A
5-Jul-15	319	N/A	2.5	0.029	N/A	N/A
12-Jul-15	326	N/A	3	0.071	N/A	N/A
19-Jul-15	333	N/A	10.3	0.023	N/A	N/A
26-Jul-15	340	N/A	7.4	<0.01	N/A	813
31-Jul-15	345	N/A	7.9	<0.01	N/A	696
11-Aug-15	356	N/A	5.5	0.011	N/A	782
22-Aug-15	367	N/A	5.8	<0.01	N/A	836
27-Aug-15	372	N/A	13.3	0.014	N/A	654
6-Sep-15	382	N/A	N/A	N/A	N/A	905
17-Sep-15	393	N/A	27.2	<0.01	N/A	942
26-Sep-15	402	N/A	3.8	0.029	N/A	1111

Table 11. NH_3, NO_3, NO_2, PO_4 and conductivity data from "BCRMT-01" bioreactor

Sampling date	Duration (day)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm
4-Sep-14	15	0.03	0.3	<0.01	0.13	492.5
13-Sep-14	24	<0.01	N/A	N/A	N/A	565
18-Sep-14	29	<0.01	<0.1	<0.01	1.14	916
23-Sep-14	34	<0.01	<0.1	<0.01	0.68	825
20-Jun-15	304	N/A	<0.01	<0.01	N/A	960
27-Jun-15	311	N/A	0.1	<0.01	N/A	822
5-Jul-15	319	N/A	0.3	0.004	N/A	694
12-Jul-15	326	N/A	0.5	<0.01	N/A	637
19-Jul-15	333	N/A	<0.01	<0.01	N/A	618
26-Jul-15	340	N/A	0.2	<0.01	N/A	728
31-Jul-15	345	N/A	0.5	<0.01	N/A	575
11-Aug-15	356	N/A	<0.01	<0.01	N/A	680
22-Aug-15	367	N/A	0.1	<0.01	N/A	645
27-Aug-15	372	N/A	0.3	<0.01	N/A	523
6-Sep-15	382	N/A	N/A	N/A	N/A	754
17-Sep-15	393	N/A	0.2	<0.01	N/A	769
26-Sep-15	402	N/A	<0.01	<0.01	N/A	938

Table 11. NH_3, NO_3, NO_2, PO_4 and conductivity data from "BCRMT-02" bioreactor

Sampling date	Duration (day)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm
4-Sep-14	15	0.11	5	<0.01	0.18	1285
13-Sep-14	24	<0.01	N/A	N/A	N/A	842
18-Sep-14	29	0.04	0.2	<0.01	0.79	1326
23-Sep-14	34	0.11	<0.1	<0.01	<0.1	1148
20-Jun-15	304	N/A	0.4	<0.01	N/A	N/A
27-Jun-15	311	N/A	1.8	<0.01	N/A	885
5-Jul-15	319	N/A	0.5	0.008	N/A	871
12-Jul-15	326	N/A	0.2	<0.01	N/A	675
19-Jul-15	333	N/A	0.9	<0.01	N/A	684
26-Jul-15	340	N/A	0.7	<0.01	N/A	822
31-Jul-15	345	N/A	1.5	<0.01	N/A	675
11-Aug-15	356	N/A	0.4	<0.01	N/A	840
22-Aug-15	367	N/A	0.3	<0.01	N/A	802
27-Aug-15	372	N/A	0.3	<0.01	N/A	575
6-Sep-15	382	N/A	N/A	N/A	N/A	840
17-Sep-15	393	N/A	0.5	<0.01	N/A	803
26-Sep-15	402	N/A	<0.01	<0.01	N/A	937

Table 12. NH_3, NO_3, NO_2, PO_4 and conductivity data from "BCRMT-03" bioreactor

