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Enlèvement des métaux des effluents miniers en climat froid par des processus biologiques et chimiques s'opérant dans des bioréacteurs semi-passifs sulfato-réducteurs

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AVANT PROPOS

Cette étude s'est concentrée sur l’utilisation de bactéries sulfato-réductrices dans des bioréacteurs passifs sulfato-réducteurs pour traiter, en climat froid, des drainages miniers contaminés par des métaux lourds.

L’objectif de ce projet de doctorat était de comprendre les mécanismes pouvant mener à la production de sulfures à partir des sulfates présents dans effluents miniers par des bactéries sulfato-réductrices indigènes du Yukon dans le but de précipiter les métaux présents dans les drainages miniers sous formes de sulfures de métaux.

La présente thèse a été structurée en quatre chapitres, dont trois chapitres représentent chacun une publication scientifique faisant l’objet d’une section des résultats de ce projet de doctorat.

- **Le Chapitre 1** présente la synthèse de cette thèse comportant une revue de la littérature, l'approche méthodologique ainsi que les principaux résultats obtenus.

- **Le Chapitre 2** présente l’étude réalisée à l’échelle laboratoire sur l’utilisation potentielle de bactéries sulfato-réductrices pour le traitement passif de drainage contaminé par des métaux (Cd, Zn) et des mécanismes qu’elles utilisent pour précipiter le Cd et le Zn en sulfures de métaux en climat froid.

  Performances of sulfate-reducing passive bioreactors for the treatment of mine drainage contaminated by Cd and Zn in cold climate. G. Nielsen, A. Janin, L. Coudert, J.F. Blais, G. Mercier. Soumis à *Mine Water and the Environment* le 24 Octobre 2016 et accepté le 6 Février 2017.

- **Le Chapitre 3** présente une revue de littérature des différentes sources de carbone utilisées jusqu’à ce jour pour la croissance spécifique de bactéries sulfato-réductrices et les résultats d’une étude menée en laboratoire où certaines sources de carbone ont été testées afin d’évaluer leur influence sur les performances d'enlèvement des métaux par précipitation sous formes de sulfures métalliques.

Influence of organic carbon sources on the reduction of sulfates from mine impacted water using sulfate-reducing bacteria bioreactors in cold climates. G. Nielsen, L. Coudert, A. Janin, J.F. Blais, G. Mercier. Prêt à soumettre à : *Artic Science*.

- Enfin, le **Chapitre 4** présente les résultats issus d'une étude pilote menée sur le site minier de Keno Hill Silver district au Yukon. Des bioréacteurs pilotes ont été installés sur le site minier et ont été échantillonnés pendant 14 mois. Une caractérisation génomique de la population microbienne de ces bioréacteurs, ainsi que de l'effluent minier a été effectuée.

Semi-passive bioreactors using native sulfate reducing bacteria at pilot scale in cold climate. G. Nielsen, A. Janin, S. Baldwin, I. Hatam, L. Coudert, J.F. Blais, G. Mercier. Prêt à soumettre à : *Applied and Environmental Microbiology.*

RÉSUMÉ

Les avancements technologiques et l'augmentation de la population mondiale font en sorte que les besoins en métaux et en minerais ne cessent de croître, conduisant à l'émergence de nombreuses mines dans le monde et dans le nord canadien. L'exploitation de ces mines implique une inévitable contamination en métaux lourds des sols et des eaux et une nouvelle gestion des drainages miniers contaminés est nécessaire.

À l'heure actuelle, la grande majorité des systèmes de traitement mis en place sont des systèmes actifs, souvent coûteux et générant des boues dont la disposition s'avère problématique, surtout en climat nordique. L'application de systèmes passifs au traitement des drainages miniers représente une solution économique et écologique prometteuse. Les systèmes de traitement passif offrent une alternative prometteuse aux systèmes de traitement conventionnels. Parmi les systèmes de traitement passif, la bioremédiation, faisant appel à des microorganismes, notamment les bactéries sulfato-réductrices (BSR), est de plus en plus utilisée pour le traitement de drainages miniers.

Même si plusieurs travaux de recherche traitent de ce sujet, peu d'études abordent l'application de traitements passifs en climat nordique, où les températures froides affectent la croissance et l'efficacité des plantes et des bactéries. Cependant, les réglementations de plus en plus strictes concernant les drainages miniers neutres et l'exploitation de nombreuses mines dans le Nord encouragent le développement de méthodes de traitement de ces drainages miniers neutres qui soient à la fois efficaces et simples d'opération.

Ce projet de doctorat est basé sur l'hypothèse de recherche selon laquelle il est possible d'appliquer des traitements passifs faisant appel aux bactéries sulfato-réductrices aux drainages miniers neutres dans des régions subissant un climat nordique.

Des essais réalisés en laboratoire à 5°C sur des périodes de 90 à 160 jours ont permis de mettre en évidence la présence de BSR indigènes dans l'effluent du site minier de Silver King situé au Yukon et en prise avec une contamination en zinc et en cadmium.

Dans les premiers travaux menés en laboratoire pendant 90 jours à 5°C (**Chapitre 2**), l'utilisation de différentes sources de carbone (mélasse, méthanol et un mélange de mélasse/méthanol) a nettement amélioré l'activité bactérienne et, par conséquent, la précipitation des métaux (Zn et Cd) sous forme de sulfures. En effet, avec le mélange mélasse/méthanol qui s'est avéré être légèrement plus efficace que la mélasse ou le méthanol seul, près de 94,8% du Zn et 99,4% du Cd initialement présents dans l'effluent minier ont été éliminés. Durant ces premiers essais en laboratoire, les sources de carbone utilisées l'ont été à deux concentrations différentes afin d'évaluer l'importance de la quantité de carbone disponible pour les BSR. Les concentrations en carbone étaient : C1 = 77 mg/L (soit 2 moles de carbone par mole de sulfate, considérée d'après la littérature comme la concentration minimale pour réduire les sulfates présents dans le drainage minier à l'étude) et C2 = 154 mg/L (soit 2 fois C1). Il a été montré qu'une augmentation significative de la quantité de carbone dans les bioréacteurs n'a pas généré d'amélioration significative des abattements en Zn ou en Cd, ce qui laisse à penser que BSR ne consomment pas plus carbone qu'elles n'en ont besoin.

La deuxième série d'essais en batch menés en laboratoire pendant 160 jours à 5°C (**Chapitre 3**) visait à étudier spécifiquement la réduction des sulfates par les BSR sous l'influence de différentes sources de carbone (sources organiques complexes telles que l'huile de pomme de terre, les résidus de brasserie, la tourbe et la paille) et sources organiques simples telles que le méthanol et l'éthylène-glycol). L'abattement des concentrations en Zn et Cd a également été suivi. Ces essais ont montré que le méthanol et l'éthylène-glycol ont entraîné une diminution des concentrations en sulfates de 71,2% et 36,9%, respectivement, tandis que la diminution des concentrations en sulfates a atteint seulement 13,8% et 5,3%, respectivement, pour la tourbe et la paille. En ce qui concerne l'abattement des concentrations en Zn et Cd, il s'est avéré qu'avant que l'activité bactérienne ne soit observée par la consommation de carbone et de sulfate, la concentration des métaux a diminué. Pour cette raison, il n'a pas été possible de corréler la diminution des concentrations en sulfate avec l'élimination des métaux. L'adsorption sur les sources de carbone pourrait être à l'origine de la diminution rapide des concentrations de Cd et de Zn.

Enfin, à la suite des travaux menés en laboratoires, des essais conduits à l'échelle pilote (**Chapitre 4**) ont permis l'étude de deux bioréacteurs sulfato-réducteurs sur une période de 14 mois dans le but de traiter

le drainage minier neutre contaminé en Zn et en Cd du Silver King mine site situé au Yukon. Une caractérisation génomique des communautés microbiennes de chaque bioréacteur, ainsi que de l'effluent minier a été entreprise. Cette caractérisation a révélé la présence dans les bioréacteurs d'une population microbienne complexe, mais stable dans le temps, ainsi que des BSR potentielles (membres des classes *Clostridia* and *Deltaproteobacteria*). Durant les 14 mois qu'ont duré l'expérience pilote, une solution de mélasse a été injectée à une concentration constante (2 moles de carbone par mole de sulfate). Comme il l'avait été conclu lors des premiers essais laboratoires, cette étude pilote a démontré que les BSR, bien que touchées par des températures froides, peuvent être efficaces pour effectuer des procédés de réduction de sulfates entraînant ainsi la précipitation des métaux sous formes de sulfures en climat froid. L'efficacité des abattements en Zn a varié entre 11,5% en hiver et 95,5% en été. L'efficacité des abattements en Cd a varié entre 21,1% en hiver et 96,6% en été.

ABSTRACT

Technological advances and the increase of world population mean that metals and minerals requirements continue to grow, leading to the emergence of mining companies across the world and in Northern Canada. Mining operations imply an ineviTable heavy metals contamination of soils and waters and an effective management of contaminated mining drainage is necessary.

Nowadays, the vast majority of treatment systems are active systems which are expensive and generate problematic sludge, especially in northern climates. Using passive systems to mine drainage represents a promising solution in terms of both economic and ecological impacts. Passive treatment systems offer a potential alternative to conventional treatment systems. Among the passive treatment systems, bioremediation, using microorganisms, in particular sulfate-reducing bacteria, is increasingly used for the treatment of mining drainage.

Although several researches have addressed this issue, only few studies have been done on the application of passive treatments in northern climates where cold temperatures affect the growth and effectiveness of plants and bacteria. However, increasingly stringent regulations on neutral mining drainage and the operation of many mines in the North encourage the development of efficient and easy to operate treating methods of these neutral mining drainage.

This PhD project is based on the research hypothesis that it is possible to apply passive treatments using sulfate-reducing bacteria to treat neutral mine drainage in northern climate areas.

Laboratory experiments conducted over 90 to 160 days at 5°C revealed the presence of native SRB in the effluent from the Silver King mine site located in the Yukon Territory struggling with Zinc and Cadmium contamination.

In the first laboratory tests conducted for 90 days at 5°C (**Chapter 2**), the use of different carbon sources (molasses, methanol and a mixture of molasses/methanol) significantly improved bacterial activity and, consequently, the precipitation of metals (Cd and Zn) as metal sulfides. Indeed, with the mix molasses/methanol mixture which proved to be slightly more effective than molasses or methanol alone, up to 94.8% of Zn and 99.4% of Cd were removed after. During these first laboratory tests, the carbon

sources used were used at 2 different concentrations to assess the amount of carbon available for SRB. Carbon concentrations were: C1 = 77 mg/L (ie, 2 moles of carbon per mole of sulfate, considered in the literature to be the minimum concentration to reduce the sulfates present in the mine drainage studied) and C2 = 154 mg/L (ie which is twice C1). It has been shown that a significant increase in the amount of carbon in the bioreactors did not result in any significant improvement in Zn or Cd removals, suggesting that SRB does not consume more carbon than what they need.

The second laboratory batch tests was conducted for 160 days at 5°C (**Chapter 3**) and aimed specifically to study the reduction of sulfates by SRB using different carbon sources (complex organic sources (potato oil, brewery residues, peat and straw) and simple organic sources (methanol and ethylene glycol)). The removal of Zn and Cd was also studied. These batch tests showed that the use of methanol and ethylene glycol as carbon sources resulted in the decreases of sulfate concentrations of 71.2% and 36.9%, respectively, while decreases of sulfate concentrations were limited to 13.8% and 5.30% for peat and straw, respectively. With regard to the decrease in Zn and Cd concentrations, it was found that before bacterial activity was observed by the consumption of carbon and sulfate, the concentration of the metals decreased. For this reason, it was not possible to correlate the decrease in sulfate concentrations with metal removals. Adsorption on carbon sources could be the cause of the rapid decrease in Cd and Zn concentrations.

Finally, following these laboratory work, a pilot scale experiment (**Chapter 4**) has been conducted to study the behavior of two pilot sulfate-reducing bioreactors over a 14-month period to treat the neutral mine drainage contaminated by Zn and Cd from the Silver King mine site, Yukon Territory. A genomic characterization of the microbial communities of each bioreactor as well as the mining effluent was performed. This characterization revealed the presence in the bioreactors of a complex but time-stable microbial population as well as potential SRB (members of Classes *Clostridia* and *Deltaproteobacteria*). During the 14-month pilot experiment, a molasses solution was injected at a constant concentration (2 moles of carbon per mole of sulfate). As it was concluded during the first laboratory experience, this pilot study demonstrated that SRB, although affected by cold temperatures, may be effective in performing sulfate reduction processes and thus precipitating metals as metal sulfides in a cold climate. The

effectiveness of Zn removal varied between 11.5% in winter and 95.5% in summer. The effectiveness of Cd removal varied between 21.1% in winter and 96.6% in summer.

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AA	Atomic adsorption
ADN	Adénosine tri phosphate
AMD	Acid mine drainage
AMOVA	Analysis of Molecular Variance
AP	Potentiel de génération acide / Generation potential
ARD	Acid rock drainage
BSR	Bactéries sulfato-réductrices
BPSR	Bioréacteur passif à sulfato-réduction
CI	Carbone inorganique
CIT	Carbone inorganique total
COD	Carbone organique dissous
COT	Carbone organique total
CSST	Commission de santé sécurité au travail
DIW	Deionized water
DMA	Drainage minier acide
DNA	Deoxyribonucleic acid
DNC	Drainage neutre contaminé
DRX	Diffractométrie de rayons X
EDM	Effluent minier
HTR	Hydraulic retention time
ICP-AES	Inductively coupled plasma - atomic emission spectroscopy
ICP-MS	Inductively coupled plasma - mass spectrometry
INRS	Institut national de la recherche scientifique
LDPE	Polyéthylène de faible densité / Low-density polyethylene
LOI	Perte au feu / Loss of ignition
MEB	Microscope à balayage électronique
MIW	Mine impacted water
NMD	Neutral mine drainage
NMDS	Non-metric multidimensional scaling
NNP	Net neutralization potential
NP	Potentiel de neutralisation / Neutralizing potential
NTK	Azote total Kjeldahl
ORP	Oxidation reduction potential
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PNN	Potentiel net de neutralisation
POR	Potentiel d’oxydo-réduction
PSRB	Passive sulfate-reducing bioreactors
PTS	Passive treatment systems
RNP	Ratio de neutralisation potentielle
SFD	Substance facilement disponible
SRPB	Sulfate-reducing passive bioreactors
SRB	Sulfate reducing bacteria
TC	Total carbon
TOC	Total organic carbon
WR	Waste rocks

INTRODUCTION

Les avancements technologiques ainsi que l’augmentation de la population font en sorte que les besoins en métaux et en minerais à travers le monde ne cessent de croître, conduisant à l’émergence de nombreuses mines à travers le monde et dans le nord canadien. L’exploitation de ces mines mène à la production d’effluents miniers contaminés en métaux lourds qui nécessitent une gestion spécifique, ainsi que la mise en place d’un système de traitement lorsque la mine est en opération et après sa fermeture. En effet, un traitement adéquat des effluents miniers est requis à court et à long terme lors de la construction, de l’opération et de la fermeture d’une mine.

À l’heure actuelle, les compagnies minières utilisent couramment des technologies de traitement actif, souvent coûteuses, pour traiter les effluents contaminés quand les drainages miniers acides ne peuvent pas être contrôlés à la source. Une démarche commune consiste à ajouter de la chaux pour neutraliser l'acidité, ce qui conduit à la précipitation des métaux sous forme d’hydroxydes (Aubé, 2005; Aubertin *et al.,* 2002; Brown *et al.,* 2002; MacDonald *et al.,* 1989). Cependant, l’utilisation des technologies de traitement actif engendre des coûts de traitement élevés qui deviennent problématiques lorsque les mines ferment. La production de boues nécessite un entreposage et une gestion sécuritaire, ce qui est également problématique, notamment dans le nord canadien. De plus, ces technologies, souvent peu durables, sont peu appropriées pour la gestion, à long terme, des effluents miniers produits sur les sites éloignés.

Les systèmes de traitement passif offrent une alternative prometteuse aux systèmes de traitement conventionnels. Parmi les systèmes de traitement passif, la bioremédiation, faisant appel à des microorganismes, notamment les bactéries sulfato-réductrices, est de plus en plus utilisée pour le traitement de drainages miniers.

Cependant, le défi reste de taille lorsqu’il est question de traitement passif dans les climats nordiques, où la température des effluents miniers souterrains n’excède jamais 5°C, même en été et où la croissance bactérienne est limitée. Ce projet s’inscrit dans le contexte d’une collaboration entre l’INRS-ETE situé au Québec, le Yukon Research Center basé à Whitehorse (Yukon) et l’entreprise Alexco Resource Corp. qui est en charge de la fermeture et de la restauration du district minier Keno Hill Silver district dans le territoire du Yukon, aux prises avec une problématique de drainage minier neutre contaminé en Cd et en Zn. Une des approches actuellement à l’étude par Alexco consiste à pomper l’eau souterraine contaminée en métaux lourds dans les anciens tunnels d’exploitation du minerai, à y mélanger une solution riche en carbone, puis à réinjecter le mélange eau-source de carbone dans le sol afin de favoriser la croissance des bactéries sulfato-réductrices (BSR) indigènes capables de précipiter les métaux.

PARTIE 1 : SYNTHÈSE

1 CHAPITRE 1 : SYNTHÈSE BIBLIOGRAPHIQUE

1.1 Revue de littérature

1.1.1 Activité minière et drainage minier

1.1.1.1. Drainage minier acide

Les problèmes de pollution provenant des sites miniers, abandonnés ou actifs, viennent principalement d'un phénomène appelé le drainage minier acide (DMA). Le DMA est produit naturellement lorsque les minéraux sulfureux réactifs contenus dans la roche, telles que la pyrite (FeS_2) et la pyrrhotite ($Fe_{1-x}S_x$), sont exposés à l'eau et à l'air. Le DMA peut être produit à partir de l'oxydation chimique ou microbiologique des stériles d'excavation (produits constitués par les sols et roches excavés lors de l'exploitation du minerai), des résidus miniers tels que les rejets de concentrateur ou des murs des exploitations à ciel ouvert ou des tunnels souterrains (SRK, 1988; Morin et Hutt, 2007; Potvin, 2009).

S'il est rejeté dans l'environnement naturel, le DMA peut être une source importante de pollution des eaux et peut avoir des effets dévastateurs sur l'environnement. En effet, il peut entraîner l'acidification des lacs et des rivières environnants (Espana *et al.,* 2006), compromettant ainsi la vie des plantes aquatiques et des organismes vivants présents dans l'eau ou sur les rives (Grout et Levings, 2001). De plus, certains métaux lourds peuvent être relâchés par lessivage acide (Lei *et al.,* 2010) et devenir bio-disponibles dans la chaîne trophique, menaçant ainsi la faune sauvage et la santé humaine (Alvarez-Valero *et al.,* 2009; El Khalil *et al.,* 2007; Zis *et al.,* 2004).

L'oxydation des minéraux sulfureux tels que la pyrite peut être représentée par les Équations 1-1 à 1-6 (Blowes et Ptacek, 1994; Evangelou et Zhang, 1995; Kleimnann *et al.,* 1981; Morin et Hutt, 1997; Nesbitt *et al.,* 1995). La première étape consiste en l'oxydation chimique directe de la pyrite (FeS_2) par l'oxygène présent dans l'air ambiant. Cette réaction produit des sulfates (SO_4^{2-}), du fer ferreux (Fe^{2+}) ainsi que de l'acidité (H^+) (Équation 1-1). Lors de la seconde étape, le fer ferreux est oxydé en fer ferrique (Fe^{3+}) comme l'indique l'Équation 1-2. Le fer ferreux peut également être oxydé en hydroxyde de fer ($FeOOH$), entraînant la production d'acidité (Équation 1-3). À un $pH > 4$, le fer ferrique (Fe^{3+}) est précipité sous forme d'hydroxyde ferrique ($Fe(OH)_3$), ce qui augmente l'acidité produite (Équation 1-4). Si le pH est inférieur à 4, le fer ferrique peut demeurer soluble et oxyder indirectement la pyrite (sans présence d'oxygène), libérant encore plus d'acidité dans l'environnement (Équation 1-5).

Équation 1.1 $2\ FeS_2 + 7\ O_2 + 2\ H_2O <=> 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+$

Équation 1.2 $2\ Fe^{2+} + 1/2\ O_2 + 2\ H^+ <=> 2\ Fe^{3+} + H_2O$

Équation 1.3 $Fe^{2+} + 1/4\ O_2 + 3/2\ H_2O <=> FeOOH + 2\ H^+$

Équation 1.4 $Fe^{3+} + 3\ H_2O <=> Fe(OH)_3 + 3\ H^+$

Équation 1.5 $FeS_2 + 14\ Fe^{3+} + 8\ H_2O <=> 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+$

À faible pH, les bactéries telles que *Acidithiobacillus ferrooxidans* peuvent également participer à la production d'acide en catalysant la réaction d'oxydation des minéraux sulfureux (Nesbitt *et al.,* 1995). En effet, l'oxydation du fer ferreux (Fe^{2+}) en fer ferrique (Fe^{3+}) à pH neutre est un processus compétitif entre l'oxydation abiotique (via l'oxygène) et l'oxydation biotique (via les bactéries ferrooxydantes) (Not, 2006). Il est connu que l'oxydation du fer ferreux par *A. ferrooxidans* diminue rapidement à un pH supérieur à 2,5. Cette inhibition de l'activité d'oxydation du fer ferreux par *A.ferrooxidans* observée à des valeurs de pH supérieures à 3,0 est partiellement liée à la formation de précipités de fer ferrique qui entravent apparemment les processus de transport à la surface cellulaire (Meruane *et al.,* 2003). Ces bactéries peuvent oxyder directement la surface du minéral ou encore favoriser la production de sulfates et la transformation des ions Fe^{2+} en ions Fe^{3+}; accélérant ainsi la vitesse d'oxydation chimique par un facteur de 20 à 1 000 (Berthelin, 1987).

L'ensemble des réactions (Équations 1-1 à 1-5) qui conduisent à la production de drainage minier acide peut être résumé par la réaction globale représentée par l'Équation 1-6. Cette réaction présente clairement le potentiel de production d'acide sulfurique et d'hydroxyde de fer à partir du minerai sulfureux tel que la pyrite dans ce cas (Potvin, 2009). Ainsi, l'Équation 1-6 met en évidence que l'oxydation d'une mole de pyrite génère 2 moles d'acide sulfurique. L'augmentation de l'acidité implique la mise en solution de métaux tels que le cuivre, le cadmium, le fer et le zinc présents dans les stériles ou les résidus miniers. Le contenu en métaux des DMA provenant des mines de métaux communs et précieux est très variable. Il dépend, entres autres, des minéraux primaires présents dans la roche oxydée.

Équation 1.6 $\mathbf{FeS_2 + 15/4\ O_2 + 7/2\ H_2O <=> Fe(OH)_3 + 2\ H_2SO_4}$

1.1.1.2. Drainage neutre contaminé

Le drainage neutre contaminé (DNC) est défini comme étant un drainage minier dont la qualité chimique est affectée par le passage à travers le rejet minier, mais qui n'est pas acide et dont le pH se situe entre 6 et 9 (Nordstrom *et al.,* 2015). Le drainage neutre contaminé peut apparaître dans différentes conditions (Calugaru, 2014):

- Les minéraux sulfureux s'oxydent et produisent de l'acide, ce qui a pour conséquence d'entraîner la mise en solution de certains métaux solubles. Toutefois, il est possible que les stériles contiennent suffisamment de minéraux acidivores pour maintenir le pH proche de la neutralité. Les réactions géochimiques (comme des précipitations) présentes dans le rejet minier n'affectent alors pas de façon significative la teneur de certains métaux plus solubles, qui demeurent à des concentrations suffisamment élevées pour avoir un impact sur l'environnement et être au dessus des limites de rejets imposées.
- Il est possible qu'il y ait lixiviation des métaux contenus dans les roches stériles sans génération d'acide. Par exemple, la sphalérite peut réagir avec l'oxygène sans générer d'acide, tout en libérant du zinc (très soluble). De plus, à pH neutre, l'accumulation d'oxydes sur la surface des minerais de sulfures entraîne une réduction significative des taux d'oxydation dans le temps. Ce phénomène implique la réduction du taux de libération des produits d'oxydation, y compris les ions hydrogène, dans les environnements où les déchets minéraux sulfurés sont exposés à l'atmosphère (Nicholson *et al.,* 1990).

- La production de drainage neutre est possible lorsque des méthodes de contrôle du DMA sont appliquées sur des rejets générateurs d'acide. Par exemple, certains rejets miniers peuvent être placés sous un recouvrement en eau qui limite l'accès à l'oxygène et, par conséquent, réduit l'oxydation des minéraux sulfureux et la génération d'acide. Cependant, la quantité d'oxygène disponible dans l'eau peut être suffisante pour qu'il y ait une oxydation parrtielle et localisée qu'un faible relargage des métaux se produise alors qu'il n'y a pas de problème d'acidité.

Les principaux éléments métalliques qui peuvent être retrouvés dans les effluents neutres contaminés sont l'arsenic, le cadmium, le chrome, le cobalt, le manganèse, le nickel et le zinc. Au Québec, les éléments métalliques les plus problématiques sont l'As, le Ni et le Zn (Bussière *et al.,* 2005).

L'impact du drainage neutre contaminé (DNC) sur les cours d'eau ou les lacs environnants a été significativement moins étudié que celui des drainages acides qui sont plus courants. D'après les travaux réalisés par Byrne (2013), même si le pH est neutre, des concentrations élevées en métaux dissous peuvent être mesurées dans les DNC ([Pb] = 269 µg L^{-1}; [Zn] = 1 044 µg L^{-1}) et peuvent causer des problèmes dans les cours d'eau. Dans cette étude, la raison pour laquelle le pH de ces drainages est neutre est liée à la présence de minéraux neutralisants qui sont présents dans les stériles ou les résidus miniers à l'origine de ces drainages. L'acide sulfurique produit lors de l'oxydation des sulfures est alors neutralisé par les carbonates ou par certains silicates au potentiel neutralisant (Kwong, 1993; Lawrence et Scheske, 1997). En effet, la calcite ($CaCO_3$) et la dolomite ($CaMg(CO_3)_2$), deux des carbonates les plus abondants dans les résidus miniers, peuvent neutraliser l'acide sulfurique généré, comme l'indiquent les Équations 1-7 et 1-8 (Lapakko, 1994).

Équation 1.7 $\mathbf{2\,CaCO_3 + H_2SO_4 <=> 2\,Ca^{2+} + 2\,HCO_3^{-} + SO_4^{2-}}$

Équation 1.8 $\mathbf{CaMg(CO_3)_2 + H_2SO_4 <=> Ca^{2+} + mg^{2+} + 2\,HCO_3^{-} + SO_4^{2-}}$

Dans le cadre de ce projet de doctorat, les drainages miniers sur lesquels le traitement *in-situ* est réalisé, ont un pH compris entre 6 et 7. Par conséquent, les effluents miniers étudiés au cours de ce projet de doctorat appartiennent à la catégorie des DNC.

1.1.2 Généralités sur les contaminants

Les métaux lourds sont des contaminants préoccupants, car contrairement aux polluants organiques, ils ne se dégradent pas avec le temps. La contamination des sols et des eaux de surface ou souterraines par les métaux est une problématique majeure rencontrée par les pays industrialisés où de très nombreux sites sont contaminés (Petruzzelli *et al.,* 2004). Certains métaux lourds peuvent être retrouvés dans l'eau, l'air et le sol dans des concentrations variant d'un site à un autre. Ces métaux ne sont pas tous nécessaires à la vie et peuvent perturber le cours normal des processus métaboliques, même à l'état de traces. Dans la nature, les métaux existent sous différentes formes (Stumm et Morgan, 1996). La spéciation et la solubilité de chaque élément métallique jouent un rôle important sur sa toxicité pour la faune et la flore ou pour les êtres humains ainsi que sa capacité de dispersion dans l'environnement (Bliefert et Perraud, 2001). La toxicité aiguë ou chronique d'un élément métallique dépend de plusieurs facteurs tels que sa spéciation, sa concentration, le mode d'absorption, le type, l'âge et l'état de l'organisme exposé.

Parmi les métaux retrouvés dans les effluents miniers neutres produits dans le district minier Keno Hill, le zinc (Zn), le cadmium (Cd) et l'arsenic (As) peuvent être présent en quantités élevées. Les principales caractéristiques physico-chimiques de ces métaux sont résumées au Tableau 1-1.

Tableau 1-1 Généralités sur les principaux métaux retrouvés dans le drainage minier neutre étudié

Paramètres	**Zn**	**Cd**	**As**
Masse atomique (g.moL^{-1})	65,4	112	74,9
Masse volumique (g.cm^{-3})	7,1	8,6	5,7
Température de fusion (^{o}C)	419	321	817 (28 bar)
Température d'ébullition (^{o}C)	907	765	613
Minerai d'origine	Sphalérite (ZnS)	Scories du Zn	Roches volcaniques

1.1.2.1 Arsenic

L'arsenic, de symbole chimique As et de numéro atomique 33, est un métalloïde. Par souci de simplification, le terme métal sera utilisé dans ce manuscrit. De masse atomique 74,9 $g.moL^{-1}$, l'As est le $20^{\text{ième}}$ élément chimique le plus présent sur Terre. Parmi les degrés d'oxydation existants de l'As, il est majoritairement présent dans l'environnement sous forme d'arsénite (+III) et d'arséniate (+V). L'arséniate (+V) est moins mobile et moins toxique que l'arsénite (+III) (Kumaresan et Riyazuddin, 2001).

L'arsenic est répertorié comme étant une substance dangereuse pour la santé (ATSDR, 2007) et potentiellement cancérigène (USEPA, 2012). En effet, son inhalation peut causer des cancers des poumons et son ingestion peut être à l'origine de cancers du foie, de la vessie, de la peau ou de la langue (Cheng *et al.,* 2009; Henke 2009; Ng *et al.,* 2003). Des effets non cancérigènes tels que des maladies cardiovasculaires ou cérébrovasculaires, du diabète (Chen *et al.,* 2007; Henke, 2009), des problèmes de la peau (hyperpigmentation ou kératoses), des lésions du système nerveux ou du système immunitaire ont également été répertoriés (Duker *et al.,* 2005; Coudert, 2013).

1.1.2.2 Cadmium

Le cadmium est un élément chimique de symbole Cd et de numéro atomique 48. Le cadmium est peu présent dans la nature. Cependant, il est possible de le trouver dans certains minerais, tels que la greenockite (CdS) et l'otavite ($CdCO_3$) et comme impuretés dans certains sulfures. L'exploitation de ces minerais ne constitue pas une source économique importante car ils sont très rares dans la nature. La production commerciale de cadmium provient principalement de l'exploitation du zinc, où le cadmium est un sous-produit du traitement des concentrés de zinc.