Sampling date	Duration (day)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm
4-Sep-14	15	0.11	3.4	<0.01	0.11	1053
13-Sep-14	24	<0.01	N/A	N/A	N/A	841
18-Sep-14	29	0.02	<0.1	<0.01	0.83	1318
23-Sep-14	34	0.03	<0.1	<0.01	1.1	1126
20-Jun-15	304	N/A	1.1	<0.01	N/A	N/A
27-Jun-15	311	N/A	1.8	<0.01	N/A	1094
5-Jul-15	319	N/A	1	0.009	N/A	860
12-Jul-15	326	N/A	0.3	0.016	N/A	711
19-Jul-15	333	N/A	4.6	<0.01	N/A	724
26-Jul-15	340	N/A	2.3	<0.01	N/A	878
31-Jul-15	345	N/A	2.1	<0.01	N/A	687
11-Aug-15	356	N/A	0.4	<0.01	N/A	903
22-Aug-15	367	N/A	0.6	<0.01	N/A	N/A
27-Aug-15	372	N/A	1.4	<0.01	N/A	616
6-Sep-15	382	N/A	N/A	N/A	N/A	866
17-Sep-15	393	N/A	1	<0.01	N/A	850
26-Sep-15	402	N/A	<0.01	<0.01	N/A	981

Table 13. NH_3, NO_3, NO_2, PO_4 and conductivity data from "BCRMT-04" bioreactor

Sampling date	Duration (day)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm
4-Sep-14	15	0.21	0.5	0.02/0.03	2.4	1461
13-Sep-14	24	<0.01	N/A	N/A	N/A	881
18-Sep-14	29	0.5	<0.1	0.03	18.4	1309
23-Sep-14	34	0.19	0.2	0.1	25.4	1133
20-Jun-15	304	N/A	0.1	<0.01	N/A	N/A
27-Jun-15	311	N/A	<0.01	<0.01	N/A	878
5-Jul-15	319	N/A	0.5	0.056	N/A	846
12-Jul-15	326	N/A	0.9	0.024	N/A	601
19-Jul-15	333	N/A	1.5	<0.01	N/A	251.8
26-Jul-15	340	N/A	<0.01	<0.01	N/A	889
31-Jul-15	345	N/A	<0.01	<0.01	N/A	733
11-Aug-15	356	N/A	<0.01	<0.01	N/A	864
22-Aug-15	367	N/A	0.2	<0.01	N/A	808
27-Aug-15	372	N/A	0.1	<0.01	N/A	585
6-Sep-15	382	N/A	N/A	N/A	N/A	849
17-Sep-15	393	N/A	0.1	<0.01	N/A	840
26-Sep-15	402	N/A	0.1	<0.01	N/A	962

3.8. TOTAL ORGANIC CARBON

Total Organic Carbon (TOC) was measured and is represented graphically in Figure 9 (numerical data is provided in Appendix 1). The data clearly indicates that the wood amended bioreactors (BCR MT 02 and BCR MT 03) were leaching organic carbon in the one month of operation in 2014, with 200 to 700 mg/L TOC measured. This correlates well with the observed decrease in pH and supports the hypothesis that wood chips are releasing organic acids (Wang et al 1995). Germain and Cyr (2003) reported the operation of a full-scale bioreactor at the Wood Cadillac site in north-western Quebec which is strictly made up of wood chips (mixed deciduous and conifer (Tasse et al. 2003)). The authors reported 99%, 95%, and 67% removal efficiency for sulfate along with the release of organic matter in the effluent of the bioreactor in Year 1 and 2 with reduction in Year 3 of operation. Hence, if wood chips are being used as part of the substrate materials, attention should be given to the release of organic matter and acidity.

Elevated TOC might not be an issue when the water treatment is composed of multiple cells in series with a final aerobic component. The integration of an aerobic treatment unit has been used successfully after an anaerobic unit allowing for polishing treatment to remove organic carbon, dissolved sulfide of carbon dioxide (Johnson and Halberg 2005).