Selon la Commission de santé sécurité au travail (CSST), l'absorption du cadmium ou de ses composés dans l'organisme se fait principalement par voie respiratoire suite à l'inhalation de fumées ou de poussières contaminées en Cd. Étant un cation bivalent, le cadmium se substitue au calcium dans le cristal osseux et en modifie les propriétés mécaniques. Le cadmium et ses composés ne sont pas métabolisés dans l'organisme. L'ion cadmium (+II) se lie à des groupements anioniques (particulièrement aux groupes sulfhydriques) présents dans les protéines (entre autres l'albumine et la métallothionéine) et dans d'autres molécules. La métallothionéine peut lier jusqu'à sept atomes de cadmium. Chez les êtres humains, il provoque notamment des problèmes rénaux et l'augmentation de la tension (CSST, 2015a).

1.1.2.3 Zinc

Le zinc est un élément chimique de symbole Zn et de numéro atomique 30. Son état d'oxydation est +II. Dans la nature, il se trouve sous la forme d'un cation : Zn^{2+}. C'est le 24ième élément le plus abondant dans l'écorce terrestre. Le principal minerai dans lequel le zinc est présent est la sphalérite, un sulfure de zinc (ZnS) (Scott et Barnes, 1971).

Le zinc est un oligo-élément indispensable pour les êtres humains. Cependant, il s'agit également de l'un des éléments traces métalliques qui représente un contaminant pour l'environnement au-delà des concentrations qui le rendent écotoxique. Il est notamment plus mobile et bio-disponible en milieu acide qu'en milieu basique. Le zinc est considéré comme étant relativement non toxique, en particulier si l'absorption est effectuée par voie orale. Cependant, les manifestations de symptômes de toxicité tels que des nausées, des vomissements, des douleurs épigastriques, une léthargie ou une fatigue extrême se produiront lors d'exposition à des concentrations extrêmement élevées en zinc (CSST, 2015b). Les organismes aquatiques, en particulier les poissons, sont particulièrement sensibles au zinc (Skidmore, 1964).

1.1.3 Règlementation Yukonnaise

Au niveau fédéral, la loi sur les pêches est à l'origine du Règlement sur les Effluents des Mines de Métaux (DORS/2002-222) qui définit les normes de rejet maximales imposées à l'ensemble des mines au Canada. Au niveau territorial, la Loi sur les Eaux du Territoire du Yukon (L.C. 1992, ch. 40) régit la délivrance des licences en lien avec l'utilisation de l'eau et/ou le dépôt de déchets dans l'eau. Ces licences sont délivrées pour un grand nombre d'activités telles que l'exploitation minière, l'utilisation d'eau municipale, l'utilisation de l'eau comme source d'énergie, dans le domaine agricole, industriel et récréatif. En vertu de la Loi sur les eaux, des licences d'exploitation spécifiques sont délivrées pour chaque exploitation minière et des normes de rejet spécifiques sont imposées en fonction de l’activité minière et du milieu récepteur. Il n’existe donc pas de normes applicables à l’ensemble du territoire du Yukon.

Les travaux menés dans le cadre de ce doctorat ont été conduits sur le site minier de Silver King mine site, qui fait partie du Keno Hill Silver district. Le site minier de Silver King est très proche du site minier Bellekeno contrôlée par la licence d’exploitation QZ12-057 délivrée le 30 Janvier 2013. C’est donc cette licence d’exploitation qui sera utilisée pour ces travaux de recherche. Les teneurs maximales en métaux dans les effluents rejetés dans le milieu récepteur selon le règlement fédéral et selon la licence territoriale applicables au site de Keno Hill sont présentées au Tableau 1-2.

Tableau 1-2 Normes de rejet des métaux lourds imposées à Bellekeno mine site

Métaux	**Concentration maximale dans un échantillon ($mg.L^{-1}$)**
Arsenic	0,5
Cadmium	0,05
Plomb	0,2
Zinc	0,5
Cuivre	0,1

1.1.4 Traitement des drainages miniers

La production de DMA et DNC amène les mines qui les produisent à mettre en place des systèmes de traitement. À l’heure actuelle, la grande majorité des systèmes de traitement mis en place sont des systèmes actifs, souvent coûteux et générant des boues dont la disposition s’avère problématique, surtout en climat nordique. La méthode de traitement actif conventionnelle consiste à ajouter aux DMA, un agent alcalin tel que de la chaux, du carbonate de calcium, du carbonate de sodium, de l'hydroxyde de sodium ou de l'oxyde de magnésium (Ritcey, 1989 ; Johnson et Hallberg, 2005). L’ajout d’un agent alcalin élève le pH de l’effluent minier, accélère la vitesse d'oxydation chimique du fer ferreux (une aération et/ou addition d'un oxydant tel que le peroxyde d'hydrogène peuvent être requis), ce qui amène un grand nombre de métaux présents en solution à précipiter sous forme d'hydroxydes et/ou de carbonates

(Johnson et Hallberg, 2005). Des procédés de traitement actif faisant appel à des bioréacteurs sulfidogéniques ont été développés au cours de ces dernières années.

Les systèmes de traitement passif, minimisant la maintenance ainsi que les coûts de traitement et contrôlant les réactions chimiques et biologiques épuratoires pour l'eau qui ont lieu naturellement dans l'environnement, représentent une solution d'avenir. Parmi les procédés passifs, nous pouvons citer les drains calcaires oxiques et anoxiques (traitement chimiques) ainsi que les traitements biologiques faisant appel aux marais aérobies, aux biofiltres passifs sulfato-réducteurs (BPSR) ainsi que les bioréacteurs avec oxydation du fer. La Figure 1-1 présente les différents types de procédés actifs et passifs pouvant être appliqués au traitement des DMA ou DNC. Cependant, dans le cadre de ce projet, nous nous intéresserons uniquement aux méthodes de traitement passif biologiques applicables aux DMA et DNC faisant appel aux bactéries sulfato-réductrices.

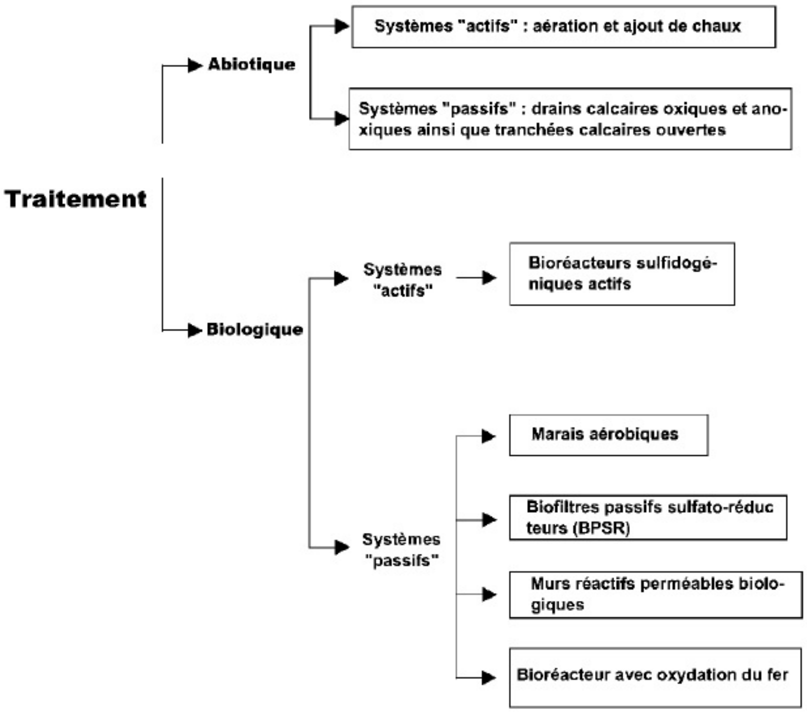

Figure 1-1 Systèmes de traitement des DMA (modifiée et traduite d'après Johnson et Hallberg, 2005; Neculita 2016)

1.1.4.1 Bioréacteurs passifs sulfato-réducteurs

Les bioréacteurs passifs sulfato-réducteurs (BPSR) représentent un système de traitement passif applicable aux DMA et DNC car ces effluents miniers ont potentiellement des concentrations élevées en sulfates dues à l'oxydation des roches sulfurées et qui sont nécessaires au développement de la microflore bactérienne. De nombreuses recherches ont été réalisées sur le développement et l'optimisation de systèmes d'assainissement biologique basés sur la réduction des sulfates présents dans les effluents miniers en sulfures, sous l'action des BSR. Ce type de procédé entraîne la consommation des sulfates tout en produisant des sulfures, augmentant ainsi le pH et réduisant les concentrations en métaux lourds par précipitation sous formes de sulfures métalliques (Egiebor et Oni, 2007; Hulshof *et al.,* 2003; Jin *et al.,* 2008; Neculita, 2008; Ziemkiewicz *et al.,* 2003).

Les Équations 1-9 et 1-10 présentent le système de fonctionnement des BSR dans un BPSR dans lequels les métaux en solution sont précipités sous forme de sulfures métalliques. Les performances de ces procédés dépendent de trois paramètres majeurs : le temps de rétention hydraulique, le mélange de BSR/substrat et les concentrations en métaux présents dans les DMA (Neculita *et al.,* 2008a).

Équation 1.9 $\mathbf{SO_4^{2-} + 2\ C\ (organique) + 2\ H_2O + BSR = H_2S_{(aq)} + 2\ HCO_3^-}$

Équation 1.10 $\mathbf{M^{2+} + H_2S = MS_{(s)} + 2\ H^+}$

En 2008, au site minier de Keno Hill Silver district (Yukon), un bioréacteur pilote à flux horizontal a été construit sur la sortie de l'adit Galkeno 900 et a été mis en opération pendant une année (Alexco Resource Corp. 2012). La fosse était composée de 3 m de roches et d'une couche de sol de 1,2 m pour prévenir la formation de glace dans le bioréacteur. Le bioréacteur a été rempli avec de l'eau non traitée provenant de l'adit. Les sources de carbone utilisées étaient le saccharose, le méthanol, des poudres de lait qui ont été ajoutées pour stimuler la croissance des BSR. Avec un temps de résidence de 21 jours (débit = 0,5 L.s^{-1}), puis optimisé à 10,5 jours (débit = 1 L s^{-1}), un abattement de 99,8% et de 80% a été observé pour le zinc et l'arsenic, respectivement. Les concentrations en Cd après traitement étaient sous les limites de détection.

1.1.4.2 Bactéries sulfato-réductrices

Les bactéries sulfato-réductrices représentent un groupe phylogénétiquement et physiologiquement diversifié qui joue un rôle important dans les milieux anaérobies (Daly *et al.,* 2000). Les BSR sont caractérisées par leur capacité à utiliser les sulfates comme accepteurs finaux d'électrons pour leur respiration dans des conditions anaérobies (Hines *et al.,* 1999). Malgré le fait que les BSR soient considérées comme des bactéries anaérobies, elles ont été détectées dans des environnements « aérotolérants » et même dans des conditions aérobies (Hines *et al.,* 1999; Ito *et al.,* 2002; Okabe *et al.,* 1999). Ces bactéries sont généralement organotrophes, elles oxydent les composés organiques de faibles poids moléculaires et réduisent, simultanément, les sulfates en sulfures (Harerimana *et al.,* 2010; Lens *et al.,* 1995). Leur métabolisme peut être schématisé par la réaction décrite à l'Équation 1-11 (Harerimana *et al.,* 2010; Neculita *et al.,* 2008a). L'Équation 1-13 résulte de la dégradation de la matière organique présentée aux Équations 1-11 et 1-12.

Équation 1.11 $CH_2O + 2\ H_2O <=> HCO_3^- + 5\ H^+ + 4\ e^-$

Équation 1.12 $SO_4^{2-} + 10\ H^+ + 8\ e^- <=> H_2S + 4\ H_2O$

Équation 1.13 $2\ CH_2O + SO_4^{2-} <=> H_2S + 2\ HCO_3^-$

Dans un BPSR, la réduction des sulfates par les BSR est le bioprocédé le plus important. Une bonne gestion des conditions physico-chimiques est donc essentielle à la croissance et à l'efficacité de ce type de bactéries. D'autres mécanismes tels que la précipitation, co-précitation ou encore la sorption peuvent être à l'origine de l'enlèvement des contaminants présents dans les DNC.

1.1.4.2.1 Influence du pH sur le développement des BSR

De nombreuses études indiquent que pour favoriser une croissance et une efficacité microbienne optimale, des gammes de pH précises doivent être respectées en fonction des bactéries sulfato-réductrices spécifiques.

Dans leur étude, Reis *et al.* (2004) ont incubé des cultures de BSR à différentes valeurs de pH et ont obtenu une croissance optimale des BSR à un pH de 6,8. Rees *et al.* (1995), quant à eux, ont mis en évidence que la bactérie *Desulfacinum infernurn* a une phase de croissance optimale pour des pH allant de 6,8 à 7,2. Rozanova *et al.* (2001) ont mis en évidence que la gamme optimale pour le développement des bactéries *Desulfacinum subterraneum* est comprise entre *7,2* et 7,4.

D'après de nombreuses études réalisées sur le développement des BSR, la gamme de pH optimum de croissance des BSR est comprise entre 6,8 et 7,5 (Rees *et al.,* 1995; Rozanova *et al.,* 2001; Sievert et Kuever, 2000; Wang *et al.,* 2013). Néanmoins, une tolérance des BSR aux milieux acides a été observée par Neculita *et al.* (2008a) lors de leur étude menée sur le traitement des DMA à l'aide de colonnes sulfato-réductrices à flux gravitaire. Fortin *et al.* (2000), quant à eux, ont même constaté la présence de BSR indigènes dans des DMA ayant un pH de 2. Avec un DMA ayant un pH de 5,5 - 5,6, des taux d'enlèvement de 81,9% pour le fer, 99,4% pour le nickel, 99,9% pour le zinc ont été observés sous l'action de BSR. Wang *et al.* (2013) ont également étudié l'efficacité de BSR à pH de 5, 7 et 9. Une fois encore, le pH de croissance optimale est de 7 et une croissance des BSR plus lente a été remarquée à pH 5. Cependant, de bons résultats d'enlèvements (80%) de l'antimoine (Sb) présent dans un drainage minier ont été obtenus à pH = 5.

1.1.4.2.2 Influence de la température sur le développement des BSR

Les températures optimales de croissance et d'efficacité métabolique varient selon les espèces de BSR observés. Les BSR peuvent tolérer une large gamme de température variant de 0 à 100°C, dépendamment si elles sont psychrophiles, mésophiles, thermophiles ou hyper thermophiles (Holland et Turekian, 2010). La plupart des espèces de BSR qui ont été isolées et décrites à ce jour sont mésophiles. Le représentant le plus important connu de cette catégorie est *Desulfovibrio* (Widdel et Bak, 1992). Il existe aussi des BSR thermophiles, telles que *Desulfacinum infernurn, Desulfacinum subterraneum ou Desulfacinum hydrothermale* dont les températures de croissance optimales sont comprise entre 37 et 65°C (Rees *et al.,* 1995; Rozanova *et al.,* 2001; Sievert et Kuever, 2000). Dans le cadre de cette étude, les basses températures impliquent une problématique importante. En effet, les températures basses, comme celles rencontrées au Yukon (température de l'eau inférieure à 7°C), pourraient fortement affecter et retarder la production de sulfures gazeux et aqueux (Huan *et al.,* 2013). En effet, à basse température, il a été démontré que l'activité métabolique bactérienne nécessite des adaptations spécifiques (Cavicchioli, 2006; Robador *et al.,* 2009). La plus importante de ces adaptations est la capacité des bactéries à synthétiser, à basses températures, des enzymes ayant des activités catalytiques élevées (Feller et Gerday, 2003; Robador *et al.,* 2009).

Même si les BSR sont généralement mésophiles et ont une température de croissance optimale aux alentours de 25°C, cela ne signifie pas que leur croissance ou leur efficacité est nulle à des températures plus froides. Les bactéries actives en dessous de 0°C mais avec une température optimale de plus de 20°C sont appelées « psychrophiles modérées » ou « mésophiles adaptées au froid » (Sagemann *et al.,* 1998). Sagemann *et al.* (1998) ont comparé le taux de réduction des sulfates présents dans des sédiments issus de régions polaires et tempérées. Bien que de meilleurs taux de réduction aient été obtenus à 25°C, ces auteurs observent que les taux de réduction des sulfates dans les régions polaires semblent être comparables à ceux obtenus pour les sédiments de climats plus tempérés. La communauté microbienne réductrice de sulfates est capable de bien s'adapter aux températures froides et ce, même en dessous de 0°C. Selon ces auteurs, il est plus probable que ce soit la disponibilité du substrat, et non pas la température, qui limite la réduction des sulfates dans les sédiments polaires. Gould *et al.* (2012) ont comparé les performances de bioréacteurs en colonnes destinés à traiter des drainages miniers à différentes températures. Bien que des différences de performances aient été observées lors de l'opération de bioréacteurs à 20°C et 4°C, notamment sur l'abattement du zinc (99% à 20°C et 60% à 4°C), ces auteurs concluent que le fonctionnement à basse température d'un système de traitement anaérobie peut être amélioré par l'addition d'une source de carbone facilement métabolisable telle que le glucose ou le lactate. L'importance de la source d'énergie utilisée par les BSR dans un BPSR est donc non négligeable.

1.1.4.2.3 Influence des sources de carbone sur le développement des BSR

Les sources de carbone utilisées pour une croissance rapide des BSR peuvent être des composés organiques simples comme le lactate de calcium, l'éthanol, le saccharose, le glucose et le lactosérum (Luptakova et Macingova, 2012). Cependant, leurs prix élevés et leur trop grande biodégradabilité favorisent l'utilisation de sources de carbone alternatives comme les composés organiques complexes contenus dans les déchets organiques (fumier, compost) et cellulosiques (foin, paille). Selon Sheoran *et al.* (2010), il existe deux principales sources de carbone organique : les substrats organiques directs/simples et les substrats indirectes/complexes. Les substrats organiques directs/simples sont ceux qui ne nécessitent pas de décomposition par d'autres micro-organismes pour fournir des nutriments aux BSR. Ces sources de carbone directes/simples comprennent des alcools (par exemple, le methanol et l'éthanol), des acides organiques (par exemple l'acétate, le lactate, le formate et le pyruvate) et des sucres (par exemple le saccharose). Ces formes simples de substrats organiques sont généralement préférées par les BSR car elles sont plus facilement accessibles et n'ont pas besoin d'être préalablement décomposées (Knee *et al.,* 2001). Les sources de carbone organique indirectes/complexes sont celles qui nécessitent des communautés microbiennes plus complexes pour dégrader la matière organique et soutenir la croissance spécifique des BSR.

La nature des sources de carbone influence de façon significative l'activité et les performances des SRB à réduire les sulfates en sulfures et donc le succès d'un BPSR. L'efficacité du processus de traitement dépend du substrat organique choisi pour fournir la source de carbone au BSR. Ainsi, les teneurs en carbone (COT, COD, CIT) et azote total Kjeldahl (NTK), de même que les teneurs en substances facilement disponibles (SFD) doivent être prises en compte (Zagury *et al.,* 2006). Une caractérisation chimique peut être utilisée pour aider à prédire l'efficacité d'une source de carbone et la sélection de donneurs d'électrons organiques pour une utilisation potentielle dans le traitement de DMA (Coetser *et al.,* 2006).

Waybrant *et al.,* (1998, 2002) ont démontré qu'une meilleure efficacité peut être obtenue lorsqu'un mélange de plusieurs sources de carbone est utilisé. Par ailleurs, l'utilisation de sources de carbone complexes, et plus ou moins facilement biodégradables, peut ralentir leurs dégradations et la production subséquente de carbone assimilable par les BSR, et ainsi soutenir les BSR sur de plus longues périodes (Drury, 2006). La disponibilité des sources de carbone dans le nord canadien introduit une problématique économique, c'est pourquoi de nombreuses sources de carbone présentes dans le grand nord canadien doivent être testées.

1.1.4.2.4 Effet du potentiel d'oxydo-réduction

Le POR optimal pour permettre aux BSR de réduire les sulfates en sulfures se situe entre -100 et -300 mV (Gloyna 1972; Gibert *et al.,* 2002; Harerimana *et al.,* 2010). L'étude réalisée par Reddy et DeLaune (2008) montre clairement que plus le POR est faible, plus il y a réduction de sulfates et donc production de sulfures. Il est donc primordial d'avoir des conditions anaérobies afin que la réduction des sulfates se produise. D'ailleurs, une relation a été établie par Delgado *et al.* (1999) et Huan (2013) mettant en jeu l'influence des valeurs de POR sur la production de sulfures (Équation 1-14).

Équation 1.14 $\mathbf{POR = A + B \log ([H_2S]_{aq} / [SO_4^{2-}])}$

Où A et B sont des constantes empiriques.

1.2 Hypothèse de recherche, originalité et objectifs du projet

1.2.1 Hypothèse de recherche

Les bioréacteurs passifs sulfato-réducteurs (BPSR) représentent un système de traitement passif applicable aux DMA et DNC car ces effluents miniers ont généralement des concentrations élevées en sulfates dues à l'oxydation des roches sulfurées et qui sont nécessaires au développement de la microflore bactérienne. De nombreuses recherches ont été réalisées sur le développement et l'optimisation de systèmes d'assainissement biologique basés sur la réduction des sulfates présents dans les effluents miniers en sulfures, sous l'action des BSR. Ce type de procédé entraîne la consommation des sulfates tout en produisant des sulfures, augmentant ainsi le pH et réduisant les concentrations en métaux lourds par précipitation sous formes de sulfures métalliques Cependant, la température influençant la croissance et l'efficacité des BSR (Cavicchioli, 2006; Robador *et al.,* 2009; Sagemann *et al.,* 1998), le choix d'une source de carbone appropriée devrait être identifiée afin de permettre aux BSR de se développer à des températures froides et par conséquent faciliter l'abattement du Zn et du Cd par précipitation sous forme de sulfures. L'hypothèse de recherche de ce projet de recherche est qu'il est possible de mettre en place en climat froid un système de traitement semi passif efficace permettant de diminuer les concentrations en Zn et Cd mesurées dans des drainages miniers et reposant sur l'utilisation des BSR. Ce type de traitement pourra alors etre utilisé pour traiter les effluents souterrains d'une mine du Yukon.

1.2.2 Originalité du projet de recherche

Au cours de ces dernières années, de nombreuses études ont été menées à travers le monde sur l'utilisation des BSR en climat chaud ou tempéré pour le traitement d'effluents aux prises avec une contamination métallique. Cependant, peu d'études ont été effectuées sur le comportement et les performances de ces BSR pour le traitement des effluents miniers en climat froid (effluent à 5°C). Il est pourtant reconnu que les basses températures affectent la croissance et l'activité bactérienne (Cavicchioli, 2006; Robador *et al.,* 2009; Sagemann *et al.,* 1998).

L’originalité de ce projet de doctorat réside dans le fait d’adapter les bioréacteurs passifs à sulfato-réduction afin de les rendre efficaces en climat nordique et de favoriser leur application aux problématiques spécifiques d’effluents miniers neutres contaminés en métaux lourds, répondant aux exigences techniques imposées en climat nordique et aux réglementations yukonnaises strictes. Le développement d’un procédé alternatif aux traitements actifs est devenu nécessaire, notamment pour permettre la gestion adaptée de sites orphelins et de mines en fermeture en régions éloignées. La possibilité d’utiliser un traitement passif *in-situ*, et sous-terrain, est certainement attrayante pour l’industrie locale et plus généralement pour le nord canadien.

1.2.3 Objectifs et projet de recherche

L’objectif visé dans le cadre de cette thèse est de développer des connaissances théoriques et pratiques permettant de mettre au point un système de traitement passif *in-situ* applicable aux drainages miniers neutres contaminés par des métaux, en climat nordique, faisant appel aux bactéries sulfato-réductrices.

Les objectifs spécifiques de ce projet de doctorat sont :

- Caractérisation chimique des roches échantillonnées sur le site minier (fusion alcaline, microscopie à balayage électronique (MEB), diffractométrie de rayons X (DRX), potentiel d’acidification et de neutralisation par le test de Sobek);
- Caractérisation physico-chimique des effluents miniers (pH, potentiel d’oxydo-réduction (POR), teneurs en sulfates et en métaux);
- Caractérisation génomique des bactéries indigènes échantillonnées dans les bioréacteurs pilotes, ainsi que dans l’effluent minier utilisé lors des essais en laboratoire et lors de l’essai pilote;
- Mise en place d’un procédé de traitement des drainages miniers neutres en conditions nordiques permettant de comprendre les mécanismes à l’échelle laboratoire;
- Étude de l’influence de différentes sources de carbone sur le développement microbien et détermination des conditions optimales;
- Réalisation d’essais à l’échelle pilote sur le site minier pour une période de 14 mois afin de confirmer les résultats obtenus à l’échelle laboratoire;

Ce projet de recherche vise donc à développer un procédé de traitement des effluents miniers viable économiquement, durable et applicable à des sites miniers éloignés dans le nord canadien, tout en utilisant la flore microbienne naturellement présente et en favorisant leur développement grâce à des sources et des concentrations de carbone adaptées.

1.3 Démarche méthodologique

1.3.1 Localisation

Ces travaux de doctorat ont été menés en partenariat avec l'entreprise minière Alexco Ressource Corp. propriétaire du site minier de Keno Hill Silver district (Figure 1-2). Une des mines de ce site : le Silver King mine site, est aux prises avec une problématique d'eaux souterraines contaminées par du Zn et du Cd. Les roches générant cette contamination et l'effluent minier issu du Silver King mine site ont servi durant les essais laboratoires et pilotes de ces travaux de doctorat afin de répliquer les conditions souterraines du site de Silver King (réservoir souterrain à travers lequel le drainage minier s'écoule).

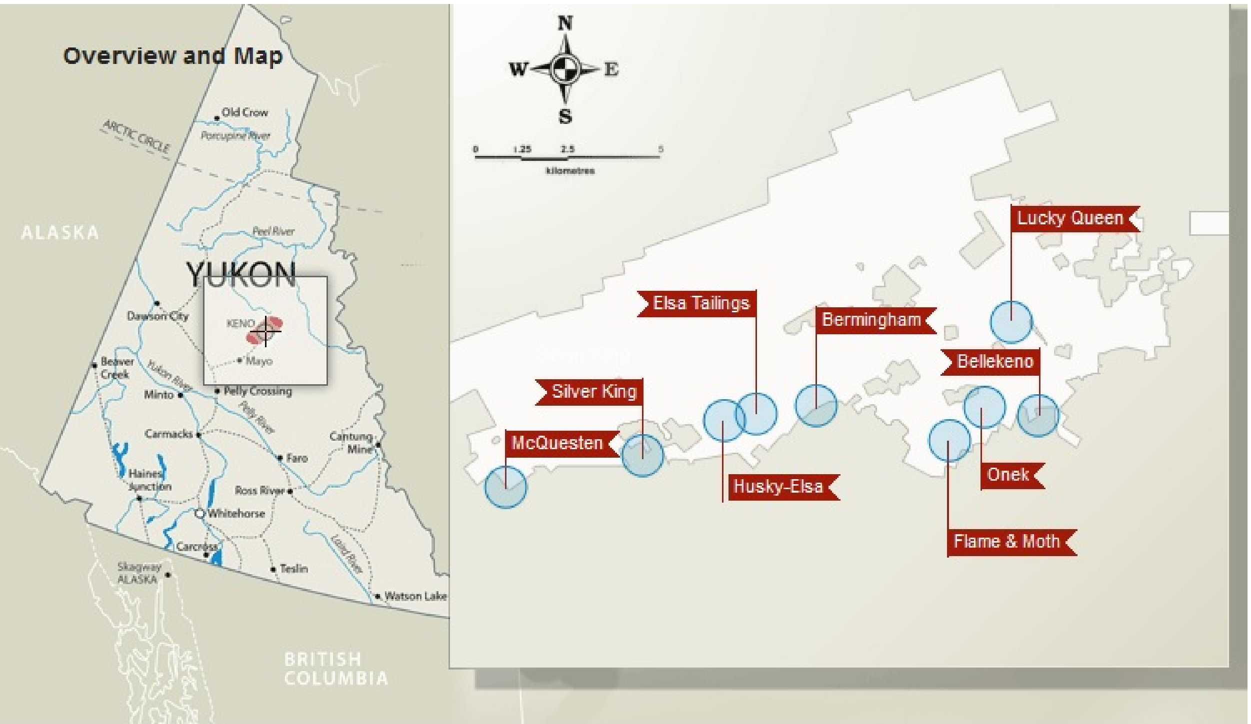

Figure 1-2 Carte du site minier de Keno Hill Silver district situé dans le territoire du Yukon

1.3.2 Caractérisation

Des effluents miniers du site de Silver King adit ont été échantillonnés mensuellement par Alexco de février 2007 à janvier 2013 et ont été analysés par le laboratoire AGAT situé à Vancouver, BC. La caractérisation des effluents prend en considération le pH, les matières en suspension, la conductivité électrique, la température, l'oxygène dissous, le POR, la concentration en sulfates et la concentration d'environ 35 métaux lourds. Cet effluent minier a été utilisé dans le cadre de ce projet pour évaluer les performances d'un traitement passif sur l'enlèvement du Cd et du Zn.

Deux types de roches (Roches #1 et Roches #2) ont été utilisées pour les expériences de traitement passif réalisées en laboratoire puis à l'échelle pilote (Figure 1-3). Ces roches proviennent du site minier de Silver King Mine site situé dans le Keno Hill Silver District (Whitehorse, Yukon). Deux tas de roches stériles ont été échantillonnés à proximité des sorties des galeries qui ont été minées dans les années 1950.

Figure 1-3 **Échantillonnage des roches 1 (a.) et 2 (b.) utilisées au cours de ce projet de recherche**

Une caractérisation physico-chimique de ces différents types de roches a été réalisée (granulométrie, granulochimie, perte au feu, fusion alcaline, microscope à balayage électronique (MEB), diffractométrie à rayons X (DRX), teneur en S, etc.) afin de déterminer les minéraux présents et la composition chimique de chaque roche. Le potentiel de génération d'acide (détermination du potentiel net de neutralisation et du potentiel d'acidité maximale) de chaque type de roches et du mélange utilisé a également été déterminé selon les méthodes définies par Lawrence *et al.* (1997a, b) et Sobek *et al.* (1978).

1.3.3 Essais en mode batch

1.3.3.1 Protocole expérimental

Afin d'évaluer l'impact des températures froides sur l'activité des BSR ainsi que sur l'abattement des concentrations en métaux (Cd et Zn) lors du traitement passif d'un drainage minier, plusieurs expériences ont été conduites en laboratoire dans les laboratoires du Yukon Research Centre et de l'INRS. Ces expériences ont été conduites en utilisant l'effluent provenant du site minier Keno Hill Silver District (Yukon) ainsi que des roches et de l'inoculum prélevés directement sur le site minier. La température des effluents miniers souterrains soumis au traitement est comprise entre 1°C et 6°C et ce, tout au long de l'année (selon des discussions avec Alexco). Par conséquent, les expériences menées en laboratoire ont été réalisées dans des chambres froides répliquant ces températures. Des essais de traitement passif ont été conduits pendant 3 mois dans les laboratoires du Yukon Research Centre afin de fournir une meilleure compréhension des procédés impliqués dans ce traitement *in-situ.* Une seconde série d'essais a été conduite pendant 90 jours dans les laboratoires de l'INRS afin d'évaluer la présence de BSR indigènes sur le site de Silver King mine site (YT) et de déterminer les conditions les plus favorables au développement des BSR et par conséquent à l'enlèvement du Cd et du Zn présents dans les effluents miniers. Une troisième et une quatrième série d'essais ont été menées, pendant respectivement 120 et 160 jours, dans les laboratoires de l'INRS pour étudier différentes sources de carbone et leur impact sur l'efficacité des BSR à réduire les sulfates en sulfures.

Pour chaque série d'essais en laboratoire, les essais ont été réalisés dans des bouteilles en polyéthylène de faible densité (LDPE) d'une capacité de 1 L dans lesquelles 850 mL d'effluent minier, 5 mL d'inoculum prélevé dans le fond d’un ruisseau du site minier, 200 mL de roches prélevées sur le site (100 mL des roches #1 et 100 mL des roches #2) et une source de carbone ont été ajoutées (Figure 1-4). De l’eau déminéralisée a ensuite été ajoutée pour compléter le niveau des bouteilles afin de s’assurer que le milieu soit anoxique.

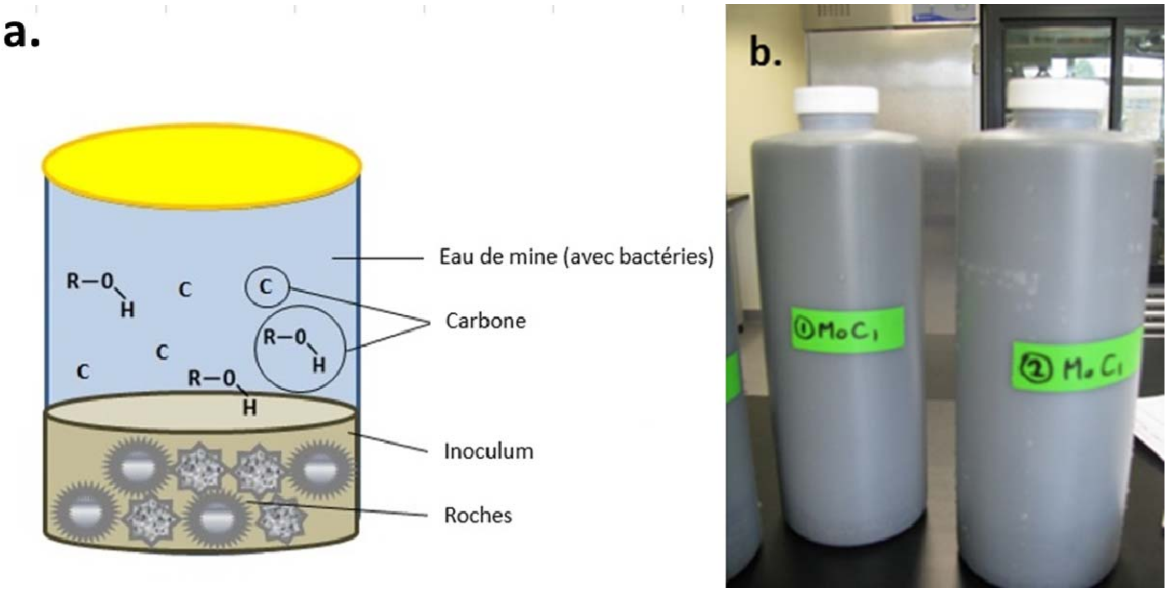

Figure 1-4 Bioréacteurs utilisés pour les essais en batch

Des prélèvements ont été réalisés tous les 15-20 jours. Les paramètres suivis sont le pH, le potentiel d’oxydoréduction (POR) ainsi que les concentrations en cadmium, en zinc, en sulfates et en carbone organique total (COT).

1.3.3.2 Premiere série d’essais en mode batch : Mise en évidence des BSR et détermination des conditions les plus favorables au développement microbien

Afin de mettre en évidence l’importance de supporter la croissance microbienne avec de la matière organique, des contrôles ont été mis en place sans ajout de matière organique. Le Tableau 1-3 présente les contrôles mis en place lors de la première série d’essais réalisés en mode batch.

Tableau 1-3 Composition des différents contrôles testés

Numéro	Effluent minie (EDM)	Eau déminéralisée	Roches	Inoculum	Air
1. EDM	1000 mL				
2. EDM + eau déminéralisée	850 mL	150 mL			
3. Roches + eau déminéralisée		800 mL	200 mL		
4. Roches + EDM	850 mL		200 mL		
5. EDM + inoculum	995 mL			5 mL	
6. EDM + air	850 mL				150 mL

Les objectifs fixés dans la première série d'expériences mise en place au Yukon étaient de démontrer la présence de BSR indigènes dans l'inoculum utilisé dans cette expérience, de comparer deux sources de carbone (mélasse, méthanol et un mélange de ces sources de carbone) à deux concentrations différentes (77 mg $C.L^{-1}$ et 154 mg $C.L^{-1}$) ajoutées au système de traitement *in-situ*, afin de favoriser les réactions de sulfato-réduction et de déterminer la consommation en carbone par les bactéries. Les quantités et volumes de chaque source ont été calculés pour que chaque bioréacteur contienne 2 moles de carbone pour une mole de sulfates (concentration C1) et 4 moles de carbone pour une mole de sulfates (concentration C2). La première série d'essais a été conduite en triplicata sur 90 jours à 4,5 ℃ et des prélèvements ont été réalisés tous les 15-20 jours. Les sources de carbone utilisées sont la mélasse, le méthanol et un mélange mélasse-méthanol à part égale (Tableau 1-4).

Tableau 1-4 Sources de carbone (Mo : mélasse et Me : méthanol) et différentes concentrations ($C1 = 77\ mg.L^{-1}$ et $C2 = 154\ mg.L^{-1}$) testées dans les bioréacteurs pour le développement microbien

Numéro de l'essai	Nom	Mélasse	Méthanol	Inoculum
1	Mo C1	x		x
2	Me C1		x	x
3	Mo + Me C1	x	x	x
4	Mo C2	x		x
5	Me C2		x	x
6	Mo + Me C2	x	x	x

1.3.3.3 Deuxième série d'essais en mode batch : étude de différentes sources de carbone

Selon le même protocole expérimental, une deuxième série d'essais en mode batch a été mise en place afin d'évaluer l'efficacité de plusieurs sources de carbone (simples et complexes) sur les BSR et leur capacité à réduire les sulfates en sulfures. Lors de cette seconde série d'essais, différentes sources de carbone facilement accessibles au Yukon ont été sélectionnées. Elles incluent des sources de carbone dites « complexes » ou « indirecte » telles que la tourbe, la paille, l'huile de friture, les résidus d'orge de brasserie et la mélasse; et des sources de carbone dites « simples » ou « directes » telles que le méthanol et l'éthylène glycol. Ces matériaux ont été testés afin de déterminer quelle source de carbone, à une concentration donnée, est la plus favorable à la croissance des BSR et à la réduction de sulfates sur une période de 160 jours. Selon la littérature, deux moles de carbone sont nécessaires aux BSR pour réduire une mole de sulfate. Mais afin de s'assurer que le carbone est fourni en excès dans cette série d'essais, il a été décidé de doubler la quantité de carbone fournie dans les bioréacteurs. Par conséquent, 20,7 mmoles de carbone ont été ajoutées dans chaque bioréacteur de 1 L, ce qui correspond à une concentration en carbone de 249 mg L^{-1}. À cette concentration, quatre moles de carbone sont disponibles pour chaque mole de sulfate initialement présente dans l'effluent de drainage minier (497 mg SO_4^{2-} L^{-1}). Selon la source de carbone (liquide ou solide), le carbone pouvait être sous forme solide ou dissoute. La masse de matière nécessaire pour atteindre la concentration souhaitée dans chaque bioréacteur a été calculée sur la base de la teneur en carbone déterminée par analyse CHNS de chaque source de carbone. Chaque source de carbone et chaque contrôle ont été testés en triplicata.

1.3.4 Essais pilotes

Des essais ont été conduits à l'échelle pilote pendant 14 mois sur le site minier de Silver King Mine Site. Ce projet pilote, réalisé *in situ,* visait à fournir des données scientifiques sur l'efficacité des BPSR pour le traitement de l'effluent minier souterrain de Keno Hill Silver Mine Site (YT) en collaboration avec la société minère Alexco Resource Corp. qui est en charge de la fermeture du district minier de Keno Hill. L'un des sites miniers de Keno Hill Silver district, la mine abandonée de Silver King, est confronté à une contamination en métaux lourds dans ses eaux souterraines. En effet, les veines souterraines issues de l'exploitation minière relarguent du Cd et Zn. Afin de traiter cet effluent, Alexco Resource Corp. a voulu mettre en place un traitement passif biochimique *in situ* afin d'atteindre les limites de rejet imposées par la licence d'utilisation de l'eau (QZ12-057 livrée le 30 janvier 2013). Comme la plupart des vieux tunnels de la mine sont inondés, le système est potentiellement suffisamment anaérobie et la concentration initiale en sulfate est suffisante pour supporter la croissance de BSR indigènes. Alexco Resource Corp. a foré un puit de 110 m de profondeur pour pomper l'eau contaminée et a commencé à injecter de la mélasse comme source de carbone dans les tunnels submergés afin de soutenir la croissance bactérienne, en particulier les potentielles BSR indigènes (Figure 1-5).

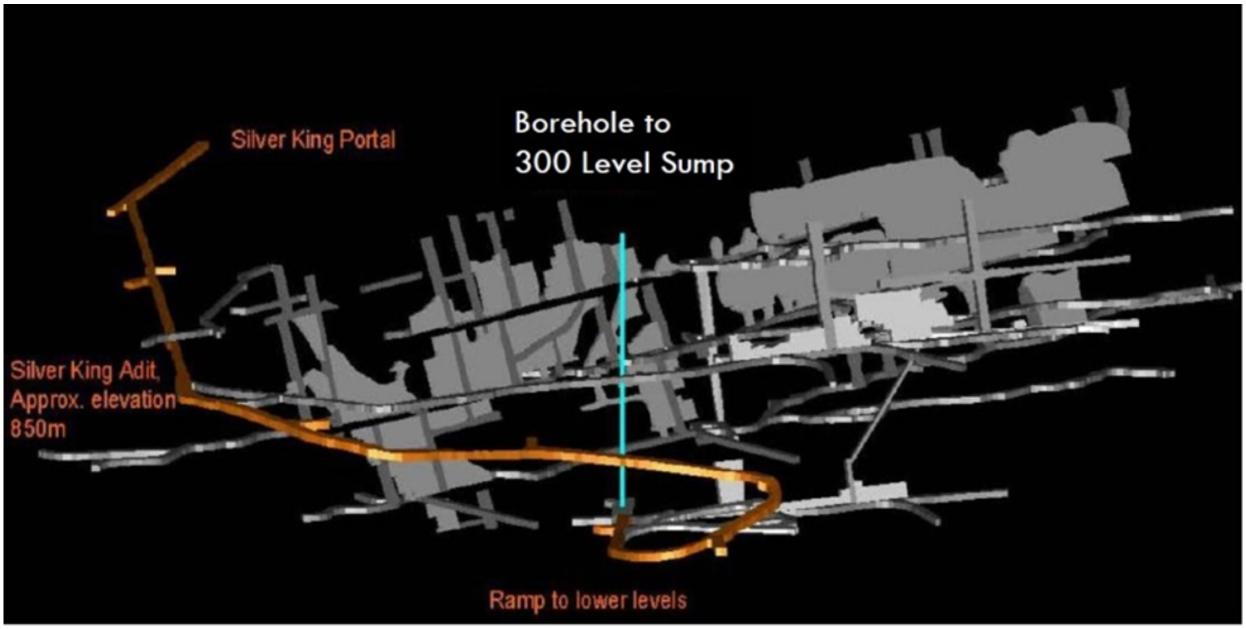

Figure 1-5 **Coupe transversale des tunnels souterrains inondés de la mine de Silver King et son puit de forage**

La présence de BSR indigènes sur le site de la mine Keno Hill a été rapportée par Londry (2013) qui a effectué une évaluation technique des rôles microbiens potentiels dans l'enlèvement des métaux pour le projet de bioremédiation Galkeno 900 mené par Alexco. Par ailleurs, les essais laboratoire menés en amont dans le cadre de ce projet de doctorat avaient aussi rapporté la présence de BSR indigènes dans l'effluent du Site de Silver King. Cette étude pilote visait aussi à fournir une caractérisation génomique complète de la communauté microbienne de l'effluent Silver King mine site et des bioréacteurs pilotes.

Afin que les conditions expérimentales soient les plus représentatives possibles des conditions réelles et afin de simuler le système de traitement *in situ* de la compagnie minière Alexco, deux bioréacteurs en polyéthylène de 55 gallons (200 L) (Figures 1-6 et 1-7) ont été installés sur le site dans un cabanon chauffé afin de maintenir la température des drainages miniers à 4-6°C durant l'hiver et d'éviter que les bioréacteurs ne gèlent (Figure 1-6). Chaque bioréacteur pilote a été rempli avec 40 L de roches stériles (20 L de roches #1 et 20 L de roches #2), ce qui correspond à 20% ($v.v^{-1}$) (Figure 1-7).

Un réservoir en polyéthylène de 1 000 L a été installé dans le cabanon afin de stocker l'effluent minier pompé depuis le puit de forage mis en place par Alexco.

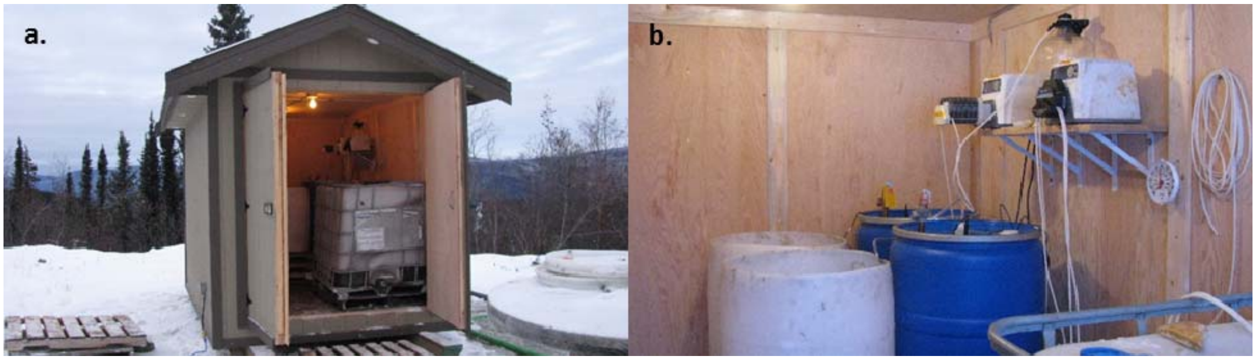

Figure 1-6 **Installation et infrastructures utilisées pour la réalisation des essais à l'échelle pilote**

La source de carbone utilisée pour les essais pilote était une solution de mélasse préparée en laboratoire et injectée à l'aide d'une pompe péristaltique Masterflex dans les bioréacteurs. Afin de respecter les quantités quotidiennes d'injection de carbone, le débit appliqué est de 6 $mL.min^{-1}$, ce qui correspond une fois encore à 2 moles de carbone pour une mole de sulfate. Un minuteur (Cole-Parmer Digital Programmable Plug-In Timer Controller; 115 VAC) déclenchait la pompe quotidiennement pendant 56 minutes au débit calculé. Une pompe péristaltique Masterflex 4-channels permettait le pompage de l'effluent depuis la cuve de 1 000 L contenant le drainage minier vers les bioréacteurs. Conformément aux calculs de temps de rétention hydraulique dans la nappe souterraine contaminée effectués par Alexco, le temps de rétention hydraulique dans les bioréacteurs a été fixé à 2 semaines afin de répliquer les conditions souterraines réelles. Considérant le volume des barils, le débit appliqué était de 9,9 $mL.min^{-1}$. L'alimentation du système s'effectuait de manière ascendante dans les bioréacteurs. L'échantillonnage hebdomadaire d'eau avant et après traitement dans l'unité pilote a permis de suivre le pH, le POR, la température, les concentrations en zinc, en cadmium, en sulfates ainsi que la teneur en COT.

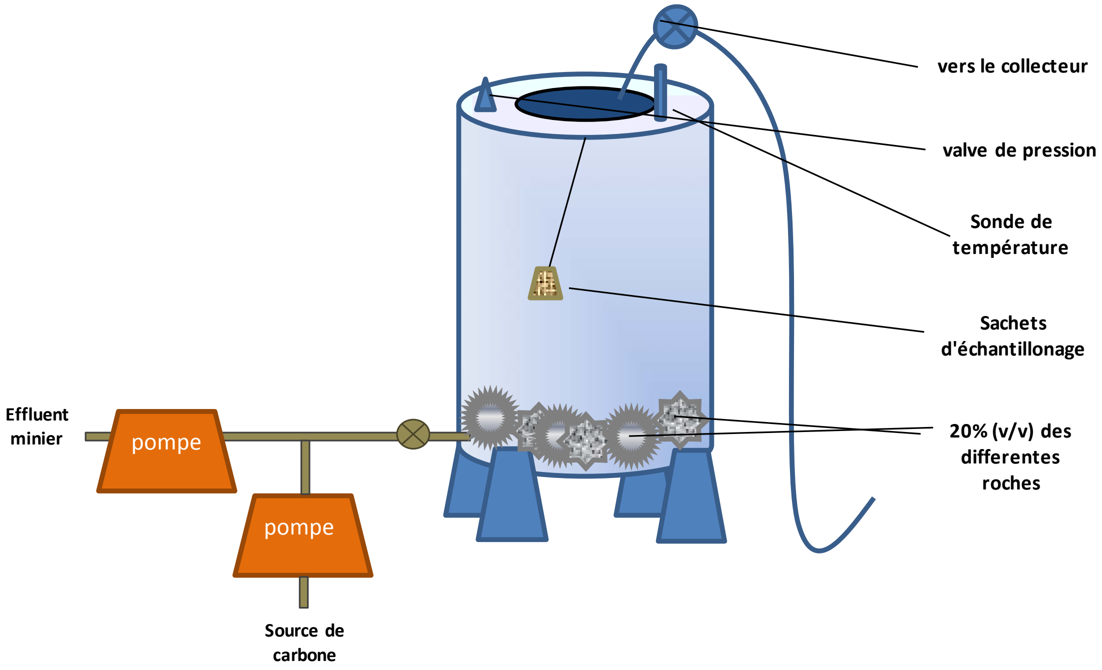

Figure 1-7 **Schéma d'un bioréacteur pilote utilisé sur le site minier de Silver King**

1.3.5 Caractérisation génomique

Un des objectifs de l'étude à l'échelle pilote était de fournir des indications sur les populations bactériennes présentes dans les effluents miniers de Silver King et dans les deux bioréacteurs pilotes.

Afin de collecter des échantillons de population microbienne et de caractériser génétiquement les bactéries présentes, des supports de croissance ont été mis en place dans les deux bioréacteurs pilotes et dans l'effluent minier avant traitement. En effet, 12 sachets d'échantillonnage ont été installés dans chaque bioréacteur et dans une chaudière collectant de l'effluent minier fraichement pompée (avant traitement) afin qu'un biofilm s'y développe et permette l'échantillonnage de bactéries (un sachet d'échantionnage prélevé par mois). Les sachets d'échantillonnage étaient constitués d'une poche en coton très fin qui renferme environ 50 mL des mêmes roches stériles que celles utilisées dans les bioréacteurs. Après échantillonnage, chaque sachet a été congelé dans de l'azote liquide (Figure 1-8) puis conservé dans un congélateur à -8 °C avant d'être envoyé, en mai 2016, à l'Université de Colombie-Britannique (Vancouver) afin une extraction ADN de réaliser une caractérisation des communautés bactériennes par profilage génomique.

a. b.

Figure 1-8 **Échantillonnage des sachets d'échantillonnage pour la caractérisation génétique de la communauté microbienne**

1.3.6 Méthodes d'analyse

Dans les laboratoires du Yukon Research Center, les métaux ont été analysés par absorption atomique avec flamme et four au graphite selon les éléments quantifiés (Perkin Elmer PinAAcle 900T). Cette méthode d'analyse chimique permet de détecter et de quantifier tous les éléments métalliques étudiés de manière simultanée. Dans les laboratoires de l'INRS, les concentrations en métaux ont été déterminées par ICP-AES sur un appareil *Varian* (modèle *Vista AX CCO Simultaneous ICP-AES* - Palo Alto, États-Unis). Des solutions de calibration ont été préparées à l'aide de solutions certifiées (SCP Science, CA) et des solutions « contrôle » (SCP Science Multi éléments, CA) ont été analysées tous les 20 échantillons afin de valider les résultats et de mettre en évidence toute dérive éventuelle de l'appareil.

Dans les laboratoires du Yukon Research Center, les sulfates présents dans l'eau ont été mesurés en utilisant la méthode de référence EPA 9038 (sulfate, turbidimétrique). La limite de détection pour cette méthode d'analyse est de 5 mg.L^{-1}. Dans les laboratoires de l'INRS, les teneurs en sulfates ont été déterminées selon la méthode colorimétrique développée par Bertolacini et Barney (1957). Dans un premier temps, l'échantillon a été mis sous agitation lente avec une résine échangeuse de cations afin d'éliminer les interférents cationiques présents en solution. Le pH de l'échantillon a ensuite été fixé à 4,0 à l'aide de solutions de NaOH ou de HCl. Ensuite, 8 mL d'échantillon, 2 mL de solution buffer (phtalate acide de potassium) et 10 mL d'éthanol (90%) ont été mélangés à 69,4 mg de chloranilate de baryum ($BaC_6Cl_2O_4$) pendant 10-15 min. Une étape de filtration sur un filtre G6 a ensuite été réalisée avant la lecture de la coloration de la solution obtenue à l'aide d'un spectrophotomètre UV-visible à une longueur d'onde de 530 nm. À chaque série d'analyses, une gamme d'étalonnage a été préparée à partir de sulfate de potassium (K_2SO_4) ainsi que des solutions « contrôle » à partir d'autres sources certifiées de sulfates, dans les mêmes conditions que celles utilisées pour les échantillons. La limite de détection pour cette méthode d'analyse est de 10 mg.L^{-1}.

Dans les laboratoires du Yukon Research Center, le Carbone Organique Total (COT) et le carbone inorganique (CI) ont été mesurés en utilisant un Skalar Formacs HT instrument. Des courbes d'étalonnage de 10-100 mg.L^{-1} pour CI et 20-200 mg.L^{-1} pour COT ont été mises en place quotidiennement afin de vérifier l'étalonnage de l'appareil. L'intervalle de confiance étant établi à ± 20%

en bas et ± 10% en haut de la gamme d'étalonnage. Les standards ont été préparés à partir de standards commerciaux achetés auprès de SCP. Dans les laboratoires de l'INRS, les teneurs en COT ont été déterminées à l'aide d'un analyseur Shimadzu par un technicien (TOC-VCPH model, Shimadzu Scientific Instruments, Columbia, MD, USA).

Les mesures de pH ont été réalisées à l'aide d'un multimètre (Fisher Acumet modèle 915) muni d'une électrode double jonction de Cole Parmer avec une cellule de référence Ag/AgCl. Une calibration du pH-mètre a été réalisée à l'aide de solutions tampon certifiées (pH 2,00 – 4,00 – 7,00 et 10,00) lors de chaque série de mesures. Les mesures de POR ont été réalisées à l'aide d'une électrode POR double jonction (59001-77).

La caractérisation génomique a été effectuée dans les laboratoires de UBC, Vancouver (BC). L'ADN a été extrait à partir de biofilms sur les sachets d'échantillonnage bactérien prélevés dans les bioréacteurs et dans l'effluent minier à différents moments (Tableau 4-1). L'extraction d'ADN a été effectuée en utilisant le kit rapide DNA^{TM} spin kit pour sol selon les spécifications du fabricant (MP bio Solon, OH, USA). Une amplification de l'ADN a été obtenue par polymerase chain reaction (PCR) et les analyses bioinformatiques, les étapes de prétraitement et de contrôle de la qualité des séquences ont été effectuées en utilisant USEARCH (v 9.0.2132, 32 bit for Linux).

1.4 Synthèse des résultats obtenus

1.4.1 Caractérisation de l'effluent minier

Le Tableau 1-5 présente les principales caractéristiques physico-chimiques mesurées dans les effluents provenant du site minier de Silver King Mine Site de Février 2007 à Janvier 2013, à savoir le pH, le POR ainsi que les concentrations en sulfates et en métaux lourds (As, Cd, Fe et Zn). En moyenne, ces effluents présentent un pH de 7,3 et des concentrations de 36 ± 11 µg $As.L^{-1}$, 12 ± 8 µg $Cd.L^{-1}$, 19 600 ± 5 160 µg $Fe.L^{-1}$ et 860 ± 197 µg $Zn.L^{-1}$. Il est à noter que la concentration moyenne en zinc dépasse la limite maximale de 500 µg $Zn.L^{-1}$ en vigueur pour la décharge de ces effluents dans le milieu récepteur au site de Bellekeno à Keno Hill (Licence QZ12-057).

Tableau 1-5 Caractérisation de l'effluent échantillonné sur le site de Keno Hill Silver district ainsi que les normes de rejet d'effluent en vigueur sur ce site

Paramètres	Nb échantillons	Valeurs	Normes
[As] ($µg.L^{-1}$)	98	36 ± 11	100
[Cd] ($µg.L^{-1}$)	98	12 ± 8	50
[Fe] ($µg.L^{-1}$)	98	19 600 ± 5 160	n.a.*
[Zn] ($µg.L^{-1}$)	98	860 ± 197	500
pH	98	7,3 ± 0,5	6,5 - 9,5
[SO_4^{2-}] ($mg.L^{-1}$)	68	426 ± 182	n.a.

**n.a : non applicable.*

1.4.2 Caractérisation des roches – stériles miniers

Les résidus de roches ont été échantillonnés à partir de deux piles de stériles du Silver King Mine Site Yukon (YT, Canada). Les deux types de roches ont été mélangés (50/50, $v.v^{-1}$) dans cette étude pour répliquer la géologie des tunnels souterrains générant le drainage minier.

La répartition granulométrique et la composition chimique des différentes roches résiduaires sont présentées dans le Tableau 2-3. Les deux types de roches utilisés ont une distribution granulométrique relativement similaire. Les gros matériaux (> 10 mm) représentent la plus grande fraction du sol, avec

45,4% pour les roches stériles appelés « WR1 » et 40,8% pour roche stériles appelles « WR2 ». Il a également été observé que les pourcentages en poids diminuent avec la diminution de la taille des particules pour les deux échantillons de roches usées. La fraction fine (< 0,125 mm) représentait 11,4% et 13,9% pour WR1 et WR2, respectivement.

Selon ces résultats, la perte au feu (LOI) variait de 0,99% à 9,59% selon l'échantillon de roche et la taille des particules. La LOI était plus élevée dans la fraction fine (< 0,125 mm) que dans les fractions grossières pour les deux échantillons de roche. En effet, pour WR1, la LOI était de 1,12% pour la fraction > 10 mm et de 9,59% pour la fraction fine (< 0,125 mm). Ces résultats indiquent que la fraction fine contient des quantités plus importantes de carbonates et de sulfures qui ont été perdus lors de la combustion à 1 000°C. La composition chimique des échantillons de roches, exprimée en oxydes, est présentée au Tableau 2-3. Il a été déterminé que le dioxyde de silicium (SiO_2) était la composante prédominante présente dans les deux échantillons de roches usées. La teneur en SiO_2 variait entre 73 et 98% pour WR1 et entre 59 et 96% pour WR2 et ce, en fonction de la fraction solide. La prédominance du SiO_2 a montré que les deux échantillons de roches collectées étudiés étaient principalement composés de quartz. De plus, à mesure que les particules sont devenues plus fines, la proportion d'autres oxydes métalliques augmente. Dans les fractions les plus fines, l'augmentation des oxydes d'aluminium et de potassium a permis de mettre en évidence la présence d'argile dans ces fractions sous forme de kaolinite ($Al_2Si_2O_5(OH)_4$) ou d'illite ($(K,H_3O)(Al, Mg, Fe)_2[(OH)_2,(H_2O)]$). Il a été remarqué que les principaux contaminants, à savoir le cadmium (Cd) et le zinc (Zn), présents dans le drainage minier, n'étaient pas présents sous forme d'oxydes en proportions importantes dans les échantillons de roches usées.

Une évaluation du potentiel de génération acide (AP) et de potentiel de neutralisation (NP) des roches utilisées dans ces expériences a été effectuée selon le test de Sobek modifié selon Price *et al.* (1997). Après titrage, le NP des deux roches mélangées a été estimé à 20 kg de $CaCO_3.t^{-1}$ et l'AP a été estimé à 9,37 kg de $CaCO_3.t^{-1}$. Le potentiel de neutralisation net (PNN) calculé était d'environ 10,6 kg de $CaCO_3.t^{-1}$ et le ratio de neutralisation potentielle (RNP) a été estimé à 2,13. Ainsi, selon Price *et al.* (1997), le RNP calculé a montré un risque « faible » pour la génération de DMA, mais cette valeur est très proche de la limite des stériles définie comme présentant un risque «possible» de génération de DMA.

1.4.3 Synthèse des résultats préliminaires

1.4.3.1 Principaux contrôles

Afin de mettre en évidence l'importance de la mise en place d'un système de traitement efficace des effluents miniers d'Alexco, les résultats des différents contrôles « Roches + Eau distillée », « Effluent minier (EDM) » et « Roches + EDM » sont présentés à la Figure 1-9.

Les résultats obtenus pour le contrôle « Roches + Eau distillée » ont mis en évidence que les roches excavées sur le site minier et plongées dans de l'eau déminéralisée pendant 90 jours relarguent des sulfates ($[SO_4^{2-}]$ = 252 mg.L^{-1} à t = 90 jours) (Figure 1-9 e), du cadmium ([Cd] = 22 µg.L^{-1} à t = 90 jours) (Figure 1-9 d.) et du zinc ([Zn] = 784 µg.L^{-1} à t = 90 jours) (Figure 1-9 c.). Une diminution du pH de 3,6 à 2,8 a également été observée; indiquant une légère acidification du milieu (Figure1-9 b.). Le POR, quant à lui, est resté stable (compris entre 310 et 400 mV) durant toute la durée de l'expérience (Figure1-9 a.).