TOC concentrations were high in the first samples collected in 2015 on June 20th and dropped dramatically by August 22nd and fell below the concentration of the influent with increasing TOC removal rates. High organic carbon consumption in a bioreactor is an indicator of a healthy community of heterotrophic microbes (Tsukamoto et al 2004, Mayes et al. 2011).

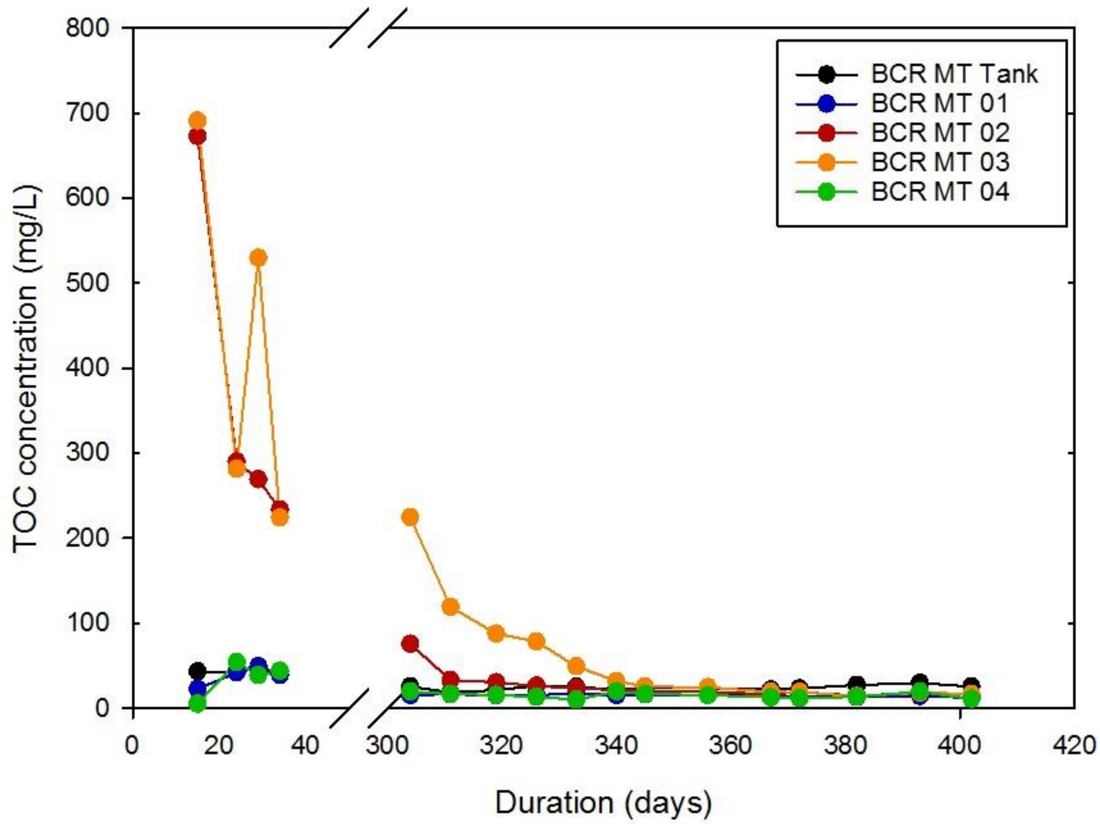

Figure 9. Total Organic Carbon in influent and effluent from the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 and September 26th 2015.

3.9. SULFATE REMOVAL

Sulfate (SO_4) in the influent and effluent were analyzed for samples taken during the summer of 2015. Sulfate concentration in influent was measured between 58.0 and 132.8 mg/l. Reduction of the sulfate concentrations was observed in the effluents for all four bioreactors. The most effective reduction of sulfate was observed in the bioreactor BCR-MT-03 which was made with wood chips and crushed gravel, followed by the bioreactor with wood chips and fluvial gravel (BCR-MT-02), the bioreactor with biochar and crushed gravel (BCR-MT-04), then control (BCR-MT-01). The average SO_4 removal rate was 91.3% for BCR-MT-03, 86.0, 55.2, and 36.4% for BCR-MT-02, BCR-MT-02, and BCR-MT-01 respectively (Figure 10).