Le contrôle « Effluent minier (EDM) » met en évidence une diminution naturelle des teneurs en cadmium et en zinc après 90 jours (10 µg.L^{-1} à t =0 versus 5 µg.L^{-1} à t = 90 jours pour Cd et 880 µg.L^{-1} à t =0 versus 480 µg.L^{-1} à t = 90 jours pour Zn) (Figure 1-9c. et d.). Une réduction de la concentration en sulfates de 9,5% (Figure 1-9 e.), ainsi qu'une diminution du POR (Figure1-9 a.) ont également été observées après 90 jours. Les résultats obtenus à ce jour ne permettent pas encore de conclure sur les mécanismes responsables, mais il est possible que la réduction des concentrations en sulfates et en métaux soit due à la production de précipités métalliques dans les réacteurs, en revanche aucun précipité n'a été observé visuellement. Il est possible également que les effluents utilisés dans cette expérience contiennent naturellement des BSR et que celles-ci soient responsables des diminutions des concentrations observées, ainsi que de la diminution du POR.

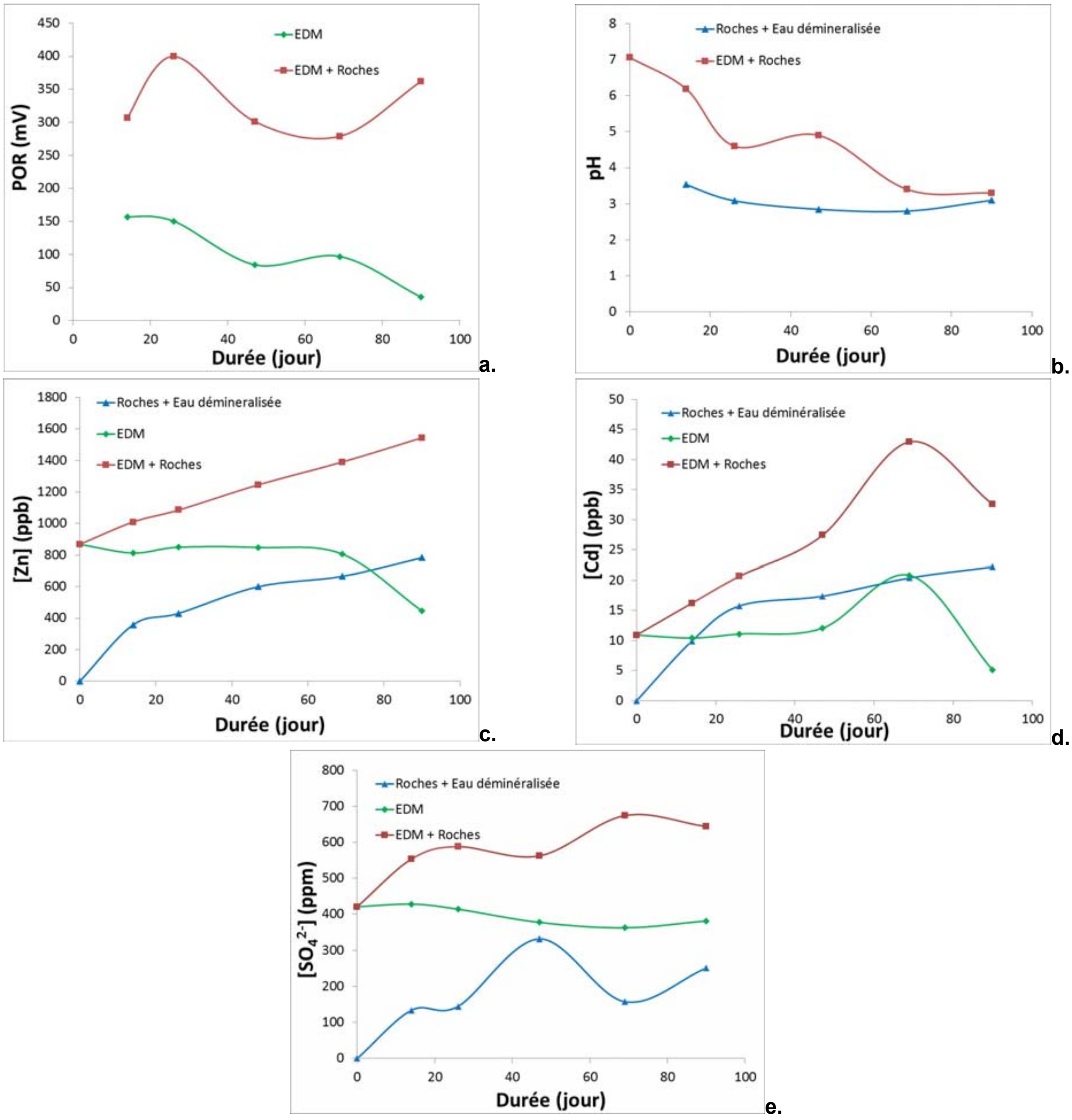

Figure 1-9 Suivi des principales caractéristiques. POR (a.), pH (b.), teneur en cadmium (µg.L^{-1}) (c.), teneur en zinc (µg.L^{-1}) (d.) et teneurs en sulfates (mg.L^{-1}) (e.) mesurées pour les contrôles lors des essais de traitement passif

Les résultats obtenus pour le contrôle « Roches + Effluent minier (EDM) » indiquent que lorsque l'effluent minier est mélangé avec les roches souterraines retrouvées sur le site afin de répliquer le système souterrain réel du site minier de Keno Hill Silver District, la réduction des sulfates liée à l'activité des BSR présentes dans l'effluent est insuffisante (augmentation de la concentration en sulfates (Figure 1-9 e.). Une augmentation de +177% (Figure 1-9 c.), de +283% (Figure 1-9 d.) et +147% $^{-}$ (Figure 1-9 e.) des teneurs en Zn, en Cd et en sulfates, respectivement, a été observée après 90 jours d'expérimentation. L'ajout de carbone afin de favoriser le développement des BSR semble donc nécessaire afin de réduire les concentrations en Cd et en Zn et de s'assurer que les effluents miniers respectent les normes de rejet en vigueur sur ce site.

Les résultats obtenus pour les trois contrôles présentés, et plus spécifiquement les concentrations résiduelles en métaux mesurées, justifient la nécessité d'une intervention humaine pour traiter les effluents miniers du site de Keno Hill Silver District afin de rencontrer les limites de rejet des effluents dans le milieu naturel selon la licence QZ12-057 en vigueur.

1.4.3.2 Essais en mode batch

Les premiers essais en bacth ont été conduits pour déterminer si les températures froides entravaient la croissance et l'activité des BSR utilisées pour traiter le drainage provenant du site minier de Keno Hill (territoire du Yukon). Le drainage minier était contaminé en Zn et en Cd lixiviés lors de l'oxydation des minéraux sulfureux présents dans roches stériles utilisées pour l'expérience. Comme ces concentrations ont dépassé les limites de relargage pour le Zn, un traitement passif efficace, basé sur l'activité de BSR, devait être développé et permettre d'atteindre les limites imposées et pour protéger l'environnement nordique. Pour favoriser la croissance bactérienne, différentes sources de carbone, telles que la mélasse, le méthanol et un mélange des deux ont été ajoutées à différentes concentrations sur la base du rapport moléculaire par rapport au sulfate. Les réacteurs d'une capacité de 1 litre ont été échantillonnés toutes les deux semaines sur une période de 90 jours. Le pH, le potentiel d'oxydo-réduction (POR) et les concentrations résiduelles de Zn, Cd, sulfates et carbone organique total (TOC) ont été mesurés. Les résultats obtenus lors des premiers essais réalisés en mode batch ont révélé la consommation totale des sources de carbone utilisées (Figure 2-2 a) et des sulfates (Figure 2-2 b) présents dans le drainage minier après 90 jours à 4,5°C, mettant ainsi en évidence la présence de BSR indigènes dans les BPSR.

Après 90 jours, la concentration en Zn et en Cd a diminué de 1 455 à 8,7 µg/L (99,4% de réduction) et de 21 à 0,6 µg/L (97,6% de réduction), respectivement, lorsque le mélange « mélasse + méthanol » a été utilisé comme source de carbone (Figure 1-10 et Figure 2-3). Ces abattements en Zn et Cd est attribuable à la réduction des sulfates en sulfures par des BSR et à leur précipitation sous forme de sulfures (ZnS et CdS). De plus, il semble que le mélange « mélasse + méthanol » ait été légèrement plus efficace pour la croissance des BSR et pour l’abattement des métaux que la mélasse ou le méthanol seul. Il a été conclu à la suite de ces premiers essais en batch menés en laboratoire que les résultats obtenus permettaient de penser que les BSR indigènes du site minier de Keno Hill au Yukon pouvaient être utilisées avec succès pour le traitement du drainage minier contaminé par des métaux lourds.

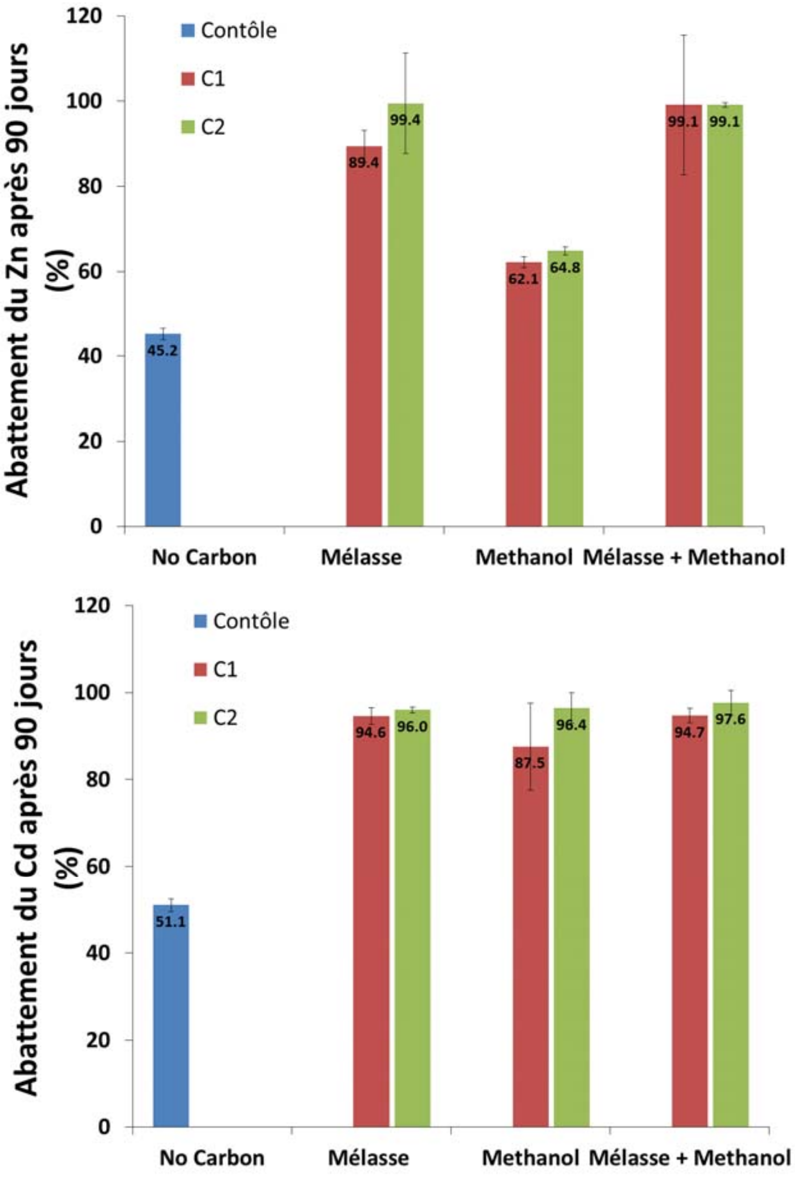

Figure 1-10 Abattements du Zinc et du Cadmium selon les différentes sources de carbone utilisées pour deux concentrations différentes (C1 = 77 mg/L et C2 = 154 mg/L) à 5ºC

Suite aux conclusions de l'étude menée sur les premiers essais en batch, une deuxième étude a été conduite afin de présenter une revue de la littérature actualisée des différentes sources de carbone utilisées au cours des dernières décennies pour soutenir les activités métaboliques des BSR et notamment réduire les sulfate en sulfures. Tout en suivant le même protocole expérimental des premiers essais en batch (utilisation des même roches stériles, du drainage minier issus de Keno Hill, YT) des bioréacteurs d'une capacité de 1 litre ont été étudiés et échantillonnés toutes les 2 semaines sur une durée de 160 jours pour tester diverses sources de carbone simples et complexes disponibles au Yukon en tant que donneur d'électrons pour la réduction du sulfate. La réduction subséquente des concentrations en Cd et Zn issus du drainage minier a aussi été suivie.

Lors de la seconde série d'essais en mode batch, une diminution des concentrations en carbone organique total (TOC) et en sulfate a été observée dans tous les bioréacteurs étudiés après 160 jours. Dans certains d'entre eux, ces consommations pourraient s'expliquer par la présence BSR. L'utilisation de sources de carbone organique simples (méthanol et éthylène glycol) et de sources de carbone organique complexes (huile de friture, résidus de brasserie, tourbe et paille) pour favoriser la croissance des BSR à 5°C a montré que le méthanol et l'éthylène glycol ont entraîné une diminution des teneurs en sulfates de 71,2% et 36,9% (Figure 3-5 a.), respectivement, tandis que la consommation des sulfates a été limitée à 13,8% et à 5,3% en présence de tourbe et de paille, respectivement (Figure 3-5 b.). Les résidus de brasserie n'ont, pour leur part, pas favorisé de façon significative la consommation des sulfates.

En accord avec la revue de littérature conduite pour cette étude, il a été révélé que des sources de carbone organiques simples, telles que le méthanol ou l'éthylène glycol, sont efficaces pour soutenir la croissance de la BSR à température froide (5 °C). Leur utilisation est fiable et permet un meilleur contrôle de la quantité de carbone injectée dans le BPSR. Néanmoins, à température froide, une longue période d'adaptation (98-112 jours) a été nécessaire avant que la consommation ne commence et l'utilisation de sources de carbone simples nécessite des modèles de BPSR plus sophistiqués qui peuvent entraîner une augmentation des coûts, en particulier pour les sites miniers éloignés. D'autre part, les sources de carbone organiques complexes ont également bénéficié la croissance de BSR. Elles ont été dégradées plus progressivement que les sources de carbone organique simples. Bien qu'elles n'aient pas permis des rendements de réduction de sulfates aussi élevés qu'avec des sources de carbone organiques simples, les sources de carbone complexes peuvent être plus appropriées pour le traitement de DMA de sites miniers éloignés. Les résultats ont montréque les sources de carbone simple ont permis de diminuer la concentration de Cd de 100% en présence de méthanol et de 99,6% en présence d'éthylène glycol après 112 jours. La concentration de Zn, quant-à-elle, a diminué de 99,7 et 98,5% en présence de méthanol et d'éthylène glycol, respectivement.

1.4.3.3 Essais pilotes

Suite aux études menés en labartatoire, une étude pilote a été conduite sur le site minier de Keno Hill au Yukon afin de confirmer les résultats prometteurs obtenus lors des essais en batch. Ainsi, deux bioréacteurs pilotes d'une capacité de 200 litres chacun ont été installés sur le site minier de Silver King faisant parti de Keno Hill Silver District. Ils ont permis l'étude des mécanismes de sulfato-réduction opérés par des BSR indigènes et leur efficacité sur l'enlèvement du Zn et du Cd sur un effluent minier réel. Durant les 14 mois que cette étude a duré (de juillet 2015 à septembre 2016), la température des bioréacteurs dans le cabanon a été maintenue aux alentours de 5°C durant les mois d'automne et d'hiver mais a significativement augmenté durant les mois d'été en raison des températures saisonnières. Une forte corrélation a été remarquée entre la température des bioréacteurs et la réduction des sulfates puisque le pourcentage de réduction des sulfates pouvait être quasi nul en hiver et atteindre près de 91,9% en été (Figure 4-1). Une solution de mélasse dont la concentration a été fixée pour atteindre un ratio moléculaire sulfate/TOC de 1/2 a été utilisée comme source de carbone. La consommation du carbone injecté dans les bioréacteurs a, elle aussi, été plus importante (de l'ordre de 95%), durant les mois d'été (Figure 4-2), lorsque la température interne des bioréacteurs était plus élevée, passant de 5ºC durant l'hiver à 18ºC durant l'été. Il a été constaté que la consommation en carbone diminuait donc en hiver mais restait relativement élevée (de l'ordre de 60%) par rapport à la réduction des sulfates qui était quasi-nulle au cours de la même période. L'hypothèse a donc été faite qu'une large communauté bactérienne (pas uniquement des BSR) avait colonisé les bioréacteurs pilotes et certaines bactéries non sulfato-réductrices restaient actives durant l'hiver.

Bien que les concentrations initiales en métaux dans l'effluent aient considérablement changé en fonction des saisons (ruissellement et crue saisonnière dans les eaux souterraines et intensité de pompage opéré par la compagnie minière Alexco, proriétaire du site miner, le BPSR a permis de réduire les teneurs en Zn (Figure 4-3 a.) et en Cd (Figure 4-3 b.). Afin de s'assurer que le phénomène prépondérant d'abattement du Zn et du Cd était bien la précipitation sous forme de sulfures, une simulation MINEQL a été réalisée sur l'effluent minier après traitement à la date du meilleur abattement en Cd et Zn obtenu le 7 juillet 2016. L'analyse MINEQL révèle que le Cd est présent sous forme de $Cd(HS)_4^{2-}$ à 97,2% et que le Zn précipite sous forme de ZnS à 87,3% (Annexe IV). Durant toute la durée de l'expérience, les concentrations résiduelles en Cd et Zn ont été maintenues en-dessous des limites de relargage imposées par la licence d'exploitation délivrée en vertu de la Loi sur les eaux. Ainsi, les meilleurs taux d'enlèvement pour le Zn et le Cd ont été observés pendant les étés 2015 et 2016 lorsque les températures étaient au-dessus de 10°C, atteignant près 95,5% d'enlèvement pour le Zn dans BR1 (juillet 2015) et 96,3% pour le Cd dans BR2 (juillet 2016). Les résultats d'abattements des concentrations en Zn et Cd obtenus à l'échelle pilote en été, où les températures internes dans les bioréacteurs étaient comprise entre 5 et 17°C, sont proches de ceux obtenus en laboratoire à 5°C. En revanche, alors que la température en hiver était maintenue à 5°C dans les bioréacteurs pilotes, les abattements du Zn et du Cd diminuaient considérablement (de l'ordre de 13 à 70%). Il peut être supposé que les températures réelles, variant selon les saisons, et la communauté bactérienne plus complexe rencontrée à l'échelle pilote ont pâti au développement et à l'activité métabolique de BSR et donc à l'abattement des métaux. De plus, le temps de résidence hydraulique (HTR) dans les bioréacteurs pilotes a été fixé à 2 semaines, quand les essais en batch étaient conduits sur 90 et 160 jours. Il est possible que ce laps de temps soit trop court à 5°C pour que les BSR puissent opérer la réduction des sulfates.

1.4.3.4 Caractérisation génomique

Les échantillons de population microbienne collectés mensuellement ont permis une caractérisation génomique des bactéries présentes dans les deux bioréacteurs pilotes ainsi que dans l'effluent minier. La caractérisation génomique de la communauté microbienne a mis en évidence la présence d'une population complexe mais stable au cours des 14 mois que l'expérience a duré. La similarité entre les structures de la communauté microbienne des deux bioréacteurs BR1 et BR2 a permis de vérifier que ces derniers ont bien agit en duplicata.

Les résultats des analyses génomiques ont confirmé la présence de potentielles BSR indigènes dans les effluents miniers. Les même « classes » de bactéries se retrouvent en abondance relative plus grande dans les échantillons des deux BPSR (Figure 4-4), ce qui laissait à penser que les bioréacteurs constituaient des environnements propices à la colonisation des BSR. Par ailleurs, les populations de bactéries présentes dans les deux bioréacteurs et dans l'effluent minier (avant traitement) étaient significativement différentes. Les différences se trouvaient principalement dans l'abondance des membres des classes *Bacilli* et *Betaproteobacteria*, qui étaient plus abondants dans les échantillons d'effluent minier et les membres des classes *Clostridia* et *Deltaproteobacteria* qui étaient plus abondants dans les échantillons de bioréacteurs (Figure 4-5) et qui sont tous deux connus comme étant capables de potentielle réduction des sulfates. De plus, aucun changement en ce qui concerne les populations microbiennes présentes dans les BPSR n'a été observé lors du changement de température mesuré entre l'hiver (5°C) et l'été (18°C).

Les données scientifiques collectées durant les travaux menés à l'échelle pilote ont permis à Alexco de mieux comprendre les mécanismes de sulfato-réduction s'opérant *in situ* par des BSR indigènes. De plus, cette étude pilote a révélé que les BSR indigènes issues du site minier Keno Hill au Yukon pouvaient être utilisées pour traiter un drainage de mine neutre contaminé en Zn et en Cd et ce, dans un climat froid. Au cours de cette expérience de 14 mois, les concentrations en Zn et en Cd à la sortie des bioréacteurs étaient toujours sous les limites de relargage imposées par la licence d'utilisation sur les eaux du Yukon. La population microbienne a considérablement affecté l'efficacité de des bioréacteurs dans l'assainissement du DNC. Cependant, ce système de traitement passif est limité par la température puisque cette dernière a joué un rôle considérable dans l'efficacité des bioréacteurs pilotes. Les différences entre la population microbienne dans l'effluent et dans les bioréacteurs suggèrent que, dans un climat froid, les bioréacteurs fournissent des conditions environnementales relativement stables et favorisent la colonisation des BSR.

1.5 Conclusion et Recommandations

Les besoins croissants en métaux impliquent l'ouverture et l'exploitation de nouvelles mines à travers le monde tandis qu'en parallèle, les règlementations environnementales évoluent dans chaque pays et deviennent de plus en plus strictes. Par conséquent, il est indispensable de développer de nouvelles approches permettant une gestion des déchets plus adéquate et de réduire les risques de contamination des cours d'eau et des environnements naturels. Les systèmes de traitement actifs traditionnels sont certes efficaces, mais ils requièrent à la fois de la main d'œuvre, de l'énergie et l'addition de produits chimiques, en plus de générer des boues dont la disposition est problématique, particulièrement en milieu nordique. De plus, ces traitements actifs sont relativement coûteux et peu appropriés à la fermeture des mines lorsque les opérateurs sont à la recherche de solution « clés en main ». Les bioréacteurs passifs faisant appel aux bactéries sulfato-réductrices apparaissent comme une solution prometteuse pour le traitement d'effluents miniers même si ces systèmes nécessitent une injection de carbone à des intervalles définis et une gestion des boues de précipitation. La main d'œuvre requise pour entretenir ces systèmes est bien moins importante que pour les traitements actifs.

Jusqu' à présent, de nombreuses études ont été conduites en climat tempéré, où la température est favorable au développement de populations microbiennes, dont les bactéries sulfato-réductrices. En revanche, trop peu de recherches ont été conduites concernant l'application de ces procédés de traitement dans les climats nordiques, comme le climat subarctique du Yukon, et sur l'influence de basses températures sur la croissance et l'activité des BSR. En plus de l'enlèvement des métaux par précipitation sous forme de sulfures, d'autres mécanismes tels que la co-précipitation et la sorption sur les précipités formés et/ou matière organique présente peuvent intervenir pour améliorer l'enlèvement des contaminants présents dans les DMA/DNC.

Les essais réalisés dans le cadre de ce projet de doctorat ont permis de mettre en évidence la présence de BSR indigènes dans les effluents du Silver King Mine Site faisant partie de Keno Hill Silver district au Yukon. Une caractérisation génomique a montré que de potentielles BSR membres des classes Clostridia *Clostridia* et *Deltaproteobacteria* se trouvaient dans les bioreacteurs pilotes étudiés sur le site. Il a été montré que ces BSR, bien qu'impactées par les températures froides, peuvent être efficaces pour opérer des processus de sulfato-réduction et donc faciliter la précipitation des métaux sous forme de sulfures en climat froid. Les essais menés en laboratoire en mode batch ont permis de prouver qu'avec une source de carbone adéquate injectée à une concentration suffisante, l'effet du froid peut être réduit et que les BSR semblent pouvoir s'adapter et fonctionner dans des BPSR à 5°C. Les travaux menés à échelle pilote sur le site de Keno Hill Silver district ont permis de mieux comprendre les mécanismes s'opérant dans les bioréacteurs *in situ*. L'efficacité d'enlèvement du Zn variait alors entre 11.5% en hiver et 95.5% en été. L'efficacité d'enlèvement du Cd variait entre 21.1% en hiver et 96.6% en été. Les différences de performances observées à l'hiver avec les essais en laboratoire pourraient s'expliquer par les difficultés à maintenir la température stable et les périodes de gel des bioréacteurs qui ont eu lieu lors des essais pilote alors que dans le cas des essais en laboratoire, la température a été maintenue à 5°C.

Dans l'éventualité de travaux futurs, quelques recommandations devraient être prises en considération :

- L'utilisation d'une source de carbone adéquate étant un élément clé pour la croissance et l'efficacité des BSR pour réduire les sulfates et sulfures, et ce surtout en climat froid, la recherche et l'étude de sources de carbone économiquement abordables, disponibles dans le Nord et utilisables par les BSR doit être poursuivie.
- Durant les essais pilotes, la température était supposée être constante et maintenue aux alentours de 5°C mais elle a considérablement augmenté durant les mois d'été. Une étude d'un système continuellement sujet à des températures froides devrait être conduite à l'échelle pilote.
- Dans les essais futurs, la mise en évidence de BSR dans les expériences menées en laboratoire, jusqu'à présent basée sur la consommation des sulfates, peut être faite par la technique du nombre le plus probable (NPP) ou d'évaluation qualitative en flacons agités. De plus, il serait intéressant d'évaluer la durée de vie du garnissage ou du biofilm formé.

- Des études concernant le design optimal des BPSR et du temps de rétention hydraulique devrait être menées. De plus, l'influence d'une augmentation de l'acidité ou des teneurs en métaux sur les performances des BPSR devrait être évaluée à l'échelle pilote.
- La stabilité des précipités de sulfures métalliques devrait être évaluée dans le temps pour déterminer le potentiel de relargage des métaux en fonction d'éventuelles fluctuations des systèmes de traitement.

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PARTIE 2 : ARTICLES

2 CHAPITRE 2

Performances of sulfate-reducing passive bioreactors for the treatment of mine drainage contaminated by Cd and Zn in cold climate

Performances des bioréacteurs sulfato-réducteurs passifs pour le traitement de drainages miniers contaminés par du Cd et du Zn en climat froid

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Note

L’étudiant est le premier auteur de cet article. Toutes les manipulations, le traitement des données ainsi que la rédaction ont été effectués par l’étudiant sous la supervision des autres co-auteurs.

Modifications exigées par le comité de lecture

L’article a été accepté avec des modifications mineures. Les commentaires les plus importants demandaient d’épurer le papier de quelques détails jugés trop techniques, de raccourcir l’introduction et de préciser certaines parties de la section Matériels et Méthodes.

2.1 Résumé

Le traitement passif, utilisant des bactéries sulfato-réductrices (BSR), est une technologie prometteuse et écologique de plus en plus utilisée comme alternative de choix pour le traitement de drainages miniers. Cependant, plusieurs défis subsistent quant à sa mise en œuvre dans les régions nordiques, où la température de l'eau reste froide toute l'année, et la croissance bactérienne est limitée en raison des basses températures. Les effets de la température froide sur l'activité des BSR et sur l'élimination du Cd et du Zn présents dans un drainage minier acide (DMA) ont été étudiés en effectuant des essais statiques à 4,5°C sur une période de 90 jours. Différentes sources de carbone ont été testées pour soutenir des BSR indigènes : la mélasse, le méthanol et un mélange de « mélasse / méthanol » à différentes concentrations. Les réacteurs d'une capacité de 1 L ont été échantillonnés toutes les deux semaines et le pH, le potentiel d'oxydoréduction (POR) et les concentrations résiduelles en Zn, Cd, sulfates et carbone organique total (COT) ont été mesurés. L'utilisation de sources de carbone a nettement amélioré l'activité bactérienne et, par conséquent, la précipitation des métaux (Cd et Zn) sous forme de sulfures. En effet, près de 94,8% du Zn et 99,4% du Cd initialement présents ont été éliminés après 90 jours, ce qui a entrainé une réduction des concentrations des métaux en-dessous des limites de rejet imposées par la mine yukonnaise qui a fourni l'effluent ([Cd] < 50 µg / L et [Zn] < 500 µg / L). Le mélange de « mélasse + méthanol » était légèrement plus efficace que la mélasse ou le méthanol seul. Ces résultats indiquent que les sources indigènes de BSR pourraient être utilisées avec succès dans le traitement des eaux relarguées par l'exploitation minière et contaminées par des métaux lourds.

Mots clé : Traitement passif, Bioremédiation, Climat froid, Cadmium, Mine, Zinc

2.2 Abstract

Passive treatment, using sulfate-reducing bacteria (SRB), is a promising and green technology that is increasingly being used as an alternative for the treatment of mine drainage as opposed to conventional methods. However, several challenges remain concerning its implementation in cold climates, where the temperature of the water remains cold year round, and bacterial growth is limited due to low temperatures. The impacts of cold temperature on the activity of SRB and the subsequent removal of Cd and Zn from acid mine drainage (AMD) were studied by conducting static tests at 4.5°C over a 90-day period. Different sources of carbon were tested to support native SRB: molasses, methanol and a mix of molasses/methanol at different concentrations. The 1-L capacity reactors were monitored biweekly, and the pH, the oxido-reduction potential (ORP) and the residual concentrations of Zn, Cd, sulfates, and total organic carbon (TOC) were measured. The use of carbon sources clearly improved the bacterial activity and consequently, the removal of metals (Cd and Zn) by precipitation as sulfide. Up to 94.8% of zinc and up to 99.4% of cadmium were removed after 90 days, resulting in a reduction in the metal concentrations below the discharge limits ([Cd] < 50 µg/L and [Zn] < 500 µg/L). The molasses + methanol mix was slightly more efficient than molasses or methanol alone. These findings indicated that native sources of SRB might be used successfully in the treatment of mine-discharged water contaminated with heavy metals.