It appeared that better sulfate reduction rates were achieved in bioreactors where wood chips were used. It is well known that wood chips release labile carbon such as organic acids and cellulosic materials (Mayes et al 2011, Hemsi et al 2005, Wang et al 1995) and the availability of carbon to the microbial community may support the growth of sulfate-reducing bacteria and explain the improved sulfate reduction in BCR-MT-03. Many other studies of bioreactors have used wood chips as a substrate materials. Further monitoring is required to assess long term effectiveness and seasonal variance on sulfate and the microbial community.

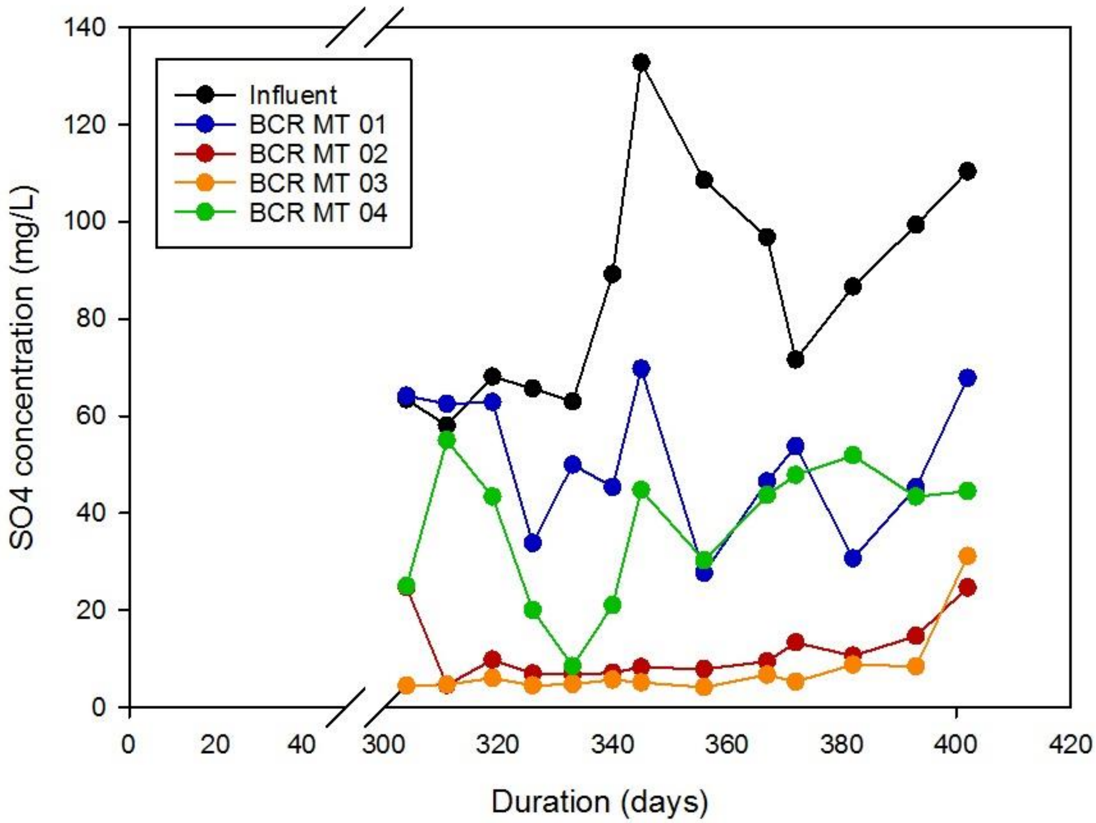

Figure 30. Total Sulfate in influent and effluent from the four bioreactors (BCR-MT-01 to -04) between August 20th 2014 to September 26th 2015.