Keywords: Passive treatment, Bioremediation, Cold climate, Cadmium, Mine, Zinc.

2.3 Abbreviations

AA	Atomic adsorption
AP	Generation potential
AMD	Acid mine drainage
ARD	Acid rock drainage
DIW	Deionized water
DNA	Deoxyribonucleic acid
HTR	Hydraulic retention time
LDPE	Low-density polyethylene
NP	Neutralizing potential
NNP	Net neutralization potential
ORP	Oxidation reduction potential
PTS	Passive treatment systems
SRPB	Sulfate-reducing passive bioreactors
SRB	Sulfate reducing bacteria
TOC	Total organic carbon
WR	Waste rocks

2.4 Introduction

Technological advances and the increasing population have resulted in an increasing need for metals and minerals, leading to the emergence of many mines across the world, especially in the north of Canada. The operation of these mines leads to the production of mine water contaminated with heavy metals that requires specific management and treatment when the mine is in operation and after its closure. Indeed, adequate management of mine effluent is required over short- and long-term periods during the construction, operation and closure of a mine to protect the North's sensitive environment.

The production of acid mine drainage (AMD) at abandoned or active mine sites has been widely studied. Indeed, the extraction and processing of base and precious metals from sulfide-ore deposits generate large quantities of fine-grained tailings and waste rocks, which are composed of the rock that surrounds the ore bodies, have a mineral content lower than the economic threshold required to process them, and need to be excavated to gain access to the ore deposit (Moncur *et al.,* 2015; Nordstrom *et al.,* 2015). Thus, AMD can be produced from tailings storage facilities, waste rock piles, underground tunnel walls or sub-aerial deposits where sulfide minerals are thermodynamically unstable (Lindsay *et al.,* 2015; Morin and Hutt, 2001; Potvin 2009).

The production of AMD is considered to be temperature limited due to the diminished activity of Fe(II)-oxidizing microbes at low temperature (Gault *et al.,* 2015). However, the heat generated by sulfide oxidation may elevate temperatures sufficiently to permit the oxidation of sulfide minerals by ferric iron even in cold environments (Bigham and Nordstrom, 2000; Ninteman, 1978). Although AMD is not specifically defined in terms of pH, most types of AMD are in the range of pH 2 to 6 (Nordstrom *et al.,* 2015). If released into the natural environment, AMD can be a major source of water pollution, having devastating effects on the environment and causing the acidification of lakes and rivers (Espana *et al.,* 2005). Currently, active mining companies commonly use active treatment technologies to treat contaminated effluent when the production of contaminated drainage cannot be stopped or controlled (Aubé 2003; Aubertin and Bussière, 2001; Johnson and Hallberg, 2005). In the Yukon Territory, active treatments are usually used to treat AMD from both active and closed mines due to the low temperatures and the lack of efficiency of some passive treatments. Indeed, passive treatment system (PTS) face challenges in cold climates that include hydraulic failure due to freezing in winter, reduced microbial activity, and increased water flows during spring melt (Ness *et al.,* 2014). However, the use of active treatment technologies results in high treatment costs and requires labour. This can be challenging when the mines close, making it a less sustainable option for long-term management of mine-impacted water, especially in remote locations.

On the other hand, passive treatment systems (PTS) offer a promising alternative to conventional active treatment systems. PTS are based on chemical and biological reactions that naturally occur in the environment and offer a potential sustainable option that is adequate for long-term mine drainage treatment during and after mine closure. PTS are defined as technologies which use natural materials to promote natural chemical and biological processes and require little or no maintenance once installed (Younger *et al.,* 2002; Johnson and Hallberg, 2005). More specifically, PTS allow the removal of contaminants from water using biological or geochemical processes. The process requires limited or no power or chemicals after construction and can last for decades with minimal human maintenance (Gusek and Wildeman, 2002). Among the PTS, bioremediation techniques using microorganisms, including sulfate-reducing bacteria, are increasingly used for the treatment of mine drainage. Some passive treatment systems require periodic active management to sustain desired conditions and processes, rendering them semi-passive (Martin *et al.,* 2009).

Sulfate-reducing passive bioreactors (SRPB) represent a passive or semi-passive treatment system applicable to mine drainage effluents as these types of drainage usually contain high concentrations of sulfates due to the oxidation of sulfidic minerals. The use of bioremediation systems based on sulfate reduction into sulfide in the presence of sulfate-reducing bacteria has been extensively studied (Hashim *et al.,* 2011; Johnson and Hallberg, 2005; Neculita *et al.,* 2007; Ness *et al.,* 2014; Nordin, 2010). Biological sulfate reduction results in the reduction of sulfate into sulfide and leads to a decrease in dissolved metal contents due to the production of metal sulfide precipitates. In addition, the production of bicarbonate can lead to an increase in pH (Egiebor and Oni, 2007; Hulshof *et al.,* 2003; Jin *et al.,* 2008; Neculita *et al*., 2008; Ziemkiewicz *et al.,* 2003). Equations 2-1 and 2-2 present the reactions of the reduction of sulfate into sulfide and the precipitation of metal as sulfide that occur in an SRPB. The performances of these treatments depend on three major parameters: the hydraulic retention time (HTR), the SRB/substrate mixture and the initial metal concentrations present in the AMD (Neculita *et al.,* 2008).

Equation 2.1 $\mathbf{SO_4^{2-} + 2\ C\ (organic) + 2\ H_2O + SRB <=> H_2S_{(aq)} + 2\ HCO_3^-}$

Equation 2.2 $\mathbf{M^{2+} + H_2S <=> MS_{(s)} + 2\ H^+}$

Dissimilatory sulfate reduction is accomplished, in the absence of oxygen, by SRB that decompose simple organic compounds using sulfate as a terminal electron acceptor. One mole of sulfate is reduced to hydrogen sulfide for every two moles of carbon oxidized following the reaction (Equation 2-3) (Hammer, 1989).

Equation 2.3 $\mathbf{2\ CH_2O^* + SO_4^{2-} <=> H_2S + 2\ HCO_3^-}$

where $^*CH_2O$ *represents a simple organic molecule such as acetate.*

In a sulfate-reducing passive bioreactor (SRPB), sulfate reduction by SRB is the most important bioprocess. Therefore, good management of physical and chemical conditions is essential to the growth and effectiveness of this type of bacteria. Few SRB have been reported as cold-tolerant, and the low temperatures encountered in the northern climate can challenge the growth of these organisms. The optimal growth temperature and metabolic efficiency vary with sulfate-reducing bacteria species. Thus far, most species of SRB that have been isolated and described are mesophilic. However, at low temperature, several researchers showed that bacterial metabolic activity requires specific adaptations (Cavicchioli, 2006; Robador *et al.,* 2009). The most important of these adaptations is the ability of bacteria to synthesize enzymes with high catalytic activities at low temperatures (Feller and Gerday, 2003; Robador *et al.,* 2009).

The efficiency of passive bioreactors depends on the activity of bacteria, which is mainly controlled by the composition of the reactive mixture. The carbon sources used for rapid growth of SRB can be simple organic compounds such as calcium lactate, ethanol, sucrose, glucose and lactoserum (Luptáková and Macingova, 2012; Tsukamoto, 1999). Alternative cheap carbon sources containing complex organic compounds such as organic waste (manure, compost) and cellulose (hay, straw) have also been studied. However, hydrolysis of the carbon substrate is required before SRB can use these sources of carbon as an energy source. Therefore, as the degradation of complex carbon sources is very slow, they potentially have the ability to support SRB growth over longer periods of time (Drury, 2006). Such an attribute can be attractive for the design of a treatment system at mine closure. Moreover, the sorption of metals (Cd, Zn) on solid organic materials may even act in a complementary manner to the decrease in the metal contents present in AMD when a carbon source such as biochar or wood chips is used (Janin and Harrington, 2013). In this study, molasses and methanol were tested for their ability to support the growth of SRB in situ and remove zinc and cadmium from mine water. Methanol is a source of carbon well-known for its ability to support bacterial growth (Tsukamoto, 1999) and is a relatively cheap bulk chemical widely used in biotechnological processes (Dijkhuizen *et al.,* 1985). Sobolewski (2010) used other liquid carbon sources, such as ethylene glycol, to provide tangible benefits for bioreactors treating mine water. According to his results, microbial activity can be maintained at temperatures lower than 4°C, allowing the operation of such bioreactors in cold climates.

Janin and Harrington (2015) monitored anaerobic column bioreactors at 3 and 6°C at a laboratory scale for more than one year to study their capacity to decrease the concentrations of metals present in water (As, Cd, Cu, Se and Zn). Their results showed that, even if the temperature affected the performance of the bioreactors, native bacteria from northern environments were adapted to the low temperatures, when liquid carbon substrate was provided to the bioreactors. Removal efficiencies in the range of 90 to 100% were obtained with initial concentrations of 521 ± 93 mg/L for Cd and 5525 ± 960 mg/L for Zn. Waybrant *et al.,* (1998, 2002) showed that greater efficiencies were obtained when a mixture of several carbon sources (wood chips, sawdust, composted municipal sewage sludge, and leaf compost) was used compared to a single organic carbon source (like methanol) (Neculita *et al.,* 2007; Zagury *et al.,* 2007). Another important parameter can significantly influence the growth and activity of SRB; according to numerous studies on the development of SRB, the optimum pH range for SRB growth is between 6.8 and 7.5 (Rees *et al.,* 1995; Rozanova *et al.,* 2001; Sievert and Kuever, 2000; Wang *et al.,* 2013). Although some SRB are known to survive in extremely low pH waters (Neculita 2008; Tsukamoto, 1999). Still, the use of SRB has some limitations and very high dissolved heavy metals concentrations in an AMD may have an inhibitory effect of on SRB (Cabrera *et al.,* 2006). Besides, the initial concentration of calcium in the AMD must be considered to. Indeed, high Ca concentrations may lead to gypsum precipitation ($CaSO_4$) or ettringite precipitation ($Ca_6Al_2(SO_4)_3(OH)_{12}\cdot 26H_2O$) with the released sulfates (Tolonen *et al.,* 2015).

The objective of this study was to examine the activity of sulfate-reducing bacteria (SRB) in the presence of different sources of carbon (molasses, methanol and a mix thereof) to enhance the performance of an *in situ* passive treatment system based on the reduction of sulfate by native SRB and the precipitation of metals as sulfides. This *in situ* PTS was intended to reduce the initial metal contamination in the Silver King Mine drainage located in the Keno Hill Silver district mine site (Yukon Territory). The experiments were conducted at a laboratory scale under cold temperature (4-5°C) to replicate the conditions of the underground mine water system. If this study demonstrates the efficiency of PSRB in cold climates, it would be applied to many northern historical mine sites which are struggled with AMD generation.

2.5 Materials and Methods

2.5.1 Mine Water, Waste Rocks and Inoculum Sampling and Characterization

This laboratory scale project is part of a larger experiment conducted with Alexco Resource Corporation mining company which has injected carbon sources in a 110 meter deep borehole located at Silver King, in the United Keno Hill Mines,YT, in order to support the growth of native SRB to lower cadmium and zinc contamination in ground water flowing through the old mining tunnels. Since this laboratory batch test experiment's purpose was to bring scientific evidences of the feasibility of Alexco's project, it had been decided to work with mine impacted water (MIW) from the borehole and wastes rocks from the Silver King Mine site.

2.5.1.1 Mine water characterization

Mine-impacted water (MIW) was collected in April 2014 from the Silver King Mine site. This water was sampled monthly between February 2007 and March 2016 by the Alexco Resource Corporation mining company, and the concentrations of the metals were determined by Agat Laboratories (Whitehorse, YT, Canada) (See section 2.2.5 for analytical methods).

2.5.1.2 Waste rock characterization

The waste rocks were collected at the Silver King Mine site in April 2014 from two different waste rock piles (WR1 and WR2) that were built when the Silver King Mine tunnels were excavated (personal discussion with Alexco, 2014). The waste rock samples were collected in a 20-L bucket and stored in cold storage. Each bioreactor used during these experiments was filled with 100 mL of the waste rocks from each pile (WR1 and WR2). The waste rocks used in the different experiments were sieved through a 3.18-mm-opening sieve (VWR Scientific, Mississauga, ON, Canada). A complete characterization (particle size distribution, metal contents, AMD production) of the waste rock samples was performed to determine the potential risk of AMD production. First, the waste rocks samples were sieved into different fractions: > 10 mm; 10-2 mm; 2–1 mm; 1–0.250 mm; 0.250–0.125 mm and < 0.125 mm to determine the particle size distribution.

Then, each waste rock sample was crushed using a jaw crusher and ground using an impact disc mill (Bleuler-Mill, NAEF, Los Angeles, CA, USA) to obtain a median grain size of 28.4 ± 1.8 µm. X-ray diffraction (XRD) analysis (Siemens D5000 diffractometer, Berlin, Germany) was then performed on each sample at Laval University (Québec, QC, Canada) to identify the major crystalline phases. Scans were taken for 2θ ranging from 5° to 65° with increments of $0.02° \, s^{-1}$ using a Cu anode.

The chemical composition of each waste rock sample was determined after heat activation in a muffle furnace (Furnatrol 133, Thermolyne Sybron Corporation, Milwaukee, WI, USA) at 1 000°C for 30 min. Solid samples were then fused using metaborate lithium prior to analysis (Corporation Scientifique Claisse, Québec, QC, Canada). Chemical analyses of liquid samples were performed using inductively coupled plasma−atomic emission spectrometry (ICP−AES) analysis (Vista AX CCO Simultaneous ICP−AES, Palo Alto, CA, USA).

A Sobek test was conducted to determine the neutralizing potential (NP) of the waste rock samples following the procedure described in the reference method US EPA-600 and in the study carried out by Sobek *et al.,* (1978). The waste rock samples were mixed in the same proportions as those used for the bioreactors, and a Fizz test was conducted with 0.5 g of waste rock samples onto which a few drops of a solution of HCl (1/3) were added. The results of the Fizz test provided information on the volume and normality of HCl required for the digestion of 2 g of waste rock for NP determination (no fizz: 20 mL of 0.1 M HCl; slight fizz: 40 mL of 0.1 M HCl; moderate fizz: 40 mL of 0.5 M HCl; strong fizz: 80 mL of 0.5 M HCl). The digestion for the Sobek test was conducted by heating the pulp (waste rock sample and the solution of HCl) at 90°C until gas bubbling stopped. Once the digestion was complete, the solution was titrated with a solution of NaOH of known normality to pH 7.0 to determine the NP of the rock sample. The acid generation potential (AP) was determined from the analysis of the percentage of sulphur present in the waste rock samples following Equation 2-4 (Sobek *et al.,* 1978).

Equation 2.4 **Acid generation potential (AP - kg/t) = % sulfur x 31.25**

The percentage of sulfur present in the waste rock samples was determined using a TruSpec® Micro apparatus (St. Joseph, Michigan, USA). To perform this analysis, between 1 and 3 mg of the waste rock samples were subjected to combustion at 1 200°C for 200 seconds under a pressure of 12 PSI.

The net neutralization potential (NNP) was then calculated by subtracting the AP from the NP according to Equation 2-5 (Sobek *et al.,* 1978), and the neutralization potential ratio (NPR) was calculated according to Equation 2-6 (Sobek *et al.,* 1978).

Equation 2.5 $$\mathbf{NNP = NP - AP}$$

Equation 2.6 $$\mathbf{NPR = NP/AP}$$

Where NNP represents the net neutralization potential; NP represents the neutralizing potential; AP represents the acid generation potential (kg/t); and NPR represents the neutralization potential ratio.

2.5.2 Sediment sampling

Sediment was collected from Crystal Creek, located in the Keno Hill mining district between Keno and Mayo (YT, Canada). The sediment had been shoveled from the bottom of the creek, collected in a 20-L bucket and stored in cold storage (15°C). The presence of SRB in this creek's sediment has been documented in baseline studies by Alexco during the remediation and maintenance of the historic mining district. Londry (2013) conducted a technical assessment of the potential microbial roles in metal removal for the Galkeno 900 bioremediation project conducted by Alexco using microbial molecular (DNA) and culturing techniques. It showed SRB were present within sediments collected in several creeks located in the United Keno Hill Mines area. The sediment collected from that study have been used as an inoculum for the experiments conducted as part of this study.

2.5.3 Experimental Design and Operation

Bioreactors were built using 1-L capacity bottles made of low-density polyethylene (LDPE) to replicate the conditions of the *in situ* water treatment system of the underground tunnels. Each bottle was filled with

200 mL of waste rocks, 850 mL of mine-impacted water, 5 mL of inoculum and variable amounts of liquid carbon (molasses, methanol and a mixture of molasses/methanol). Deionized water was then added to make up the volume of the bottles to ensure that the systems were anoxic. The bioreactors were stored at 5°C and monitored over 90 days. All bioreactors were prepared in triplicate to ensure the quality of the results and to compare the performances of the different sources of carbon with respect to the bacterial activities and metal removal using statistical tests. The duration of the experiment was not predetermined. However, the experiment was stopped when the TOC was completely depleted, which was achieved after 90 days of incubation.

The sources of liquid carbon used in this study were molasses (Crosby's 100% Natural Fancy Molasses; Crosby Molasses Company Limited, St. John, NB, Canada), methanol (certified ACS; Fisher Scientific, Fair Lawn, NJ, USA) and a mix of both (50/50, v/v). The experiments were carried out using two different concentrations of the carbon sources: 77 mg/L (C1) or 154 mg/L (C2). The C1 concentration was determined according to Equation 2-1 and accounted for two moles of carbon for each mole of sulfate present in the mine drainage effluent. The C2 concentration was fixed at twice the concentration of C1 to assess the effect of carbon concentration on the activity of bacteria and on the removal of metals from the mining water.

Table 2-1 provides an overview of the experimental design used in this study for the preparation of the bioreactors with the C1 and C2 concentrations.

Table 2-1 **Carbon sources (Mo: molasses, Me: methanol) and concentrations (C1 = 77 mg/L and C2 = 154 mg/L) during the experiments to enhance the microbial growth**

Batch test Name	Molasses	Methanol	Inoculum
Mo C1	x		x
Me C1		x	x
Mo+Me C1	x	x	x
Mo C2	x		x
Me C2		x	x
Mo+Me C2	x	x	x

Various control bioreactors were monitored during this study (Table 2-2) to assess the impact of the presence of waste rocks in the mine water (CT4) or DIW (CT3), and the presence of air (CT6) and inoculum (CT5) in the bioreactors. Two other controls were prepared to allow us to evaluate the evolution of the bioreactors when nothing was added (CT1 and CT2). All controls were prepared in duplicate except CT4 “Waste rocks + MIW”.

Table 2-2 **Composition of the various controls used during the experiments**

Assay	MIW (mL)	DIW (mL)	Waste rocks (mL)	Inoculum (mL)	Air (mL)
1. MIW	1 000	-	-	-	-
2. MIW + DIW	850	150	-	-	-
3. Waste rocks + DIW	-	800	200	-	-
4. Waste rocks + MIW	850	-	200	-	-
5. MIW + inoculum	995	-	-	5	-
6. MIW + air	850	-	-	-	150

2.5.4 Batch Bioreactor Monitoring

During these experiments, various parameters were followed in both the bioreactors and control samples to determine the influence of the source of carbon and its concentration on the SRB activity and consequently, the removal of metals (Cd and Zn). The oxido-reduction potential (ORP) was the first parameter determined in each bioreactor prior to sampling. This parameter was quantified without mixing the water to reduce the variability of the ORP measurement. Samples for the analysis of sulfate were collected using a 20-mL capacity bottle and stored in a freezer until analysis. Samples for carbon determination were collected in a 20-mL capacity bottle and acidified with H_2SO_4 (2%, v/v) before analysis. The pH was measured in the 20-mL carbon samples prior to the acidification of the samples to avoid the risk of contamination between each sample. For the determination of the Zn and Cd contents, 20-mL samples were collected and stored in a bottle at 4°C after acidification using HNO_3 (2%, v/v). For each sampling, the total sampling volume collected was approximately 60 mL. After each sampling, the 1 L-bioreactors and controls were filled to the top using DIW, to eliminate air and maintain anoxic conditions. The sampling period started at t = 0 days and continued biweekly over a 90-day period (14, 26, 47, 69 and 90 days).

2.5.5 Analytical Methods

The metals present in the effluents emerging from the bioreactors and controls were analyzed by atomic absorption (AA) using flame for Zn and graphite furnace for Cd (Perkin Elmer PinAAcle 900T, Waltham, MA, USA). At the beginning of each series of measurements, a calibration curve, prepared from SCP Science Cd AA Standard and SCP Science Zn AA Standards, was analyzed. Control standards were prepared from a mixed standard (PerkinElmer GFAAS, Waltham, MA, USA) to ensure the quality of the results. The AA detection limits were determined as 0.05 µg/L for Cd and 2 µg/L for Zn. For rock characterization, metal concentrations were determined by ICP-AES using a Varian (model AX CCO Vista Simultaneous ICP-AES, Palo Alto, CA, USA). Calibration solutions were prepared using certified solutions (SCP Science, Baie-D'Urfé, QC, Canada), and control standards (SCP Science Multi items, Baie-D'Urfé, QC, Canada) were analyzed every 20 samples to validate the results.

The concentration of sulfates present in the water was determined using the EPA 9038 (sulfate turbidimetric) reference method. The detection limit for sulfate using this method was estimated as 5 mg/L. Total organic carbon (TOC) was measured using a Skalar Formacs HT instrument (Skalar, Breda, Netherlands). Calibration curves for TOC were controlled daily using a TOC control (AccuSPEC

TOC Standard 1 000 mg/L, AccuSPEC TIC Standard 1 000 mg/L, SCP Science, Baie-D'Urfé, QC, Canada). The detection limit of the TOC measurement was estimated as 2 mg/L.

pH measurements were performed using a pH meter (Epoxy body pH electrode, Oakton, Brisbane, Australia) equipped with a double junction Ag/AgCl electrode (Cole Parmer Canada, Montréal, QC, Canada). Calibration of the pH meter was performed daily using certified buffer solutions (buffer solution pH = 10.00; pH 7.00; pH 4.00, Fisher Scientific, Montréal, QC, Canada). ORP measurements were performed using a double junction electrode ORP (ORP Electrode tested. 59001-77, Cole Parmer, Montréal, QC, Canada). Calibration of the ORP meter was performed using a certified solution (ORP Standard, Thermo Fisher Scientific, Waltham, MA, USA). The pH and ORP probes were rinsed with DIW between each sample.

2.5.6 Statistical Analysis

The experiments carried out with molasses, methanol and a mix of both as carbon sources were performed in triplicate (*n* = 3). Statistical tests were used to determine whether the carbon sources had a significant impact on metal removal. The mean values and standard deviation were calculated for each batch of experiments carried out on the same carbon source sample under the same experimental conditions. Using a *t*-test, the normalized value (*t*-value) was determined using the mean values ($\bar{Y}_a$ and $\bar{Y}_b$).

2.6 Results and Discussion

2.6.1 Mine-Impacted Water and Waste Rock Characterization

2.6.1.1 Composition of the Waste Rock Samples

Waste rocks were sampled from two different waste rock piles at the Keno Hill mine site, Yukon Territory (YT, Canada). The two types of waste rocks were mixed (50/50, v/v) in this study to represent the geology of the underground tunnels generating mine drainage. The particle size distribution and the chemical composition of the different waste rocks are presented in Table 2-3. The two waste rock samples had a similar particle size distribution, with coarse material (> 10 mm) accounting for the largest fraction of the soil, i.e., 45.4% for WR1 and 40.8% for WR2. It was also observed that the weight percentages decreased with a decrease in particle size for both waste rock samples. The smallest fraction (< 0.125 mm) represented 11.4% and 13.9% for WR1 and WR2, respectively.

According to our results, the loss on ignition (LOI) varied from 0.99% to 9.59% depending on the waste rock sample and the particle size. LOI was higher in the fine fraction (< 0.125 mm) than in the coarse fractions for both waste rock samples. Indeed, for WR1, LOI was 1.12% for the > 10 mm fraction and 9.59% for the fine fraction (< 0.125 mm). These results indicated that the fine fraction contained larger amounts of carbonates and sulfurs that were lost during the ignition at 1 000°C. According to the chemical composition of the waste rock samples expressed as oxides presented in Table 2-3, silicon dioxide (SiO_2) was the predominant component present in the two waste rock samples. The SiO_2 contents varied between 98% and 73.2% for WR1 and between 96.3% and 59.1% for WR2, depending on the solid fraction. The predominance of SiO_2 showed that the two waste rock samples studied were mainly composed of quartz. Moreover, as particles became finer, the proportion of other metal oxides increased. In the finer fractions, the increase in aluminum and potassium oxides highlighted the presence of clay in these fractions as kaolinite ($Al_2Si_2O_5(OH)_4$) or illite ($(K,H_3O)(Al,Mg,Fe)_2(Si,Al)_4O_{10}[(OH)_2,(H_2O)]$). It was noticed that the main contaminants, cadmium (Cd) and zinc (Zn), present in the mine drainage were not present as oxides in large proportions in the waste rock samples.

Table 2-3 **Particle size distribution (%, w/w) and chemical composition [a] (%, w/w) of WR1 and WR2**

Fraction (mm)	>10	10 – 2	2 – 1	1 – 0.250	0.250 – 0.125	<0.125	Total
WR 1							
Fraction proportion	45.4	27.3	7.88	5.48	2.52	11.4	100
Al_2O_3	2.29	4.55	8.77	9.92	11.3	16.8	5.72
CaO	0.21	0.34	0.29	0.27	0.29	0.34	0.27
CdO	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr_2O_3	0.01	0.01	0.01	0.01	0.02	0.02	0.01
Fe_2O_3	0.49	0.91	1.40	1.44	1.84	6.70	1.47
K_2O	0.52	0.91	1.61	1.78	2.10	2.97	1.10
MgO	0.11	0.29	0.35	0.36	0.39	0.59	0.25
Na_2O	0.09	0.18	0.28	0.30	0.33	0.48	0.19
PbO	0.01	0.39	0.31	0.19	0.13	0.68	0.23
SiO_2	98.0	84.3	88.4	73.2	85.5	73.5	89.0
ZnO	0.01	0.01	0.01	0.01	0.01	0.02	0.01
LOI[b]	1.12	2.09	3.30	4.30	4.48	9.59	-
WR 2							
Fraction proportion	40.8	27.4	9.52	4.94	3.46	13.9	100
Al_2O_3	2.56	6.48	9.82	10.4	15.0	22.7	7.94
CaO	0.04	0.08	0.06	0.08	0.11	0.14	0.07
CdO	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr_2O_3	0.00	0.01	0.01	0.01	0.02	0.03	0.01
Fe_2O_3	0.88	1.69	2.66	3.06	4.20	6.73	2.30
K_2O	0.53	1.34	2.04	2.21	3.08	4.80	1.66
MgO	0.12	0.30	0.39	0.42	0.59	0.94	0.34
Na_2O	0.04	0.14	0.18	0.21	0.39	0.54	0.17
PbO	0.04	2.37	0.74	0.76	0.89	1.53	1.02
SiO_2	96.3	88.2	82.5	80.6	74.8	59.2	86.1
ZnO	0.00	0.02	0.03	0.03	0.04	0.07	0.02
LOI[b]	0.99	2.47	3.20	3.70	4.93	7.99	-

[a] *Only major elements are presented.* [b] *LOI = loss on ignition.*

2.6.1.2 Mine-Impacted Water Characteristics

Table 2-4 presents the main parameters examined in the characterization of the mine-impacted water. This characterization was spread over five years (from February 2007 to March 2014) and a total of 98 samples for both metal concentrations and pH determination and 68 samples for sulfate concentration were collected. The concentration limits for the discharge of the effluent into the environment that applied to the Keno Hill Silver district (Water Use Licence QZ12-057 delivered January 30th 2013) are also presented in Table 2-4 for comparison.

Table 2-4 Characterization of the mine water from Keno Hill Silver district (5 years sampling, 98 samples for metals and pH, 68 samples for SO_4^{2-})

Parameters	Value	Discharge limitsa
[As] (ug/L)	36 ± 11	500
[Cd] (ug/L)	12 ± 8	50
[Fe] (ug/L)	19 600 ± 5 160	n.a.b
[Zn] (ug/L)	860 ± 197	500
pH	7.3 ± 0.5	6.5 – 9.5
[SO42-] (mg/L)	426 ± 182	n.a.

[a] *Water Use Licence QZ12-057 delivered January, 30th 2013.*

[b] *n.a : non applicable.*

During this time period, the average arsenic concentration was calculated to be 36 ± 11 µg/L, which was below the discharge limit of 100 µg/L. The average iron and sulfate concentrations were calculated to be 19.6 ± 5.2 mg/L and 426 ± 182 mg/L, respectively. The average Cd concentration was calculated to be 12 ± 8 µg/L and the average Zn concentration was calculated to be 860 ± 197 µg/L, exceeding the discharge limit of 500 µg/L for Zn.

The MIW had an initial Ca concentration of 152.3 ± 42.7 mg/L. The calcium present in the mine water collected at Silver King may contain some minor gypsum or ettringite precipitation but to a limited extent that is unlikely to affect the overall process (Fajtl *et al.,* 2002).

The MIW was neutral, with a pH of approximately 7.3 ± 0.5 over the five-year period. It can be assumed that carbonate minerals were also present in the old mining tunnels, and that they helped to reduce acidity and maintain the pH at a near neutral value.

As the MIW is groundwater from the Silver King Mine site, the MIW's temperature is stable all year long and is around 5°C (Alexco's data).

2.6.2 Evaluation of the Potential of Metal Leaching from Waste Rock Samples

The Sobek test was conducted to evaluate the NP of the waste rock samples. The strength of the effervescence roughly corresponded to the amount of reactive carbonates present and was considered as a "slight" fizz. After titration, the NP of the mixed waste rocks was estimated as 20 kg $CaCO_3$/t, and the AP was estimated as 9.37 kg $CaCO_3$/t. The NNP was approximately 10.6 kg $CaCO_3$/t, and the NPR was estimated as 2.13. According to Price *et al.,* (1997), a rock sample is "likely" to generate ARD when the NPR value is lower than 1. If the NPR value is between 1 and 2, the sample can "possibly" generate ARD, especially if the NP is insufficiently reactive or is depleted at a faster rate than sulfides. Between values of 2 and 4, the ARD risk is "low" and the sample is not considered as potentially ARD generating unless there is significant preferential exposure of sulfides along fracture planes, or extremely reactive sulfides in combination with insufficiently reactive NP are present in the rock sample. Finally, when the NPR is higher than 4, the sample is not considered as having ARD-generating potential. Hence, with a NPR value of 2.13 measured for the waste rock samples studied here, the calculated NPR showed a "low" risk for ARD generation according to Price *et al.,* (1997), but this value is very close to the limit of waste rock defined as having a "possible" risk of ARD generation.

Figure 2-1 presents the residual concentrations of Zn (a), Cd (b), and SO_4^{2-} (c) and the pH measured in the control samples during the entire exposure time of the experiments. The concentrations of both Zn and Cd measured in the control "Waste Rocks + DIW" indicated that the combined waste rocks (WR1 and WR2) released 784 ± 121 µg/L of Zn and 22.2 ± 4.0 µg/L of Cd after 90 days, whereas the concentration of sulfates was approximately 252 ± 19 mg/L and the pH was reduced from 7.0 to 3.1 ± 0.3.

The release of metals, sulfates and the production of acidity from waste rocks is typical of AMD, characterized by low pH and high concentrations of heavy metals, sulfates and other toxic elements and occurs when sulfide-bearing material is exposed to oxygen and/or water (Akcil and Koldas, 2006). Hence, the waste rocks used in the present study showed a good potential to produce AMD as the concentrations of both Zn and Cd exceeded the discharge limits and the pH was very acidic. This observation was in agreement with the results from the Sobek tests, which were at the limit between "low" and "possibly" acid generating, and the elevated concentrations of Zn (860 ± 197 µg/L) and Cd (12 ± 8 µg/L) observed over five years during the monitoring of the quality of the well water from the old tunnels.

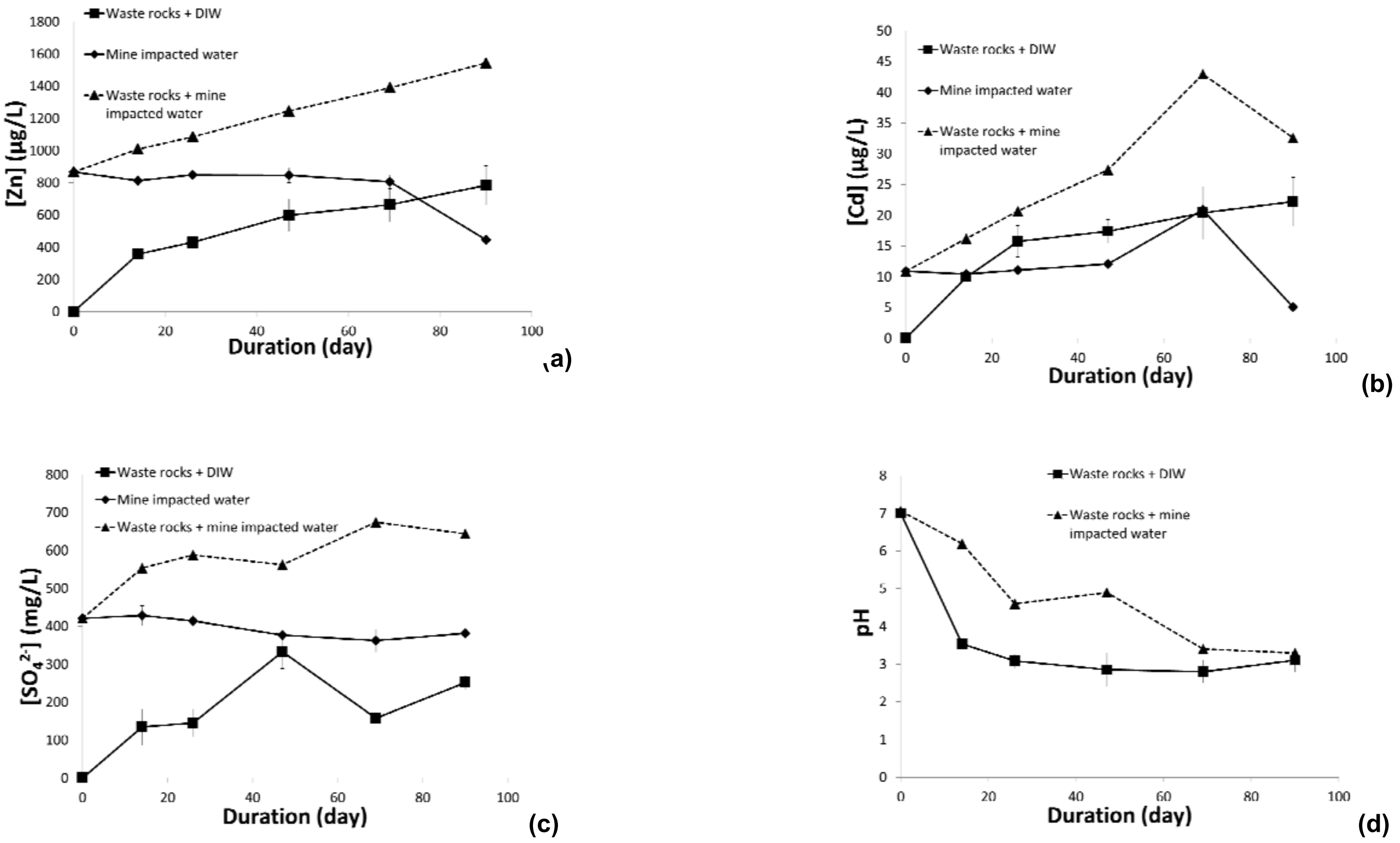


Figure 2-1 Main parameters for the controls during the batch test experiment. Zn concentration (µg/L) (a.), Cd concentration (µg/L) (b.), Sulfate concentration (mg/L) (c.), and pH (d.)

In the control “MIW only”, when no creek sediment was used, the initial concentration of Zn decreased from 868 µg/L to 446 ± 9 µg/L after 90 days. A decrease was observed in the Cd concentration from 10.9 µg/L to 5.1 ± 0.1 µg/L, as well as a decrease in the ORP from 157 mV to 35 ± 11 mV during the same period. When mine-impacted water was not in contact with the waste rocks, pH remained neutral during the 90 days experiment, anoxic conditions were generated along with a reduction in metal concentrations and a 9.5% decrease in sulfate concentration (421 mg/L to 381 ± 12 mg/L). Because gypsum and ettringite precipitation is unlikely to occur with low initial Ca concentration, the decrease of both metals and sulfate concentration seemed to suggest that native SRB were likely present in the mine water and may have been able to grow, consume sulfate to some extent and to allow the precipitation of metal as sulfides in 90 days. Moreover, negative redox potential values were measured in this control, which seemed to be optimal for the growth of SRB according to several authors (optimal values around -100 to -200 mV) (Abhilash *et al.,* 2015; Gibert *et al.,* 2002; Hao *et al.,* 1996). These results were in accordance with those obtained by several authors who reported the presence of native SRB in AMD. Indeed, the presence of native SRB in AMD has also been reported by Groudev *et al.,* (1978) and Ledin and Pedersen (1996). Londry (2013) also reported the presence of many species of SRB in the Keno Hill mine site. Meanwhile, Fortin *et al.,* (2000) found native SRB in AMD with a pH lower than 2. It is noteworthy that the concentrations of both Cd and Zn were passively reduced below the discharge limits at the Bellekeno site, without the addition of any chemicals. It must be noticed that, as DIW was used after each sampling to fill the bioreactors in order to maintain anoxic conditions, the reduced concentrations of Zn and Cd observed in control 'MIW only' may be partly due to dilution.

On the other hand, when the MIW was in contact with the waste rocks (CT4), the potential action of the native SRB observed would not have been sufficient to reduce the concentrations of Zn and Cd to acceptable levels. Indeed, the concentrations of Zn and Cd actually increased by 177% (from 868 µg Zn/L to 1 546 µg Zn/L) and by 283% (from 10.9 µg Cd/L to 32.6 µg Cd/L), whereas the concentration of sulfates increased by 147% (from 421 mg SO_4^{2-}/L to 644 mg SO_4^{2-}/L). The increase in the sulfate content and the pH decrease from 7.0 to 3.4 in this control might be an indicator of the oxidation of sulfide-bearing minerals present in the waste rock samples (WR1 and/or WR2). Moreover, it appeared that the ORP was high (approximately 362 mV) after the experimental period (90 days). Not surprisingly, this control

(“Waste Rocks + MIW”) clearly indicated that if the mine water was in contact with the waste rocks, similar to the ground water flowing through the old tunnels, the leaching of both Cd and Zn is likely to occur, releasing detrimental amounts of contaminants in water. This showed that as long as the water flows freely through the tunnels in contact with mine rocks, mine water treatment will be required. Diverting the water from the tunnels is not considered as a viable option because the tunnels were driven into groundwater, and a long-term mine water treatment will be required to remove metals from this particular mine-impacted water.

2.6.3 Performances of Carbon Sources with respect to SRB Activity and Metal Removal from Mine Drainage

2.6.3.1 Impact of Carbon Sources on SRB Activity

As described in Table 2-1, different carbon sources, i.e., molasses and methanol and a mix of both, were tested to assist bacterial growth. The first series of experiments were carried out with a carbon concentration defined by the molar ratio of sulfate to carbon according to Equation 2-1. Figure 2-2 presents the residual concentration of carbon and sulfate during the 90-day experiment when molasses, methanol or a mix of molasses and methanol was added to the bioreactors at the same initial concentration of carbon (C1 = 77 mg/L).

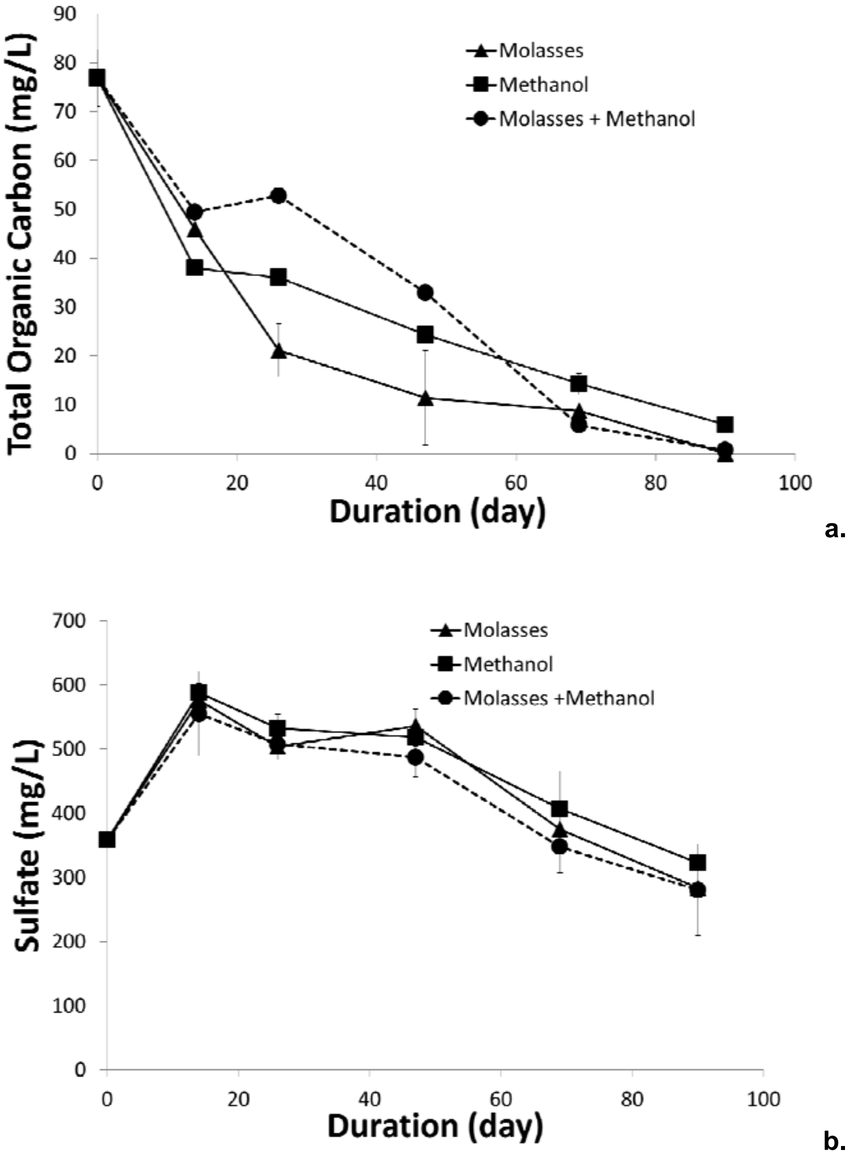


Figure 2-2 Carbon (a.) and sulfate (b.) concentration (mg/L) during the batch test

Complete carbon depletion was observed after 90 days when molasses was used, either on its own or mixed in with methanol (Figure 2-2 a.). When methanol was used as the sole carbon source, the concentration of carbon was only reduced from 77 mg/L to 5.9 mg/L after 90 days. According to these results, we noticed than the consumption of carbon was slightly faster for the experiments carried out in the presence of molasses alone than for the experiments conducted with methanol alone or a mix of methanol/molasses. Indeed, after 47 days, the *t*-values calculated when comparing the experiments molasses alone versus methanol alone and molasses versus molasses/methanol were 3.27 and 4.92, respectively. Methanol is successfully used as an electron donor in denitrification (Germonpre *et al.,* 1991; Weijma, 2000), and this chemical is well-known to be a reliable carbon source that supports bacterial growth. However, it can also be toxic to some microbes, or they might require an acclimation period (Tsukamoto, 1999). Indeed, alcohols are toxic to microorganisms at high concentrations, presumably because they damage the cell membrane and inhibit glycolytic enzymes (Dürre *et al.,* 1988; Weijma, 2000). As alcohol toxicity towards bacteria decreases with a decrease in the length of the carbon chain, it may be speculated that methanol toxicity will not occur at concentrations below 10 g/L (Weijma, 2000). In the present study, the methanol concentration used was 77 mg/L, which is far below the toxicity limits defined by Weijma (2000). Therefore, the most probable reason that can explain why the consumption of methanol was slower than molasses after 47 days is that sugars are generally consumed more rapidly by bacteria. Indeed, SRB are able to metabolize low molecular weight organic compounds such as organic acid, alcohol and amino acids (Gould *et al.,* 2012). Moreover, the most critical limiting factor for microbial activity is the amount of labile metabolizable carbon available from an added organic carbon source, such as molasses (Gibert *et al.,* 2004; Gould *et al.,* 2012; Neculita *et al.,* 2007; Zagury *et al.,* 2007).

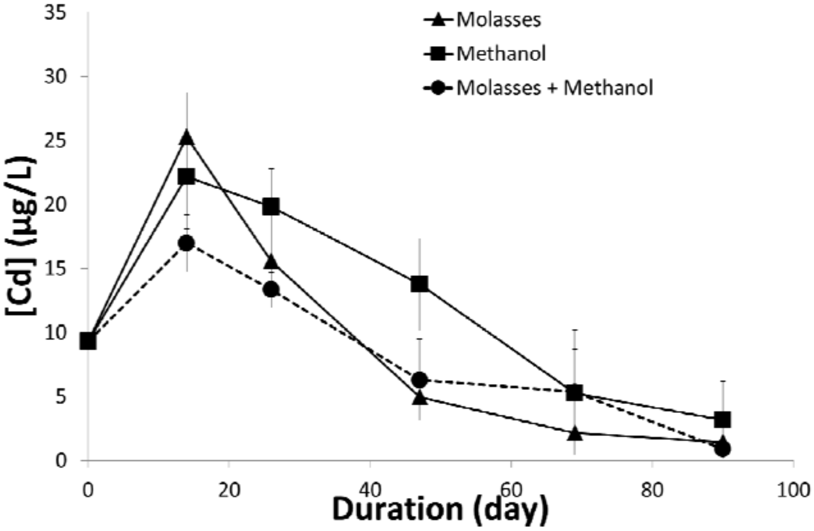
According to the results presented in Figure 2-2 b., the concentration of sulfate increased from 358 mg/L to 545-626 mg/L during the first two weeks of the experiment in all bioreactors. This release of sulfate might be due to oxidation of the sulfidic rocks in the early stages of the experiments, which was also supported by high ORP values, in the range of 249 to 346 mV (data not shown). Similar observations of sulfate release from the waste rocks were conducted during the monitoring of the control “Waste Rocks = DIW”, as described earlier (Figure 2-1 c.). After 14 days, the concentrations of sulfate started to decrease and continue to decrease over the experimental duration (90 days). Indeed, the concentration of sulfates decreased from 576 mg/L to 284 mg/L (50.7%) when molasses was used as a carbon source, from 588 mg/L to 322 mg/L (45.2%) when methanol was used and from 556 mg/L to 280 mg/L (49.6%) when the molasses and methanol mix was used. In the literature, sulfate consumption can be faster in similar batch test bioreactors, but experiments are generally conducted at a temperature optimal (T = 25°C) for the growth of SRB (Tsukamoto and Miller, 1999; Glombitza 2001). Indeed, Tsukamoto and Miller (1999) observed a diminution of 88% of the sulfate in column bioreactors after 180 days of experiments at 23-26°C whereas Glombitza (2001) reported a diminution of 90% of the sulfates after 20 days (T = 30°C).

The decrease in sulfate concentrations observed in all bioreactors might be due to the reduction of sulfates into sulfides in the presence of SRB in mine drainage and in the inoculum introduced into all bioreactors. Indeed, SRB are a ubiquitous group of microbes, and native SRB were found in the Keno Hill mine area (Londry, 2013) and a bioreactor was constructed and operated in the Keno Hill Silver District at the Galkeno 900 adit in May 2008. Organic substrate was initially added to support microbial growth until sulfate reduction by SRB became the predominant microbial activity in the reactor (Alexco, 2012).

Moreover, an obvious decrease in ORP values was observed between day 14 and day 69 (data not shown). The minimum ORP values reached -68.3 mV for the experiments carried out with molasses, -15 mV for those with methanol and -130 mV for those with the mix of molasses and methanol. The decrease in ORP to negative values indicated that the media became anoxic and that sulfides were formed in the presence of sulfate-reducing bacteria (SRB). The decrease of ORP values in the bioreactors was a good indicator of the presence of SRB in the systems. However, SRB's activity was also highlighted by laboratory observations including the visible formation of black precipitates (FeS) and the classic strong smell of H_2S (rotten eggs) as in the study conducted by Zhang and Wang (2013) who used SRB bioreactors supported by lactate or ethanol to treat mine water. When SRB are present in a low oxygen environment, they are able to catabolize sulfates, releasing sulfides under optimal ORP values ranging from -100 to -300 mV (Gloyna, 1972; Gibert *et al.,* 2002; Harerimana *et al.,* 2010). After 69 days of experimentation, the ORP values increased in every batch test, reaching positive values for some of them (in the case of molasses and methanol). This sudden increase in ORP values may be due to the low concentrations of carbon remaining in the bioreactors and that could be consumed by other bacteria. On the other hand, in every batch test, the values of pH remained between 7.0 and 5.2, which also represented optimal pH conditions for SRB activity (Wang *et al.,* 2013). All of these results (consumption of both carbon and sulfate, decrease in ORP values and favorable pH conditions) supported the hypothesis related to the presence of SRB in mine drainage and/or inoculum used in all bioreactors (Luptakova 2007; Wang *et al.,* 2013). Moreover, these results seemed to indicate that the SRB activity was more favorable when using molasses alone compared with methanol alone or a mix of molasses and methanol, due to the faster and higher consumption of carbon and sulfate throughout the duration of the experiments.

2.6.3.2 Impact of Carbon Sources on Cadmium and Zinc Removal Using SRB

Figure 2-3 presents the residual concentrations of Cd and Zn measured in each experiment over the 90 days. According to these results, it appeared that both Cd and Zn concentrations initially increased from 9.29 µg/L to 25.3 µg/L and from 738 µg/L to 1 533 µg/L, respectively, during the 14 first days of the experiments. These results are in accordance with the evolution of Cd and Zn concentrations observed in the controls (Figure 2-1), which was probably due to the oxidation of the sulfide minerals present in the waste rock samples and the leaching of metals, sulfate and acidity as defined by AMD production and highlighted with the CT4 control.



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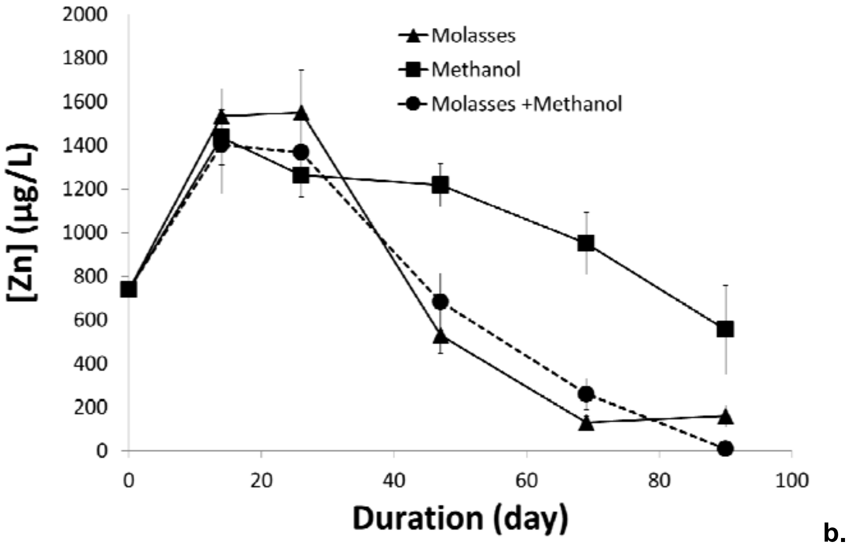


Figure 2-3 **Metal concentrations in each batch test. Cd concentration (µg/L) (a.), Zn concentration (µg/L) (b.)**

After 14 days of experimentation, the concentrations of both Cd and Zn started to decrease, irrespective of the source of carbon used. Indeed, after 90 days, the residual concentrations of Cd present in the effluent were approximately 1.4 µg/L, 3.2 µg/L and 0.9 µg/L for the assays performed in the presence of molasses, methanol and molasses/methanol, respectively. In addition, the residual concentrations of Zn measured after 90 days were approximately 160 µg/L, 550 µg/L and 9.0 µg/L in the presence of molasses, methanol and molasses/methanol, respectively. This decrease might be due to the reduction of sulfate to sulfide by SRB (Figure 2-2 b.) and the precipitation of Cd and Zn as sulfide precipitates. After 90 days of experimentation, the residual concentrations of Cd and Zn were lower than the discharge limit set by regulations ([Cd] < 50 µg/L and [Zn] < 500 µg/L), indicating that the addition of a liquid source of carbon was useful to facilitate a significant decrease in both Cd and Zn concentrations initially present in the mine drainage.

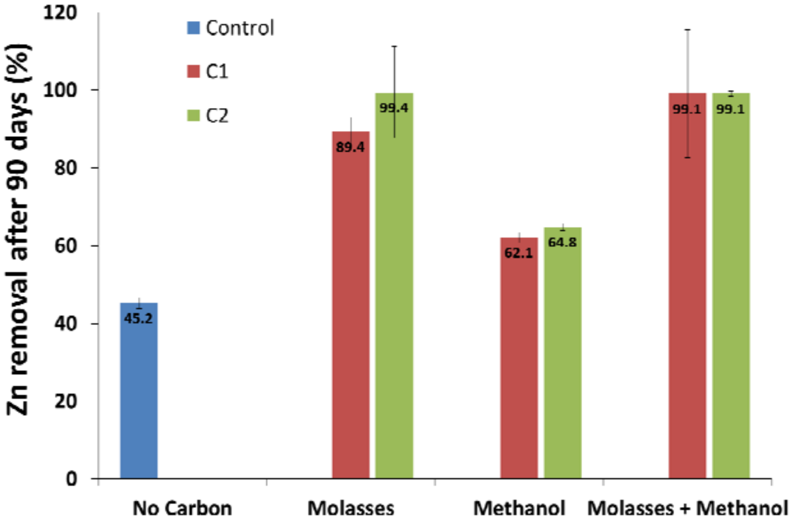
However, when comparing the results obtained with respect to the sources of carbon used, it appeared that the best removal yields were obtained for the experiments performed with the mix of molasses and methanol, allowing the removal of 94.6% of Cd and 99.1% of Zn after 90 days (residual concentration of

0.9 µg Cd/L and 9.0 µg Zn/L). According to the *t*-Student tests performed (results not shown), the sources of carbon used did not appear to have a significant influence on the removal of Cd and Zn after 90 days. However, it appeared that the sources of carbon used significantly affected the kinetics of metal removal from the mine drainage. Indeed, after 47 days of experimentation, the removal of Zn from the mine drainage was significantly higher for the experiments performed in the presence of molasses/methanol compared to molasses alone (*t*-value of 4.06) and methanol alone (*t*-value of 3.77). These results were in accordance with the observation made by Mayes *et al.,* (2011). Indeed, in their column experiments, Mayes *et al*., (2011) added methanol to the complex organic carbon sources (waste shells) to reduce Zn levels which were increasing. Similar to the present study, Gandy and Jarvis (2012) conducted a laboratory-scale column at a cold temperature of 8.4°C, which is far from the optimal temperature for mesophilic SRB's growth. Their columns contained a mixture of carbon sources (comprising limestone gravel, compost, wood chips and activated sludge) to treat mine water with an initial zinc concentration of 2–2.5 mg/L. Gandy and Jarvis (2012) removed approximately 96 % of zinc from 2–2.5 mg/L to an average of 0.14 mg/L total zinc and 0.08 mg/L dissolved zinc in the effluent water with an initial hydraulic residence time of approximately 19 h. There was an immediate decrease in effluent dissolved Zn after the addition of methanol. According to these results, the molasses/methanol source significantly reduced the retention time required in the bioreactor, which is an important parameter to consider for the design of an efficient and economically viable sulfate-reducing passive bioreactor.

2.6.3.3 Influence of the Concentration of Carbon Added to the SRPB on Cd and Zn Removal Efficiencies

Additional experiments were carried out in triplicate to determine the influence of the concentration of the carbon source on the SRB activity and therefore on the removal of Cd and Zn from the mine drainage. Figure 2-4 presents the removal yields obtained for Cd and Zn after 90 days for the experiments performed with different concentrations of carbon (C1 = 77 mg/L and C2 = 154 mg/L). In addition, these results were compared with the removal yields measured for the control CT1, which was conducted under the same conditions but without the addition of carbon, to determine how metal removals were affected when native SRB were not supported by any additional source of carbon.

When no carbon was added, only 45.2% of Zn and 51.1% of Cd were removed after 90 days of experimentation. On the other hand, when molasses was used at the C1 concentration, the concentration of Zn decreased from 1 533 µg/L to 160 µg/L after 90 days, corresponding to 89.4% removal. During the same period, the concentration of Cd decreased from 25.3 µg/L to 1.4 µg/L, corresponding to a removal of 94.6%. When molasses was used at the higher concentration (C2), the concentration of Zn decreased from 1 455 µg/L to 8.7 µg/L after 90 days, corresponding to 99.4% Zn removal, whereas the concentration of Cd decreased from 19 µg/L to 0.8 µg/L (96% removal). According to these results, it appeared that the addition of molasses at the C2 concentration was slightly more efficient for the activity of SRB and therefore for the removal of Cd and Zn by precipitation as sulfides compared with the C1 concentration. Moreover, the concentration of sulfate was reduced from 576 mg/L to 284 mg/L under C1 and from 545 mg/L to 363 mg/L under C2 using molasses. At day 69, the ORP values were estimated as -68 mV under C1 and as -180 mV under C2, which might indicate higher SRB activities (Gloyna, 1972; Harerimana *et al.,* 2010). This would explain why metal removal was slightly better with C2 than C1.



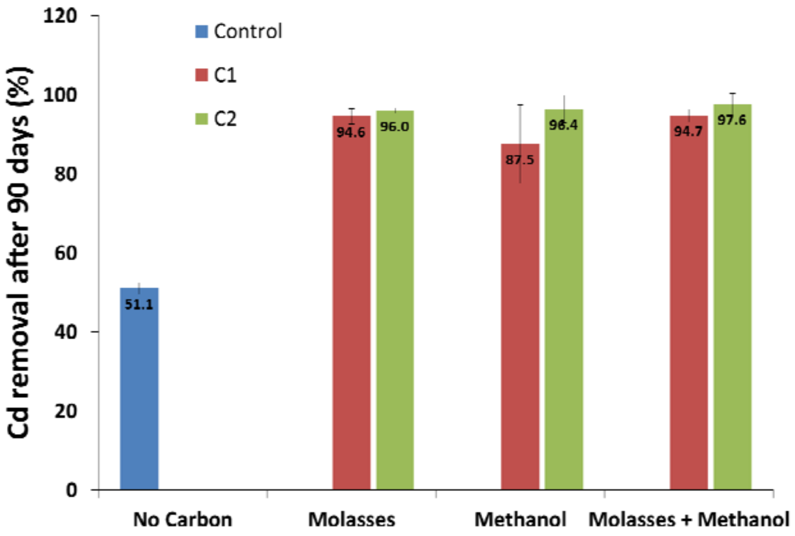


Figure 2-4 **Metal removal under C1 and C2**

Similarly, when methanol was added at the C1 concentration, the Zn concentration decreased from 1 436 µg/L to 554 µg/L after 90 days, reaching 62.1% Zn removal, whereas the concentration of Cd decreased from 22 µg/L to 3.2 µg/L, resulting in 87.5% removal. When methanol was added at the C2 concentration, the removal yields obtained for both Cd and Zn were slightly better (64.8% for C2 versus 62.1% for C1 for Zn and 96.4% versus 87.5%, respectively, for Cd). Indeed, after 90 days of experimentation, the concentrations of Zn and Cd decreased from 1 572 µg/L to 559 µg/L and from 23 µg/L to 0.9 µg/L, respectively. During the same period, the concentration of sulfate was reduced from 588 mg/L to 322 mg/L under C1 and from 618 mg/L to 264 mg/L under C2. On day 69, the ORP values were approximately -15 mV (C1) and -98 mV (C2). According to these results, it appeared that the concentration of methanol used in these experiments (C1 = 77 mg/L and C2 = 154 mg/L) did not influence the activity of SRB and therefore the removal of Cd and Zn from the mine drainage by passive precipitation.

Finally, when the mix of molasses and methanol was used, the concentration of Zn decreased from 1 403 µg/L to 9 µg/L at the C1 concentration and from 1 505 µg/L to 13 µg/L at the C2 concentration.

According to these results, up to 99.1% of Zn was removed from the mine drainage after 90 days, irrespective of the carbon concentration used. The Cd concentration decreased from 17 µg/L to 0.9 µg/L at the C1 concentration and from 21 µg/L to 0.6 µg/L at the C2 concentration. At the C1 concentration, more than 94.7% of Cd was removed from the mine drainage, whereas up to 97.6% of Cd was removed in response to the C2 concentration. These results were in accordance with the evolution of the residual concentration of sulfates, indicating that the consumption of SO_4^{2-} was higher under C2 than C1. Indeed, the concentration of sulfate was reduced from 555 mg/L to 280 mg/L under C1 and from 626 mg/L to 171 mg/L under C2. On day 69, the ORP values were approximately -65 mV (C1) and -93 mV (C2). These results revealed that the use of the C2 concentration for the molasses and methanol mix was more favorable for the SRB activities, leading to a slight but not significant improvement in Cd and Zn removal from the mine drainage.