3.10. BIOREACTOR COMPARISON

Preliminary results acquired from two periods of operation (1 month in 2014 and 4 months in 2015) with a halt of operation during freezing temperatures, allowed an evaluation of the trends and performances of the bioreactors. The first observation is that reduction in selenium and copper was achieved in all four reactors in the first month. The bacterial system apparently sustained during the winter, and improved in mine water remediation in the second summer-fall season.

Selenium removal proved to be highly efficient and steady in all four reactors during the one month operational period in 2014 and the four month operational period in 2015. Cu removal was also efficient during the one month of operation in 2014, and was improved in the subsequent operating period (2015). Dissolved Cu was significantly lower than total Cu in most cases and a large proportion of particulate Cu was observed in the outlet from the bioreactors (between 23 and 65% on average). Similar observation was made by Gammons et al. (2000) in effluents produced by a constructed wetlands for which the presence of copper sulfide particles was suspected.

Woodchips in anaerobic bioreactors has frequently been used to provide organic matter to the microbial community and to encourage long term reducing conditions (Neculita et al 2010; Germain and Cyr 2003, Wilmoth 2002; Bless et al 2008; etc.). In this study, bioreactors amended with wood chips (BCR-MT-02 and BCR-MT-03) performed well in terms of Cu and Se removal with concentrations below WUL discharge limits. However, it is suspected that the wood chips released organic acids, which decreased the pH below the allowable pH range for discharge at the beginning of the operation. Hence, integration of wood chips in bioreactors might be an issue in the short term. During the 2015 operational period, after 8 months of operation pause, pH was higher and fit within the discharge limit. This supports reliability of the bioreactors with wood chips in the long term.

Bioreactors with wood chips showed a good SO_4 removal during the second year, and by the end of the operation in 2015, organic carbon was reduced by up to 45 and 49% respectively by BCR-MT-02 and BCR-MT-03, likely through consumption by microorganisms.

Successful Se and Cu removal as well as moderate SO_4 reduction were achieved in the biochar-amended bioreactor BCR-MT-04 while having only a slight impact on pH. There wasn't evidence of a significant amount of organic carbon leaching from the bioreactor with biochar. Evidence of bacterial activity was not as strong as wood chips amended reactors. Integration of wood biochar in bioreactors was tested by Amelie Janin in a previous study and showed promising results with improved performances in cold climate which was attributed to biochar's sorption capacity (Janin and Harrington 2015; Janin and Harrington 2014). However, in this study, metal adsorption in the biochar bioreactor did not seem to be significant compared to control, and was therefore unable to evaluate the biochar's benefit of adsorptive character in this study. This study was initially designed with wood biochar (same granulometry as wood chips) however delays in shipment of wood char to Yukon College resulted in a change of plans and instead willow and bone meal biochar were used. The granulometry of this biochar is more powder-like than wood chips, with a higher potassium and phosphate content. This change may have affected the results and explained the release of suspended particulates and phosphate in the first month as indicated by low visibility and black color of the effluent. As well, the lowest flow was observed and might be due to possible occlusion of the flow by fine particles.

4. CONCLUSION

The objectives of this project, which included setting up pilot bioreactors on-site and the preliminary assessment of the potential for copper and selenium treatment by anaerobic bioreactors at the Minto mine were fulfilled. Successful removal of copper and selenium were achieved in all four bioreactors studied with Se concentration consistently below 3 ug/L and Cu concentrations below 50 ug/L except on 4 sampling events. Metal removal sustained through the decreasing temperatures in the fall. Sulfate and carbon reduction also suggest that sulfate-reducing bacteria were active in the bioreactors although no test were performed to confirm their presence.