In conclusion, increasing the concentration of carbon from 77 to 154 mg/L in the bioreactors improved metal removal by 10% (when molasses was used as the carbon source), 2.7% (when methanol was used) and 0% (when the molasses + methanol mix was used) for Zn and by 1.4% (when molasses was used), 8.9% (when methanol was used) and 2.9% (when the molasses + methanol mix was used) for Cd. It is interesting to note that a significant increase in the amount of carbon added to the bioreactors did not generate a significant improvement in the removal of either Cd or Zn. Hence, SRB do not consume more carbon than they need according to Equation 2-1.

2.7 Conclusion

Passive treatment systems applied to mine drainage represent a promising alternative to conventional neutralization techniques. They may be employed during the mine operation as well as in the post-closure period to allow a secure and economically viable treatment of contaminated mine drainage. Batch test bioreactors were designed at a laboratory scale and operated for 90 days at 4.5°C. This was done to determine whether cold temperatures hindered the growth and activity of sulfate-reducing bacteria used to treat mine drainage originating from the Keno Hill mine site (Yukon Territory) that was contaminated by Cd and Zn. To promote bacterial growth, different carbon sources such as molasses, methanol and a mix of both were added to each batch test at different concentrations based on the molecular ratio relative to sulfate.

According to our results, the mine drainage initially contained 10.93 µg Cd/L and 868 µg Zn/L, leached during the oxidation of sulfide minerals present in the waste rock samples. As these concentrations exceeded the discharge limits for Zn, an efficient passive treatment, based on the activity of SRB, must be developed to allow the discharge and to protect the Nordic environment. The complete consumption of the carbon sources used and of the sulfates present in the mine drainage observed during these experiments highlighted the presence of native SRB in SRPB. The addition of liquid carbon sources to SRPB led to a decrease in both Cd and Zn concentrations in mine drainage to below the discharge limits established for the Silver King Adit mine site (< 50µg Cd/L and < 500 µg Zn/L). After 90 days, the concentration of Zn and Cd decreased from 1455 to 8.7 µg/L (up to 99.4% reduction) and from 21 to 0.6 µg/L (up to 97.6% reduction), respectively, when molasses + methanol was used as the carbon source. These reductions in Cd and Zn concentrations might be due to the reduction of sulfates to sulfides by SRB and their precipitation as sulfides (ZnS and CdS). Moreover, it appeared that the molasses/methanol mix was slightly more efficient for both SRB growth and metal removal than molasses or methanol alone. A significant increase in the amount of carbon (C1 = 77 mg/L and C2 = 154 mg/L) in the bioreactors did not generate a significant improvement of either Cd or Zn removal from the mine drainage, indicating that SRB do not consume more carbon than they need. These findings indicated that native sources of SRB might be successfully used for the treatment of mine water contaminated with heavy metals.

2.8 Acknowledgments

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3 CHAPITRE 3

Influence of organic carbon sources on the reduction of sulfates from mine impacted water using sulfate-reducing bacteria bioreactors in cold climates

Influence de différentes sources de carbone organique sur la réduction des sulfates issus d'un drainage minier acide par des bactéries sulfato réductrices en climat froid

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3.1 Résumé

Le drainage minier acide (DMA) est actuellement l'un des principaux défis environnementaux auquel fait face l'industrie minière en régions nordiques. Les technologies classiques actuellement utilisées dans le traitement de DMA sont coûteuses tant en termes de coûts d'exploitation que de coûts d'investissement et il existe une demande croissante pour développer des traitements passifs fiables et durables à des coûts raisonnables. Les bioréacteurs passifs sulfato-réducteurs (BPSR) offrent une technologie écologique et prometteuse qui repose sur l'utilisation de bactéries sulfato-réductrices (BSR). Cette étude vise à présenter une revue de la littérature des différentes sources de carbone utilisées au cours des dernières décennies pour soutenir les activités métaboliques des BSR et réduire les sulfates en sulfures. Des expériences ont été menées en laboratoire pour tester diverses sources de carbone simples et complexes disponibles au Yukon comme donneur d'électrons pour la réduction des sulfates afin d'optimiser l'élimination subséquente du Cd et du Zn présents dans le DNC produit au Yukon. Les réacteurs, d'une capacité de 1 L, ont été échantillonnés toutes les deux semaines à 5°C sur une période de 162 jours. Les paramètres suivis étaient : le pH, le potentiel d'oxydoréduction (POR), les concentrations résiduelles de Zn, Cd, sulfates et carbone organique total (COT). Après 162 jours, une diminution des concentrations en COT et en sulfates a été observée dans tous les bioréacteurs. Une longue période d'adaptation a été nécessaire avant que la consommation de la source de carbone ne commence, ce qui pourrait être attribué aux températures froides. Les sources organiques simples de carbone (méthanol et éthylène glycol) et les sources organiques complexes de carbone (huile de pomme de terre, résidus de brasserie, tourbe et paille) utilisées pour soutenir la croissance des BSR à 5°C ont montré que le méthanol et l'éthylène glycol ont entraîné une diminution des concentrations en sulfates de 71,2% et 36,9%, respectivement. Tandis que la diminution des teneurs en sulfates ont été limitées à 13,8% et à 5,3% pour la tourbe et la paille, respectivement. L'utilisation de sources organiques simples de carbone semble fiable et permettait de mieux contrôler la quantité de carbone injectée dans les BPSR, mais pourrait exiger des conceptions plus sophistiquées pour les bioréacteurs passifs, ce qui pourrait entraîner une augmentation des coûts, en particulier pour les sites miniers en régions éloignées.

L'utilisation de sources de carbone complexes peut, cependant, être utile car elles sont généralement moins chères et sont dégradées plus progressivement que les sources organiques simples de carbone.

Mots clés : Climat froid, Traitement d'effluent minier, Traitement biologique passif, Bactérie sulfato-réductrice, Métaux, Source de carbone

3.2 Abstract

Acid mine drainage (AMD) is currently one of the major environmental challenges facing the mining industry in northern climates. Conventional active technologies used in the treatment of AMD are expensive, in terms of both operating and capital costs, and there is increasing demand to develop reliable, cost-effective, and sustainable passive treatments. Passive sulfate-reducing bioreactors (PSRB) offer a promising and green technology that relies on the use of sulfate-reducing bacteria (SRB). The aim of this study was to present an updated literature review of the different carbon sources used in recent decades to support metabolic activities of SRB and to reduce sulfate to sulfide. Laboratory scale experiments were conducted to test various simple and complex sources of carbon available in the Yukon as electron donors for sulfate reduction, to allow the subsequent removal of Cd and Zn from Yukon NCD. The 1-L capacity reactors were monitored biweekly over a 162-day period, at 5 °C. The pH, the oxidation-reduction potential and the residual concentrations of Zn, Cd, sulfates, and total organic carbon were measured. After 162 days, a diminution of both TOC and sulfate concentrations was observed in all bioreactors. A long adaptation period was necessary before the consumption of the carbon source started, which might be due to the cold temperature. The simple organic sources of carbon (methanol and ethylene glycol) and complex organic sources of carbon (potato oil, brewery residue, peat and straw) used to support SRB growth at 5 °C showed that methanol and ethylene glycol led to a diminution of sulfate concentrations by 71.2% and 36.9%, respectively. The decrease of sulfate concentrations was limited to 13.8% and 5.3% when using peat and straw, respectively. The use of simple organic sources of carbon seemed to be reliable and allowed better control of the amount of carbon injected in the PSRB. However, more sophisticated designs for passive bioreactors may be required, which could lead to increased costs, especially for remote mine sites. The more complex carbon sources may be useful because they are usually cheaper and are degraded more gradually than simple organic sources of carbon.

Keywords: Cold climate; Mine water treatment; Passive biological treatment; Sulfate-reducing bacteria; Metal removal; Source of carbon.

3.3 Abbreviations

AA	Atomic adsorption
AMD	Acid mine drainage
AP	Generation potential
ARD	Acid rock drainage
DIW	Deionized water
DNA	Deoxyribonucleic acid
HTR	Hydraulic retention time
LDPE	Low-density polyethylene
MIW	Mine impacted water
NNP	Net neutralization potential
NP	Neutralizing potential
ORP	Oxidation reduction potential
PSRB	Passive sulfate-reducing bioreactors
PTS	Passive treatment systems
SRB	Sulfate reducing bacteria
SRPB	Sulfate-reducing passive bioreactors
TC	Total carbon
TOC	Total organic carbon
WR	Waste rocks

3.4 Introduction

Passive Treatment Systems (PTS) have been considered as a promising alternative to replace active treatment systems to treat Mine Impacted Water (MIW) from abandoned or closed mining sites for many years. Passive sulfate-reducing bioreactors (PSRB) are one of the options available among the PTS for the decontamination of sulfate and metal enriched effluents (Martins *et al*. 2009). The performances of these processes depend on the understanding of the microorganisms involved and their microbial activity. The role of the microbial community must be well-understood to optimize the design and the performance of these passive systems (Johnson and Hallberg 2005; Kaksonen *et al*. 2004a; Sheoran *et al*. 2010).

A group of anaerobic bacteria called sulfate-reducing bacteria (SRB) can reduce sulfate to sulfide using an organic source as an electron donor and sulfate as a terminal electron acceptor (Gibert *et al*. 2002; Postgate 1979). The Equations 1 and 2 present the reaction of the reduction of sulfate to sulfide and the precipitation of metals present in solution as metal sulfides, respectively. These reactions occurred during the operation of a PSRB (Neculita *et al.* 2008).

Équation 3.1 $SO_4^{2-} + 2\ C\ (\text{organic}) + 2\ H_2O + SRB \leftrightarrow H_2S_{(aq)} + 2\ HCO_3^-$

Équation 3.2 $M^{2+} + H_2S \leftrightarrow MS_{(s)} + 2\ H^+$

The efficiencies of these processes rely on three major parameters: the concentration of metals present in the acid mine drainage (AMD), the presence of both SRB and substrate (source of carbon) and the hydraulic retention time (Neculita *et al*. 2008).

The reduction of sulfate using SRB is an energy-consuming process (Barnes 1998). According to the Equation 1, one mole of sulfate is reduced to hydrogen sulfide, consuming two moles of carbon which are oxidized (Hammer 1989; Neculita *et al.* 2008). However, the concentrations of dissolved organic carbon present in AMD are typically very low (< 10 mg/L) (Kolmert and Johnson 2001; Sheoran *et al*. 2010) and are usually not high enough to accomplish biological sulfate reduction (Martins *et al*. 2009). Thus, the addition of a significant amount of an energy-rich reductant is necessary and the choice of the adequate source of carbon will greatly influence the efficiency and the economic viability of PSRB. According to several authors, the most critical factor limiting the growth and the activity of bacteria and therefore, the performances of PSRB is the availability of carbon (Gibert *et al*. 2004; Neculita *et al*. 2007; Zagury *et al*. 2007). The composition of the organic matter used in the PSRB must be carefully considered when developing the process, as it ultimately determines the performances of metal removals (Gibert *et al*. 2002).

This study aims to evaluate the performance of six simple (methanol and ethylene glycol) and (potato oil, peat, brewery residue and straw) complex sources of carbon available in northern Yukon to support the growth of native SRB in groundwater contaminated by metals and where the temperature is around 5°C all year long.

3.5 Literature review

The literature reported a wide variety of natural organic substrates that were tested as a carbon nutrient source for SRB growth. Several authors classified 2 types of substrates, which can be used as carbon sources for microbial growth: “direct” or “simple” organic substrates and “indirect” or “complex” organic substrates (Knee *et al*. 2001; Sheoran *et al*. 2010). Table 1 summarizes the performances of various PSRB using simple or complex sources of carbon to reduce sulfates.

3.5.1 Direct/simple organic carbon sources

According to Sheoran *et al.* (2010), direct/simple organic substrates do not require decomposition by other microorganisms to provide nutrients to SRB. Those direct/simple carbon sources include alcohols (e.g., methanol and ethanol), organic acids (e.g., acetate, lactate, formate and pyruvate) and sugars (e.g., sucrose). Usually, simple forms of organic substrates are preferred by SRB as carbon sources because they are readily accessible without needing to be decomposed beforehand (Knee *et al*. 2001). The use of direct substrates allows a better control of the amount of carbon injected in the PSRB but requires the design of more sophisticated reactor and may not be suitable for remote mine sites (Zaluski *et al*. 2006).

Table 3-1 Performances of sulfate reduction by SRB from effluents using a PBSR – A review

Source of carbon	System	$[SO_4^{2-}]$ (ppm)	RT* (days)	Temperature (°C)	Sulphate removal yield (%)	Reference
Simple organic carbon						
Methanol	Column	300	0.27	23 - 26	88	Tsukamoto and Miller (1999)
	Pilot plant	1,900 – 2,100	0.3 - 2.6	30	90	Glombitza (2001)
	Batch	576	90	5	51	Nielsen *et al.,* (2016)
Ethanol	Fluidized bed reactor	2,000	1	35	90	Sahinkaya *et al.,* (2011)
	Batch	3,000	40	25	65	Pagnanelli *et al.,* (2011)
	Batch	1,600	17.7	30	45	Luptakova and Macingova (2012)
Lactate	Batch	2,000	0.42	35	99	Zhao *et al.,* (2008)
	Column	300	0.27	23 - 26	69	Tsukamoto and Miller (1999)
	Batch	3,000	9	25	65	Pagnanelli *et al.,* (2011)
Sodium lactate	Batch	1,600	17.7	30	48	Luptakova and Macingova (2012)
Calcium lactate	Batch	1,600	17.7	30	48	Luptakova and Macingova (2012)
Saccharose	Batch	1,600	17.7	30	30	Luptakova and Macingova (2012)
Glucose	Batch	1,600	17.7	30	28	Luptakova and Macingova (2012)
Cellulose	Batch	4,800	125	n.s**	<99.8***	Waybrant *et al.,* (1998)
Complex organic carbon						
Mushroom compost	Pilot scale continuous bioreactor	1,002	5 - 17	18 - 24	17	Dvorak *et al.,* (1992)
Mushroom compost composed of composted Straw, Hay horse and Poultry manure, Ground corncobs, Gypsum, Limestone (mix)	Column	2,000	0.5	n.s	75*** (with lactate addition) 0 (no lactate addition)	Hammack and Endenborn (1992)
Cow manure and Whey (mix)	Batch	3,307***	203	14-19	19-27	Christensen *et al.,* (1996)
Sheep manure, sawdust and leaf mulch (mix)	Batch	1,200	26	n.s	<99.2***	Waybrant *et al.,* (1998)

Methanol is one of the most commonly used simple sources of carbon which has the advantage of having a low freezing point and low viscosity (Glombitza 2001; Tsukamoto and Miller 1999). Tsukamoto and Miller (1999) studied the reduction of sulfate by SRB in the presence of methanol or lactate during 100 days of experiments performed at room temperature (23 – 26°C). According to their results, it appeared that methanol was slightly better for sulfate reduction than lactate with reduction yields reaching 88% and 69%, respectively. Glombitza (2001) also used methanol to treat MIW. A decrease in the concentration of sulfate of about 90% was observed within 20 days in the presence of immobilized microorganisms (T = 30°C).

Another common source of carbon, ethanol, has been widely studied over the last decades (Sahinkaya *et al*. 2011; Nagpal *et al*. 2000; Pagnanelli *et al*. 2012, Widdel 1988). For example, Pagnanelli *et al*. (2012) compared the performances of both ethanol and lactate for the growth of SRB and therefore the reduction of sulfates. Their results demonstrated that ethanol and lactate both lead to a sulfate reduction rate of 65 ± 5%. However, ethanol proved to be a substrate more competitive than lactate in kinetic terms at room temperature (25°C). Sahinkaya *et al.* (2011) reached 90% of sulfate removal using ethanol in a real AMD at 35°C. According to several authors, ethanol is usually converted to acetate by the SRB (Mechalas and Rittenburg 1960; Nagpal *et al*. 2000; Szewzyk and Pfennig 1990).

Lactate is a well-known simple source of carbon that has been extensively investigated and shown to be one of the most preferred electron donors by SRB (Hammack *et al*. 1994; Kaksonen *et al*. 2004; Song *et al*. 1998). Zhao *et al*. (2008) highlighted that sulfate removal yields can reach 99% at temperature varying from 24 to 30°C.

Many other low molecular weight organic compounds that can support SRB growth have been studied over the last decades as described in Table 1. The performances of poly lactic acid, a biodegradable aliphatic compound, to support SRB activity and therefore the reduction of sulfate have been investigated by Endenborn (2004). This organic compound is used as a long-term source of lactic acid to enhance bacterial growth, sulfate reduction activity and therefore, to promote the precipitation of heavy metals initially present in zinc smelter tailings. The use of direct substrates seems to be efficient to reduce sulfate to sulfide and allow the precipitation of metals as sulfides. However, the application of some direct substrates, such as ethanol, at remote mine sites can be complicated due to some public safety concerns related to more complicated reactor design (Zaluski *et al*. 2006).

3.5.2 Indirect/complex organic carbon sources

Indirect/complex organic carbon sources are those requiring decomposition by other microorganisms to provide nutrients to SRB. These substrates require complex microbial communities to degrade the organic matter and support SRB growth (Sheoran *et al.* 2010). Indirect/complex organic carbon sources are generally more appropriate for long-term operating systems than direct organic substrate as they will be degraded more slowly than simple organic carbon sources; thus supporting the SRB over longer periods (Drury 2000) and require lower maintenance. Therefore, complex organic sources of carbon are more appropriate for the treatment of mine impacted water from mining sites, located far from urban areas (Coetser 2006; Hiibel *et al*. 2011; Knee *et al.* 2001; Zaluski *et al*. 2006). Those indirect/complex sources of carbon include organic compost (e.g., spent mushroom compost, leaves compost, municipal compost, composted yard waste), wood chips, sawdust, cellulose, paper wastes, food production by-products (e.g., molasses, cheese whey, potato processing waste, fishmeal), agricultural products (e.g., hay, straw), manure (e.g., cow, horse, dried poultry waste, chicken litter) and sewage waste (e.g., digested sludge, sewage sludge, wastewater sludge) (Chang *et al.* 2000; Eger et Lappako 1988).

For example, Waybrant *et al.* (1998) used complex organic carbon sources to treat groundwater contaminated by metals derived from oxidized mine waste. They tested different sources of carbon including sewage sludge, leaf mulch, wood chips, sheep manure, maple sawdust, cellulose in eight organic-carbon reactive mixture of those carbon sources. The results obtained from batch tests conducted using simulated mine drainage indicated that, within 40 - 70 days at room temperature, sulfate concentrations decreased from 1200 - 4800 mg/L to concentrations lower than 10 mg/L for four of the mixtures tested. The highest sulfate-reduction yield was obtained with the reactive mixture containing five different organic sources (sewage sludge, leaf mulch, wood chips, sheep manure and sawdust) whereas the lowest measured removal yield of sulfate was observed with sewage sludge only. Indeed, it appeared that, after the acclimation period, sulfate reduction yields were generally higher in the reactive mixtures that contained a variety of organic sources (Waybrant *et al.* 1998, 2002).

Spent mushroom compost had been used, at pilot scale, as a complex carbon source in a sulfate-reducing bioreactor at ambient temperature (18 - 24°C) to treat acidic and iron-contaminated drainage (Dvoark *et al*. 1992). The compost, used in this study, consisted of a composted mixture of manure, hay, straw, corn cobs and wood chips that were conditioned with gypsum and limestone used for cultivating mushrooms.

Whey can also be used as a complex source of organic carbon (Christensen *et al*. 1996; Drury 1999). Whey contains fermentable water-soluble proteins, lactose and lactic acid (Drury 1999; Hargrove and Alford 1974; Scott 1981). Fermentation products and lactic acid supply SRB growth with a suitable source of carbon and energy (McInerney and Beaty 1988; Widdel 1988). Drury (1999) found that sulfate removal rates decreased over time from 250 to 120 mol/m^3.d with the addition of whey.

Zaluski *et al.* (2006) investigated the performances of new available and cheap organic mix consisted of English walnut shells and corn stover to support SRB activity. Walnut shells contained a high percentage (56%) of total organic carbon (TOC) and corn stover contained a high percentage of organic carbon in the form of cellulose that is used by cellulose-degrading bacteria to produce substrates, easily accepted by SRB. Wastes from the food industry were investigated by Martins *et al.* (2009). Wastes from the wine industry and cheese whey were used as carbon sources to support the growth of dissimilatory sulfate-reducing bacteria at 21°C. Cheese whey was mainly composed of fat (1.75%), proteins (0.30%), lactose (4.52%) and total solids (6.37%). The waste from the wine contained 53.5 g/L of ethanol. Results showed a decrease of the concentration of sulfates estimated at 50% and 95% within 20 days in the presence of cheese whey and waste from wine, respectively. Martins *et al.* (2009) concluded that waste from the wine industry and cheese whey seemed to be promising sources of carbon to promote SRB activity, if supplemented with calcite tailing that acts as a neutralizing and buffer material. Sulfate reduction process in the presence of cheese whey was considerably slower compared to the waste from wine industry. Coetser *et al.* (2006) have tested wood products (fresh and old pine chips, fresh pine prunings, fresh or old blue gum chips, fresh blue gum prunings, wattle chips, fresh wattle prunings and old wattle sawdust), grasses (dry and fresh Buffalo grass, dry and fresh Eragrostis grass, fresh Eragrostis grass, dry and fresh wild type grass) and other products such as molasses and bagasse. These authors concluded that the most readily biodegradable source of carbon contained high content of protein and low content of lignin (e.g. different grass types). Moreover, they observed that the experiments performed in the presence of the source of carbon with the higher carbohydrate and crude fat contents led to the higher capacity to reduce sulfate; whereas the lowest capacity to reduce sulfate was observed when using the source of carbon with the higher crude fiber content. There is significant indication that the performance of sulfate reduction can be enhanced by the use of mixtures composed of natural organic substrate compared to a single substrate (Amos and Younger 2003; Cocos *et al*. 2002; Waybrant *et al*. 1998). According to all these studies, the nature of the carbon sources significantly influences the SRB activity and therefore the success of a PSRB. A chemical characterization can be used to assist in predicting sulfate reduction capacity of a carbon source and the selection of organic electron donors for potential use in AMD treatment (Coetser *et al.* 2006). The selection of the organic carbon source is usually made on the basis of availability and costs (Sheoran *et al*. 2010).

Another important parameter that can affect the activity of SRB and therefore, the performances of PSRB is the temperature (Neculita *et al*. 2008). Most of the studies carried out on the efficacy and bioavailability of carbon sources were conducted at optimal temperature for SRB growth, which is between 37°C and 65°C (Rees *et al.* 1995; Rozanova *et al.* 2001; Sievert and Kuever 2000). The optimal growth temperature and metabolic efficiency vary with sulfate-reducing bacteria species. Thus far, most species of SRB that have been isolated and described are mesophilic (Widdel and Bak 1992). Few SRB have been reported as cold-tolerant and the low temperatures encountered in the northern climate (≤ 5°C) can challenge the growth of these organisms. However, at low temperatures, several researchers showed that bacterial metabolic activity requires specific adaptations (Cavicchioli 2006; Robador *et al*. 2009). The most important of these adaptations is the ability of bacteria to synthesize enzymes with high catalytic activities at low temperatures (Feller and Gerday 2003; Robador *et al*. 2009). Hamai *et al.* (2015) used rice husk and rice bran as complex carbon sources to support SRB in anaerobic bioreactor which was not temperature-controlled. These authors showed that the concentration of sulfate continued to decrease, although the internal temperature dropped to approximately 5°C during the winter.

3.6 Material and Methods

The laboratory scale experiment is intended to bring scientific evidences for the larger project conducted by Alexco Resource Corporation mining company which owns the Keno Hill Mine Site, YT. Alexco planned to inject carbon sources in a 110 deep borehole in its mine site to enhance the growth of native SRB and therefore to lower Cd and Zn contamination in groundwater. A previous work showed native SRB was present in groundwater and could be used to treat this mine drainage if molasses, methanol or a mix of both was provided but no other carbon sources were tested so far (Nielsen *et al*. 2016).

3.6.1 Mine water, waste rocks and inoculum sampling and characterization

3.6.1.1 Waste rock characterization

The waste rocks (WR) were sampled from two different waste rock heaps (WR1 and WR2) in April 2014 at the Keno Hill mine site. The rocks were dug out during the exploitation of the Silver King tunnels (personal discussion with Alexco Resources, 2014). The WR were sampled in a 20-L bucket and stored at 15°C. Each waste rocks samples were sifted through a 3.18-mm-opening sieve before being placed in the bioreactor. A complete characterization (metal contents, particle size distribution and Sobek test) of the WR samples was conducted to determine the potential risk of AMD production (Nielsen *et al*. 2016).

3.6.1.2 Mine impacted water characterization

Mine-impacted water (MIW) was collected from the Keno Hill mine site in June 2015 and stored in a cold storage. This water was monitored every month by the Alexco Resource Corporation mining company between February 2007 and March 2015, and the metals concentrations and sulfate concentration were analyzed at Agat Laboratories (Whitehorse, YT, Canada).

3.6.1.3 Sediment sampling

A sediment, collected from Crystal Creek which is located in the Keno Hill mining district between Keno and Mayo (YT, Canada), was used as inoculum in the present study. The inoculum had been shoveled from the bottom of the creek, collected in a 20-L bucket and stored in cold storage (15°C). SRB in the sediment used in this study were not enumerated however past experience and knowledge of the area by the mining company strongly suggest that SRB are indeed present in the sediment of this particular creek. Londry (2013) conducted a technical assessment of the potential microbial roles in metal removal during the bioremediation project Galkeno 900 conducted by Alexco Resources Corporation using microbial molecular (DNA) techniques. The report demonstrated that SRB were present in sediments collected within the United Keno Hill mining district in several creeks. The sediment collected and used in this study is referred as an “inoculum” based on the assumption that microorganism are presents.

3.6.1.4 Carbon source characterization

Substrates used in this study were potato oil (oil used to boil potato fries, from Ashton, Quebec, QC), peat (collected from Keno Hill mine site, YT), brewery residue (Brewery company La Barberie, Quebec, QC), straw (Wilko Pet Bedding Harvest Straw), methanol (CH_3OH, certified ACS; Fisher Scientific, Fair Lawn, NJ, USA) and ethylene glycol ($C_2H_6O_2$, Sigma Aldrich, Saint-Louis, MO, USA). The percentage of total carbon (TC) in dry substrate was determined by CHNS (Leco TruSpec Micro, Mississauga, ON, Canada) using 1-3 mg of grinded substrate sample heated at 1200°C for 200 seconds at 12 PSI. Peat, brewery residue and straw were previously grinded into powder using a Black & Decker Coffee Grinder (Towson, Maryland, USA). To evaluate pH variation due to each substrate and to assess any eventual metal leaching from the studied substrates, 500 mg of grinded substrates, previously dried at 60°C for 24 hours, were immersed in 1L of De-Ionised Water (DIW) for 10 days at room temperature and shacked in a TCLP rotatory apparatus. After 10 days, pH measurements were done and samples were collected in a 20 mL vials to determine the concentrations of Zn and Cd using an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) apparatus (Varian, Vista-AX CCO, Palto Alto, CA, USA).

3.6.2 Experimental design and operation

Bioreactors consisted on 1-L low density polyethylene bottles filled with 200 mL of WR (100 mL of WR1 and 100 mL of WR2), 5 mL of inoculum, 850 mL of MIW and variable amounts of carbon sources (potato oil, peat, brewery residue, straw, methanol and ethylene glycol) based on each initial carbon contents. DIW was added to fill up the bottles to support the development of anoxic conditions. The bioreactors were stored in a cold chamber at 5°C and monitored over 162 days. All bioreactors were prepared and monitored in triplicate to compare the performances of the different sources of carbon with respect to the bacterial activities.

According to the stoichiometry in Equation 1, two moles of carbon are required to reduce one mole of sulfate. Based on the concentration of sulfate in MIW sample and to ensure that carbon is provided in excess, it had been decided to double the amount of carbon provided into the bioreactors. Hence, 20.72 mmoles of carbon were added in each bioreactor, corresponding to a carbon concentration of 248.7 mg/L. In this instance, there are four moles of carbon available for each mole of sulfate initially present in MIW (497 mg SO_4^{2-}/L). Depending on the carbon source (liquid or solid), carbon could be either in dissolved or solid form. The mass of material needed to reach the desired concentration in each bioreactor was calculated based on the carbon content determined by CHNS analysis of each substrate.

Various control bioreactors were monitored during this experiment to study the behavior of MIW with no treatment (Control C1: MIW), to assess the role and effects of the WR in DIW (Control C2: WR + DIW) and in MIW (Control C3: WR + MIW). The control C4 (WR + MIW + MeOH and without inoculum) was monitored to assess the impact of inoculum. Finally, the control C5 had no carbon added (WR + MIW + inoculum) and was monitored to assess the impact of the addition of carbon. All controls were prepared in triplicates.

3.6.3 Batch bioreactor monitoring

During this laboratory experiment, various parameters were monitored in both bioreactors and control samples to determine the influence of the different carbon source and their impact on SRB's activity and metal removals. Oxido-reduction potential (ORP) was quantified right before sampling to reduce the variability of the ORP measurement. ORP measurements were performed using a double junction electrode ORP (ORP Electrode tested. 59001-77, Cole Parmer, Montréal, QC, Canada). Calibration of the ORP meter was performed using a certified solution (ORP Standard, Thermo Fisher Scientific, Waltham, MA, USA). The pH was measured in the 20-mL carbon samples prior to the acidification of the samples. pH measurements were performed using a pH meter (Epoxy body pH electrode, Oakton, Brisbane, Australia) equipped with a double junction Ag/AgCl electrode (Cole Parmer Canada, Montréal, QC, Canada). Calibration of the pH meter was performed daily using certified buffer solutions (buffer solution pH = 10.00; pH 7.00; pH 4.00 and pH = 2.00, Fisher Scientific, Montréal, QC, Canada). The pH and ORP probes were rinsed with DIW between each sample. Samples for the analysis of sulfate were collected using a 20-mL capacity bottle and stored in a freezer (-17°C) until analysis. Samples for carbon determination were collected in a 20-mL capacity bottle and acidified with H_2SO_4 (2%, v/v). For the determination of the Zn and Cd contents, samples were collected and stored in a 20-mL bottle at 4°C after acidification using HNO_3 (2%, v/v). Considering the volume needed for each analysis, the total collected sampling volume was around 30 mL. After each sampling, the 1 L-bioreactors and controls were filled to the top using DIW, to eliminate air and maintain anoxic conditions. The sampling period started at t = 0 and continued biweekly for 112 days (28, 42, 56, 70, 84, 98, 112). The last sample was taken after 162 days of experiments.