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APPENDIX 1 – MONITORING DATA

Table 1. Measured water quality parameters in effluent from bioreactor BCR-MT-01

Sampling date	Duration (day)	pH	Se-T (ug/L)	Se-D (ug/L)	Cu-T (ug/L)	Cu-D (ug/L)	TOC (mg/L)	SO_4 (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm	Temp. °C
4-Sep-14	15	7.9	<0.7	<0.7	7.22	7.74	22.4	N/A	0.03	0.3	<0.01	0.13	492.5	4.7
13-Sep-14	24	8.0	<0.7	1.11	6.05	4.67	42.0	N/A	<0.01	N/A	N/A	N/A	565	7.9
18-Sep-14	29	9.0	0.78	0.72	7.90	4.79	48.1	N/A	<0.01	<0.1	<0.01	1.14	916	4.3
23-Sep-14	34	9.1	0.80	<0.7	18.45	4.05	38.7	N/A	<0.01	<0.1	<0.01	0.68	825	2.7
20-Jun-15	304	8.1	<0.9	<0.9	27.33	18.20	15.2	64.2	N/A	<0.01	<0.01	N/A	960	21.8
27-Jun-15	311	8.4	<0.9	<0.9	17.43	14.64	16.3	62.5	N/A	0.1	<0.01	N/A	822	15.6
5-Jul-15	319	8.6	<0.9	<0.9	28.44	13.96	15.0	62.9	N/A	0.3	0.004	N/A	694	22
12-Jul-15	326	8.1	<0.9	<0.9	11.24	14.76	15.5	33.8	N/A	0.5	<0.01	N/A	637	13.4
19-Jul-15	333	8.4	<0.9	<0.9	14.00	7.26	17.1	50.0	N/A	<0.01	<0.01	N/A	618	13.1
26-Jul-15	340	8.7	<0.9	<0.9	12.07	13.76	15.2	45.4	N/A	0.2	<0.01	N/A	728	20.6
31-Jul-15	345	8.3	<0.9	<0.9	13.23	3.55	16.0	69.8	N/A	0.5	<0.01	N/A	575	13.3
11-Aug-15	356	8.6	<0.9	<0.9	9.48	8.35	15.5	27.7	N/A	<0.01	<0.01	N/A	680	14.7
22-Aug-15	367	8.7	<0.9	<0.9	5.85	4.15	14.3	46.5	N/A	0.1	<0.01	N/A	645	10.1
27-Aug-15	372	8.4	<0.9	<0.9	12.44	3.70	13.2	53.8	N/A	0.3	<0.01	N/A	523	10.8
6-Sep-15	382	8.2	<0.9	<0.9	10.50	5.41	12.9	30.6	N/A	N/A	N/A	N/A	754	5.7
17-Sep-15	393	8.5	<0.9	<0.9	6.96	7.78	14.1	45.3	N/A	0.2	<0.01	N/A	769	6.6
26-Sep-15	402	8.7	<0.9	<0.9	6.34	3.31	12.4	67.9	N/A	<0.01	<0.01	N/A	938	2.5

Table 2. Measured water quality parameters in effluent from bioreactor BCR-MT-02

Sampling date	Duration (day)	pH	Se-T (ug/L)	Se-D (ug/L)	Cu-T (ug/L)	Cu-D (ug/L)	TOC (mg/L)	SO_4 (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm	Temp. °C
4-Sep-14	15	6.1	1.30	0.94	9.27	1.76	673.0	N/A	0.11	5	<0.01	0.18	1285	8.5
13-Sep-14	24	6.4	<0.7	<0.7	8.41	2.12	289.8	N/A	<0.01	N/A	N/A	N/A	842	8.2
18-Sep-14	29	6.2	<0.7	<0.7	9.09	0.82	268.9	N/A	0.04	0.2	<0.01	0.79	1326	5.5
23-Sep-14	34	6.3	<0.7	<0.7	24.70	1.62	233.4	N/A	0.11	<0.1	<0.01	<0.1	1148	7.1
20-Jun-15	304	N/A	<0.9	<0.9	19.63	10.38	75.6	24.7	N/A	0.4	<0.01	N/A	N/A	N/A
27-Jun-15	311	8.0	<0.9	<0.9	23.43	11.80	32.7	4.6	N/A	1.8	<0.01	N/A	885	17
5-Jul-15	319	7.4	<0.9	<0.9	11.85	4.76	30.3	9.8	N/A	0.5	0.008	N/A	871	20.9
12-Jul-15	326	7.3	<0.9	<0.9	14.21	4.92	26.2	6.9	N/A	0.2	<0.01	N/A	675	13.4
19-Jul-15	333	7.7	<0.9	<0.9	11.95	8.54	23.2	6.7	N/A	0.9	<0.01	N/A	684	13.4
26-Jul-15	340	8.1	<0.9	<0.9	11.20	4.42	21.4	7.1	N/A	0.7	<0.01	N/A	822	20.8
31-Jul-15	345	7.3	<0.9	<0.9	8.15	2.15	20.7	8.2	N/A	1.5	<0.01	N/A	675	13.5
11-Aug-15	356	7.8	<0.9	<0.9	206.90	18.48	19.3	7.9	N/A	0.4	<0.01	N/A	840	13.1
22-Aug-15	367	7.9	<0.9	<0.9	8.58	4.21	16.2	9.4	N/A	0.3	<0.01	N/A	802	10.8
27-Aug-15	372	7.6	<0.9	<0.9	7.99	4.16	18.7	13.3	N/A	0.3	<0.01	N/A	575	10.4
6-Sep-15	382	8.0	<0.9	<0.9	5.08	6.63	14.9	10.6	N/A	N/A	N/A	N/A	840	4.2
17-Sep-15	393	7.7	<0.9	<0.9	16.26	3.56	17.7	14.7	N/A	0.5	<0.01	N/A	803	7.8
26-Sep-15	402	8.1	<0.9	<0.9	12.61	6.51	15.6	24.6	N/A	<0.01	<0.01	N/A	937	3.1

Table 3. Measured water quality parameters in effluent from bioreactor BCR-MT-03

Sampling date	Duration (day)	pH	Se-T (ug/L)	Se-D (ug/L)	Cu-T (ug/L)	Cu-D (ug/L)	TOC (mg/L)	SO_4 (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm	Temp. °C
4-Sep-14	15	6.1	1.55	1.01	42.70	11.00	691.0	N/A	0.11	3.4	<0.01	0.11	1053	6.0
13-Sep-14	24	6.2	1.40	<0.7	19.85	4.62	281.2	N/A	<0.01	N/A	N/A	N/A	841	7.9
18-Sep-14	29	5.8	<0.7	<0.7	15.66	1.75	529.6	N/A	0.02	<0.1	<0.01	0.83	1318	5.1
23-Sep-14	34	6.3	<0.7	<0.7	22.64	1.24	223.9	N/A	0.03	<0.1	<0.01	1.1	1126	3.8
20-Jun-15	304	N/A	<0.9	<0.9	164.86	9.02	224.5	4.5	N/A	1.1	<0.01	N/A	N/A	N/A
27-Jun-15	311	7.3	<0.9	<0.9	24.73	15.10	119.2	4.7	N/A	1.8	<0.01	N/A	1094	14.8
5-Jul-15	319	6.8	<0.9	<0.9	9.43	4.51	87.7	6.0	N/A	1	0.009	N/A	860	18.4
12-Jul-15	326	7.2	<0.9	<0.9	7.79	1.31	78.2	4.5	N/A	0.3	0.016	N/A	711	12.9
19-Jul-15	333	7.0	<0.9	<0.9	18.36	0.90	49.1	4.8	N/A	4.6	<0.01	N/A	724	13.2
26-Jul-15	340	7.4	<0.9	<0.9	11.16	1.28	31.8	5.7	N/A	2.3	<0.01	N/A	878	19.1
31-Jul-15	345	6.9	<0.9	<0.9	8.97	0.95	25.4	5.1	N/A	2.1	<0.01	N/A	687	11.9
11-Aug-15	356	7.6	<0.9	<0.9	12.29	19.02	24.5	4.1	N/A	0.4	<0.01	N/A	903	13.0
22-Aug-15	367	N/A	<0.9	<0.9	3.94	1.51	19.1	6.7	N/A	0.6	<0.01	N/A	N/A	N/A
27-Aug-15	372	7.3	<0.9	<0.9	20.39	3.81	18.8	5.3	N/A	1.4	<0.01	N/A	616	10.5
6-Sep-15	382	7.7	<0.9	<0.9	11.49	12.00	13.9	8.8	N/A	N/A	N/A	N/A	866	5.7
17-Sep-15	393	7.4	<0.9	<0.9	12.17	4.04	18.5	8.4	N/A	1	<0.01	N/A	850	7.5
26-Sep-15	402	8.1	<0.9	<0.9	7.67	1.56	16.6	31.1	N/A	<0.01	<0.01	N/A	981	3.9

Table 4. Measured water quality parameters in effluent from bioreactor BCR-MT-04

Sampling date	Duration (day)	pH	Se-T (ug/L)	Se-D (ug/L)	Cu-T (ug/L)	Cu-D (ug/L)	TOC (mg/L)	SO_4 (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	PO4 (mg/L)	Cond. uS/cm	Temp. °C
4-Sep-14	15	8.5	0.83	0.94	128.00	90.40	5.0	N/A	0.21	0.5	0.02/0.03	2.4	1461	9.0
13-Sep-14	24	8.1	<0.7	<0.7	23.89	22.01	54.1	N/A	<0.01	N/A	N/A	N/A	881	8.2
18-Sep-14	29	9.4	<0.7	<0.7	23.21	55.94	38.5	N/A	0.5	<0.1	0.03	18.4	1309	5.7
23-Sep-14	34	9.3	<0.7	<0.7	37.35	21.78	43.9	N/A	0.19	0.2	0.1	25.4	1133	7.0
20-Jun-15	304	N/A	<0.9	<0.9	26.52	17.09	19.6	24.9	N/A	0.1	<0.01	N/A	N/A	N/A
27-Jun-15	311	8.6	<0.9	<0.9	45.50	12.36	16.6	55.0	N/A	<0.01	<0.01	N/A	878	16.1
5-Jul-15	319	8.4	<0.9	<0.9	12.12	8.54	15.5	43.4	N/A	0.5	0.056	N/A	846	19.7
12-Jul-15	326	8.8	<0.9	<0.9	17.79	16.65	13.3	20.1	N/A	0.9	0.024	N/A	601	12.6
19-Jul-15	333	8.5	<0.9	<0.9	75.78	18.77	10.1	8.5	N/A	1.5	<0.01	N/A	252	11.7
26-Jul-15	340	8.8	<0.9	<0.9	12.51	9.42	19.8	21.0	N/A	<0.01	<0.01	N/A	889	22.6
31-Jul-15	345	8.5	<0.9	<0.9	5.33	4.43	16.9	44.7	N/A	<0.01	<0.01	N/A	733	14.0
11-Aug-15	356	8.5	<0.9	<0.9	9.68	4.37	15.1	30.3	N/A	<0.01	<0.01	N/A	864	13.9
22-Aug-15	367	8.8	<0.9	<0.9	3.98	3.15	12.6	43.7	N/A	0.2	<0.01	N/A	808	10.7
27-Aug-15	372	8.6	<0.9	<0.9	6.14	2.90	11.6	47.8	N/A	0.1	<0.01	N/A	585	10.6
6-Sep-15	382	8.0	<0.9	<0.9	3.75	3.26	13.5	51.9	N/A	N/A	N/A	N/A	849	4.6
17-Sep-15	393	8.7	<0.9	<0.9	6.59	6.80	19.3	43.4	N/A	0.1	<0.01	N/A	840	8.5
26-Sep-15	402	9.0	1.17	<0.9	16.13	7.66	11.0	44.5	N/A	0.1	<0.01	N/A	962	4.5