3.6.4 Analytical methods

The metals present in the effluents emerging from the bioreactors and controls were analyzed using an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) apparatus (Varian, Vista-AX CCO, Palto Alto, CA, USA). A calibration of the ICP-AES was performed before each series of measurements using calibration standards prepared with certified solutions (SCP Science, Lasalle, QC, Canada). Control solutions (multielements standard, 900-Q30-002, SCP Science, Lasalle, QC, Canada) were analyzed after each series of 20 measurements to ensure the conformity of the apparatus measurements.

The concentration of sulfates present in the water was determined using a modified version of the method described by Bertolacini *et al.* (1957) and a UV-Vis spectrophotometer (Varian Cary® 50, Varian, Inc., Mississauga, ON, Canada). A certified control solution (Multi-element Ion Chromatography standard sol II in H_2O, Fluka, Sigma Aldrich, Canada) was used to confirm the accuracy of the measurements. Total organic carbon (TOC) was measured using TOC-analyzer (TOC-VCPH model, Shimadzu Scientific Instruments, Columbia, MD, USA).

3.7 Results and Discussion

3.7.1 Characterization of the components used in the bioreactors

3.7.1.1 Mine-impacted water (MIW) and waste rock (WR)

The waste rocks and mine impacted water used in this study have been extensively described in Nielsen *et al*., 2016 and were found to be AMD generating. Wastes rocks used in this study are from two piles located next to the Silver King adit in the Keno Hill mine site, YT. MIW is ground water running in old mining tunnels and pumped from the Silver King 110 m borehole. The characteristics of the WR and MIW used are presented in Table 2 from Nielsen *et al.* (2016). According to this previous study, WR released acidity (pH = 3.1 ± 0.3), sulfates (252 ± 19 mg/L), Zn (784 ± 121 µg/L) and Cd (22.2 ± 4.0 µg/L) after 90 days into deionized water (Nielsen *et al.* 2016). The potential for acid generation from the WR samples was evaluated using a Sobek test in the previous study. According to our results, the calculated neutralizing potential ratio (NPR = 2.13) showed a “low” risk for ARD generation according to Price *et al.* (1997).

3.7.1.2 Carbon material characterization

Complex organic carbon sources including peat, brewery residue and straw were grinded into powder and let to settle to the bottom of the bioreactors before to start the experiments by the addition of the MIW. Potato oil has hydrophobic properties and was floating on the water’s surface of the bioreactors. It appeared that the simple organic sources of carbon, methanol and ethylene glycol, were homogeneously mixed in the MIW.

In order to calculate the mass of each carbon sources needed to reach the desired concentration of carbon to reduce sulfate (Equation 1), TC concentrations initially present in the substrates had been determined by CHNS. According to these results, the source of carbon with the highest TC content was the potato oil (71.5 ± 6.0 mg/kg of dry weight), followed by the brewery residue (46.7 ± 2.0 mg/kg of dry weight), straw (42.6 ± 2.0 mg/kg of dry weight) and peat (35.0 ± 3.2 mg/kg of dry weight). After 10 days in DIW under agitation, the pH of the potato oil, brewery residue, and straw was about neutral whereas a decrease of the pH from 7.0 to 5.3 was observed for peat. During the 10 days of agitation in DIW, neither potato oil or brewery residue released significant amounts of Cd (< 0.4 µg/L) or Zn (< 15 µg/L), whereas straw released 26.7 µg Zn/L and peat released 335 µg Zn/L and 3.80 µg Cd/L. Hence, some carbonaceous materials leached out Cd and Zn. It is worth noting that peat was collected in the vicinity of the mining district. Therefore, it could have been contaminated by heavy metals from the past mining activities nearby.

3.7.2 Metals leaching and removal

3.7.2.1 Metal leaching from the waste rocks (controls)

Figure 1 presents the concentrations of Zn and Cd (Fig. 1a) and the concentration of sulfate and pH (Fig. 1b) measured for the control C1 (MIW) and the control C3 (Waste Rocks + MIW) along the monitoring period. The differences seen between C1 and C3 highlight the fact that WR released Zn and Cd in MIW through leaching. Up to 987 ± 189 µg Zn/L were released at day 98 in C3 compared to 68.8 ± 35.6 µg Zn/L in C1 at the same date. Similarly, 23.4 ± 8.6 µg Cd/L were measured at day 112 in C3 when C1 had a concentration of Cd lower than 0.4 µg Cd/L at the same date. In C3, sulfate concentration remained stable, between 476 ± 13 mg/L and 552 ± 2 mg/L whereas the control C2 (WR + DIW) (not shown) indicated that the WR released sulfate in DIW up to 435 ± 93 after 162 days. Sulfate leaching from the WR was expected in C3 but was not observed. One of the reasons might be that sulfate was released but consumed in the MIW by native SRB. Indeed, SRB are present in anoxic habitats (Muyzer and Stams 2008) and have been found in AMD (Fortin *et al*. 2000). In C3, pH also dropped from 6.14 ± 0.11 to 3.55 ± 0.22 during the 162 days of experiments, while it stayed neutral in C1. Metals and sulfate releases and the decrease of pH values indicated that, as expected, the WR were AMD generating.

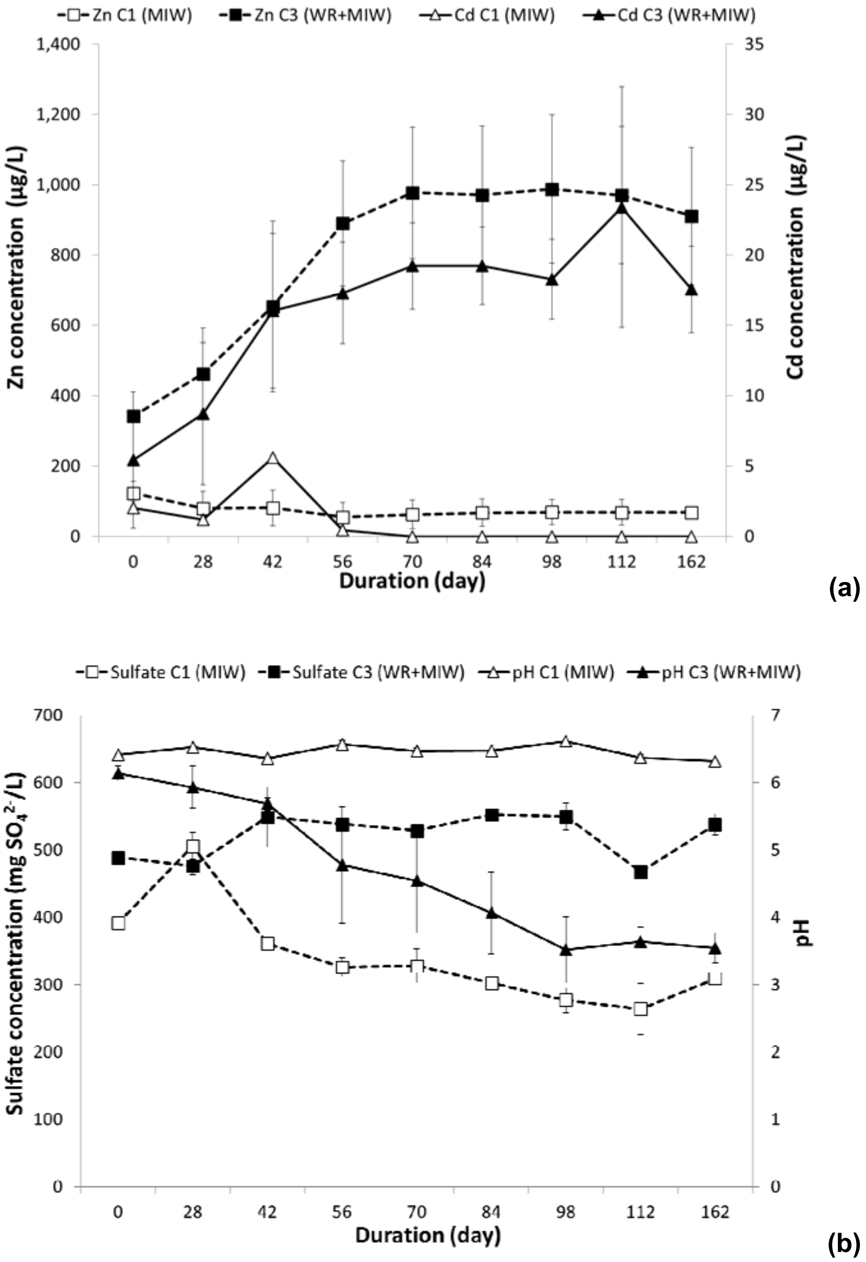

Figure 3-1 Evolution of the concentrations of Zn, Cd (a.) and Sulfate and pH (b.) measured in the control C1 (MIW) and C3 (WR + MIW) during the experimental period

3.7.2.2 Performance of Zn and Cd removal using simple organic carbon sources

Figure 2 presents the evolution of the concentrations of Zn (a.) and Cd (b.) measured in the bioreactors operated at 5°C in the presence of simple organic sources of carbon, methanol (MeOH) and ethylene glycol. The controls C3 (WR + MIW) and C4 (WR + MIW + Methanol but without inoculum) are shown to assess the impact of methanol and inoculum. As shown in Figure 2, the concentration of Zn increased in the presence of ethylene glycol from 289 ± 9 µg/L to 1,875 ± 485 µg/L after 56 days of experiment. This increase of the concentration of Zn following the same trend as those observed with control C3 during the first 56 days and was possibly due to the leaching of Zn from WR. For the experiments performed in the presence of methanol, Zn concentration remained stable during the first 42 days before to slowly decrease and be depleted at day 149. On the other hand, in the presence of methanol and ethylene glycol, the concentrations of Cd increased from 2.1 ± 0.4 µg/L to 6.5 ± 0.5 µg/L and to 8.6 ± 1.5 µg/L, respectively, during the first 40 days of experiment. However, independently of the source of simple organic carbon used, Cd concentrations increased but still remained below the concentration measured in the control C3 (WR + MIW) where no treatment was applied. This indicated that some removal of Cd was achieved in the bioreactors in the presence of simple organic carbon sources. After 42 days of experiments, Cd concentration decreased with both simple carbon sources to be completely depleted after 70 days for methanol and 84 days for ethylene glycol.

The control C4 showed the importance of the presence of inoculum. Indeed, when no creek sediment was added to the bioreactor filled up with MIW and in the presence of WR and methanol, Zn concentration increased up to 1,175 ± 110 µg/L at day 112 and Cd concentration increased up to 31.2 ± 3.7 µg/L at day 112, following the same trend as C3. Those increases in Zn and Cd concentrations observed in C4 are probably due to the fact that no SRB added through the inoculum can help with the precipitation of metals as metal sulfides.

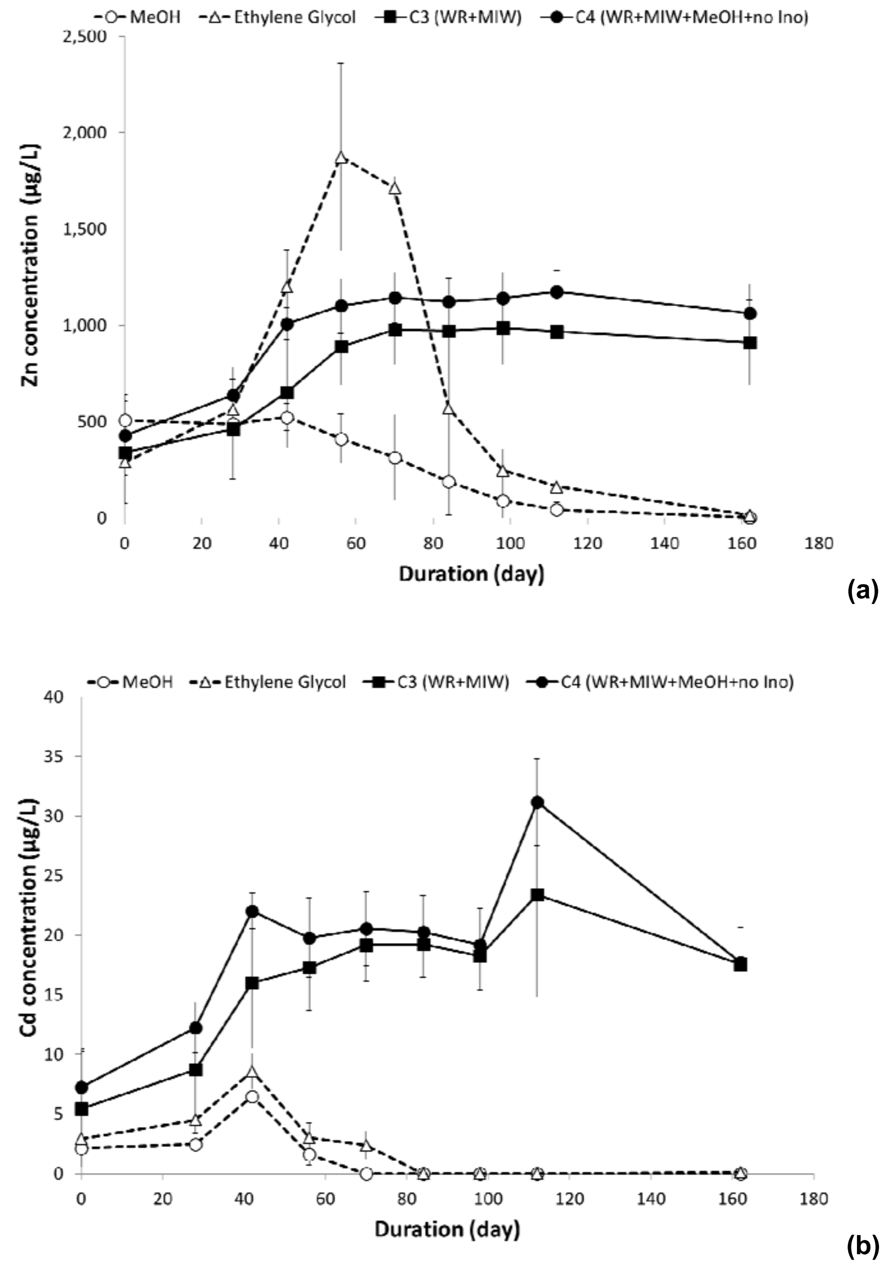

Figure 3-2 Evolution of the concentrations of Zn (a.) and Cd (b.) measured in the bioreactors for the experiments performed in the presence of MeOH and Ethylene glycol and in controls C3 (WR + MIW) and C4 (WR + MIW + MeOH) during the experimental period

These results highlighted that both simple sources of carbon studied, in the presence of inoculum, allowed the diminution of Cd concentration with Cd removal yields reaching 100% with methanol and 99.6% with ethylene glycol after 112 days of experiments compared to the Cd measured in the C3 control. For Zn, 99.7% were removed in the presence of methanol and 98.5% in the presence of ethylene glycol compared to the maximum Zn concentration released by control C3 after 98 days of experiment. However, the kinetic of Cd and Zn removals from MIW seemed to be quicker in the presence of methanol (28 days) compared to ethylene glycol (56 days). Kinetic is an important aspect to consider as it affects both, the performances and operation costs of PBSR.

3.7.2.3 Zn and Cd removal with complex organic carbon sources

Figure 3 presents the evolution of Zn and Cd concentrations in bioreactors conducted at 5°C in the presence of complex organic carbon sources (potato oil, peat, brewery residue and straw) along with the control C3 (WR and MIW). It appeared that the initial Zn concentrations at t = 0 were around 520 µg/L for potato oil, brewery residue and straw. However, the initial concentration of Zn measured in the presence of peat was a lot higher than those observed for the other complex sources of carbon studied with an initial concentration of 1 482 ± 480 µg/L. This higher initial concentration may be explained by the high Zn leaching capacity of peat as highlighted in Section 3.1.2. Independently of the complex source of carbon used, the concentration of Zn decreased below the concentration measured in C3 after 54 days and below 28 µg/L after 84 days of experiments. In comparison, the concentration of Zn was measured in the range of 890 to 987 µg/L between 54 and 160 days of operation for the control C3, where there was no inoculum and no sources of carbon to support microbial activity. Hence, this demonstrated that addition of a complex carbon source and inoculum supported the removal of Zn in the bioreactors.

The control C3 showed that the initial concentration of Cd in MIW was around 5.4 ± 4.8 µg/L. When potato oil and brewery residue were used as complex carbon sources, the initial Cd concentrations were lower, with values of 2.6 ± 0.7 µg/L and 3.4 ± 3.7 µg/L, respectively. With peat and straw, the initial Cd concentrations (8.5 ± 1.3 µg/L and 7.6 ± 1.7 µg/L, respectively) were higher than those measured in C3.

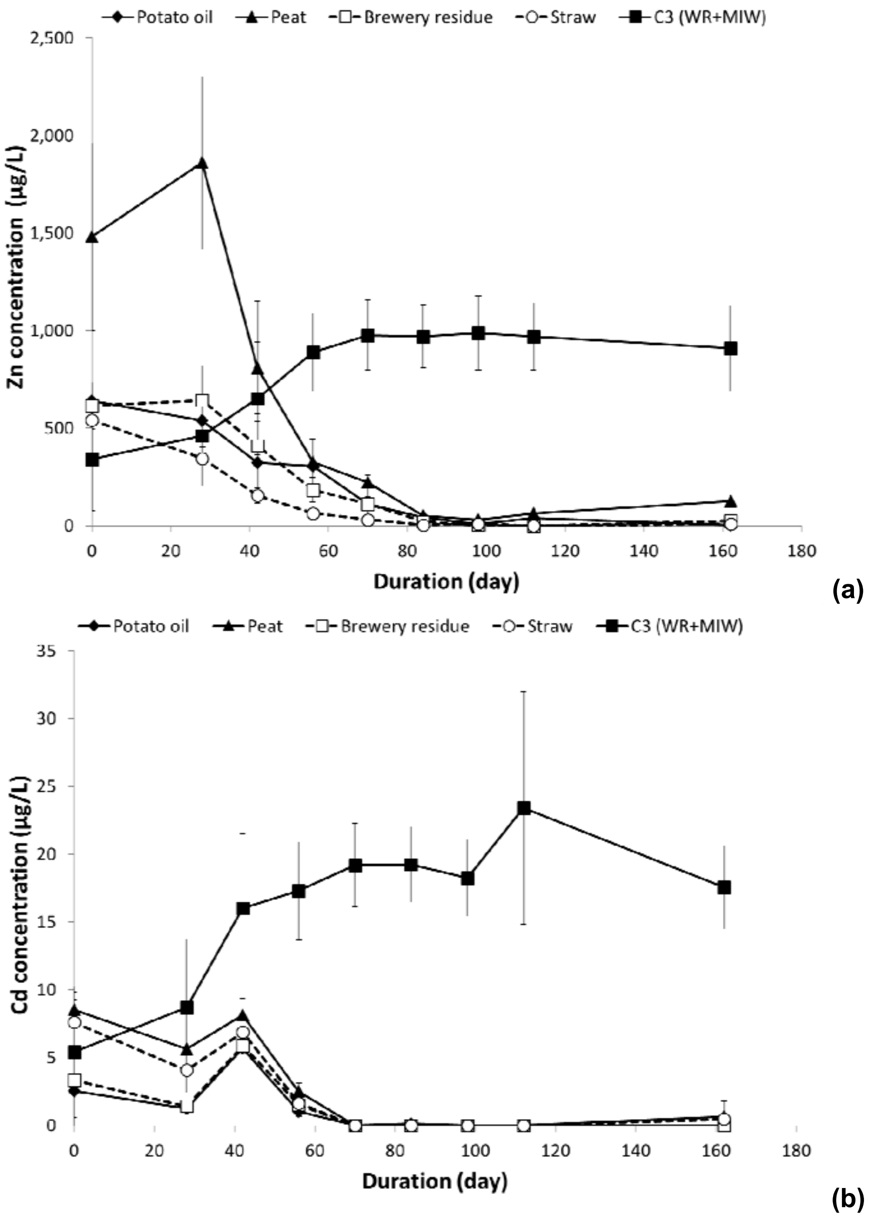

Figure 3-3 **Evolution of the concentrations of Zn (a.) and Cd (b.) measured in the bioreactors for the experiments performed in the presence of Potato oil, Peat, Brewery residues and Straw and C3 (WR + MIW) during the experimental period**

As discussed earlier, peat had the ability to leach Cd in DIW as demonstrated in Section 3.1.2. This possibly explained the higher concentration of Cd initially present in the bioreactors containing peat. However, it is unclear why the bioreactors featuring straw also displayed concentrations higher than expected. Regardless of the complex source of carbon used, the concentrations of Cd decreased during the first 70 days of experiments and remained below detection limits until the end of the experiments. By opposition, the concentrations of Cd measured in the Control C3 (WR + MIW) where no treatment was applied increased from 5.4 ± 4.8 μg/L (at t=0) to 23.4 ± 8.6 μg/L (at day 112). Again, the results reinforced the fact that complex carbon sources played a critical role in Cd removal from the MIW. The sorption of metals on the solid organic materials may be a complementary mechanism leading to the decrease of Zn and Cd concentrations in MIW. Several authors have suspected the coexistence of several mechanisms including sulfide precipitation and metal sorption. Low-cost wood-based materials such as sawdust and bark are well-known for their metal adsorption capacity (Argun *et al*. 2008; Keng *et al*. 2013). Janin and Harrington (2013) have used wood chips and biochar to promote metal sorption and obtained Zn and Cd removal efficiencies in the range of 90% at room temperature.

3.7.3 Utilization of organic carbon

The use of sources of carbon is required to support the growth of native SRB in groundwater contaminated by metals where the temperature is around 5°C all year long. Moreover, the control C5 (not shown) (WR + MIW + inoculum but without any addition of carbon) showed that when the SRB population is boosted with inoculum but without any addition of carbon, the concentrations of metals still increased in a similar manner as did the concentrations measured with the C3 control (WR + MIW), ranging from 238 ± 57 μg/L to 965 ± 21 μg/L for Zn and from 3.4 ± 2.4 μg/L to 17.4 ± 9.8 μg/L for Cd. Sulfate concentration in C5 (not shown) remained stable during the 162 days of experiments (around 500 mg/L).

Figure 4 showed the TOC concentration measured in the MIW in the presence of simple organic substrates (Fig. 4a) or complex organic sources of carbon (Fig. 4b) and in the control C3 and C4 during the 162 days of experiments.

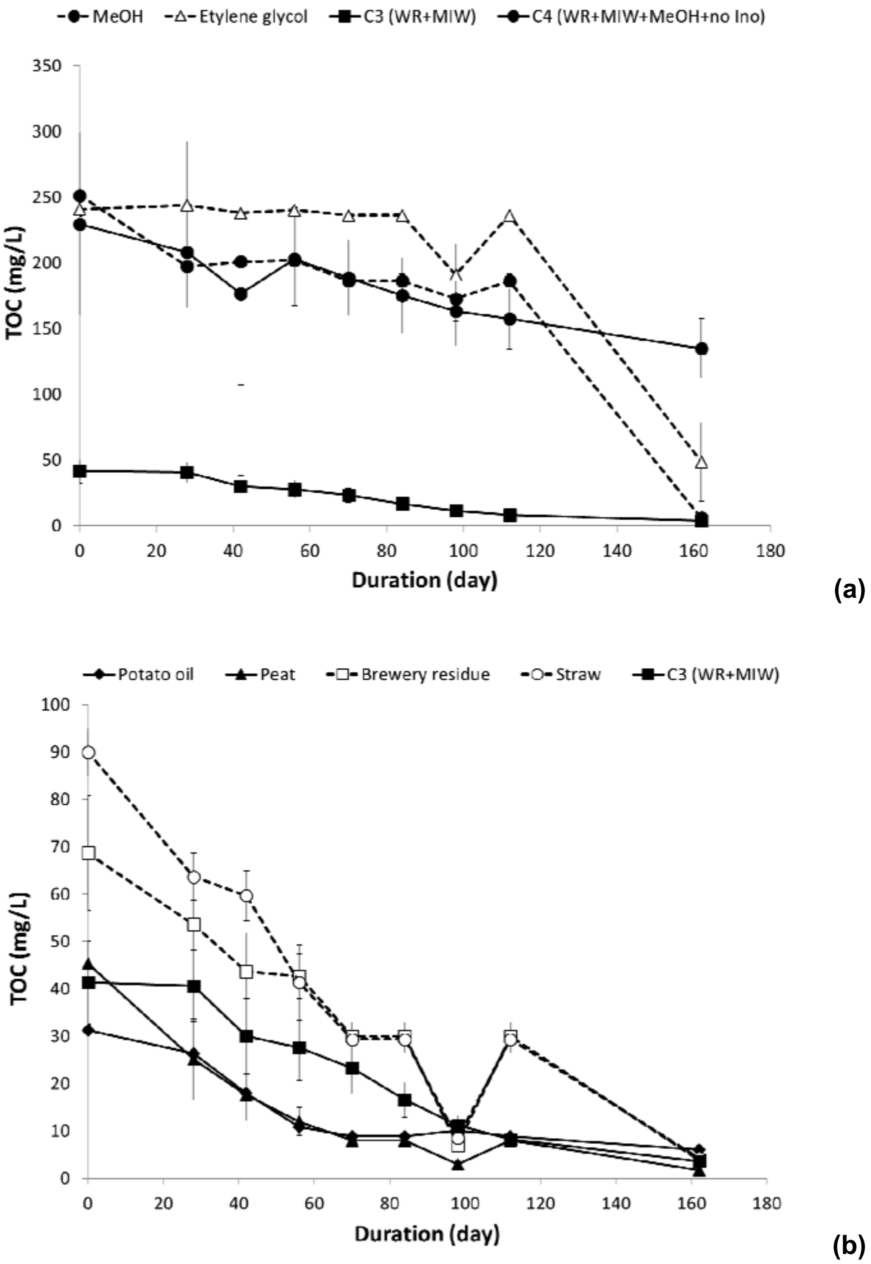

Figure 3-4 **Evolution of the TOC content measured in the bioreactors for the experiments performed in the presence of simple (a.) or complex (b.) sources of carbon during the experimental period**

3.5.3.1. Carbon consumption in the presence of simple organic carbon sources

According to the results presented in the Fig. 4a, the initial concentrations of TOC in liquid phase were very close to the calculated carbon concentration (248 mg of C/L) based on the Equation 1 with values of 251 ± 19 mg/L, 241 ± 5 mg/L and 229 ± 69.5 mg/L for methanol, ethylene glycol and control C4 which contained methanol but no inoculum, respectively. As mentioned by Zaluski *et al*. (2006), simple sources of carbon allow a better control of the amount of carbon injected in the PSRB as is the case in this study.

Not surprisingly, carbon concentration in C3 was very low (41.3 ± 8.7 mg/L) because no carbon source was added in this control. For the bioreactors using methanol and inoculum, carbon consumption started quickly, with 21.6% of TOC consumed after the first sampling on day 14. TOC concentration then slowly decreased between day 14 and day 98 after which it became almost depleted with less than 5.8 ± 1.0 mg/L remaining by day 149. When ethylene glycol, another simple carbon source was used, the TOC consumption remained stable and close to the initial concentration. The carbon concentration started to go down after 84 days of experiments. At the end of the experiments, the final TOC concentration measured in the presence of ethylene glycol was 48.7 ± 30.0 mg/L, corresponding to 79.8% consumption of TOC. On the other hand, when methanol was used, the concentration of TOC decreased to 97.7% of the initial value. In control C4, which contained methanol but no inoculum, TOC was gradually consumed during the 162 days of experiments from 229 ± 70 mg/L to 135 ± 23 mg/L. This 41.1% of TOC consumption may be caused by native microorganisms present in MIW but remained much lower than the efficiency observed when inoculum was used (97.7%). This emphasised that the presence of inoculum and a variety of microorganisms is required to degrade the organic matter and support SRB growth (Sheoran *et al.* 2010; Drury 2006). In the present study carried out at 5°C, the consumption of carbon by SRB started after about 3 months for simple carbon sources such as methanol and ethylene glycol, which seemed to be a long time. According to Sheoran *et al*. (2010), simple organic sources of carbon tend to be readily available to support SRB metabolic activities and several authors highlighted that methanol is one of the most used simple carbon sources to support SRB activity (Glombitza 2001; Tsukamoto and Miller 1999). In this study, methanol and ethylene glycol were not largely consumed before 98 days of experiments. This delay in carbon consumption may be due to the fact that cold temperature affects the growth of SRB and their affinity for that specific source of carbon.

3.5.3.2. Carbon consumption in the presence of complex organic carbon sources

According to the results presented in Fig. 4b, it should be noted that all complex sources of organic carbon did not dissolve in MIW. Indeed, the initial TOC concentrations measured in the liquid phase of the bioreactors consist only in the carbon which dissolved from the potato oil, peat, brewery residue and straw and thus the initial concentrations were low with values of 31.3 ± 0.9 mg C/L, 43.7 ± 0.5 mg C/L, 68.7 ± 12.1 mg C/L and 90.0 ± 5.0 mg C/L respectively. These concentrations were 2.7 to 8.0 times lower than those observed in the presence of simple sources of carbon and the targeted value of 248 mg C/L (Equation 1). However, after 162 days of experiments, the concentrations of all dissolved carbon from complex substrates decreased to reach 6.0 ± 1.0 mg C/L for potato oil, 1.8 ± 0.2 mg C/L for peat, 3.9 ± 0.4 mg C/L for brewery residue and 4.3 ± 0.2 mg C/L for straw. As mentioned in the literature, the degradation of complex carbon sources by bacteria should be slower than with simple carbon source as the carbon is less readily bioavailable. On the other hand, a slow carbon degradation allows for a steady support of SRB over longer periods. As discussed by Drury (2000), this can provide some advantages for utilization over the long term and therefore these sources of carbon represent an interesting option to treat AMD from remote mines where labor and maintenance are hard to provide

3.7.4 Sulfate consumption

Interestingly, the control C2 (WR + DIW, not shown) released sulfates with concentrations reaching up to 435 ± 93 mg SO_4^{2-}/L at day 162. On the other hand, the control C3 (WR + MIW, Fig. 5a) started with a concentration of 489 ± 7 mg/L and it stayed almost stable over the monitoring period, ending up at 537 ± 15 mg/L at day 162. Hence, either there is no oxidation of the WR and no release of sulfate and acidity or, the release of sulfate was compensated by the consumption of the sulfate. Because the monitoring of pH during the experiments showed a decrease in pH indicating a release of acidity, it is more likely that sulfate was produced too, but consumed. As observed in a previous study, it is very likely that native SRB are naturally present in the MIW collected on the mine site (Londry 2013).

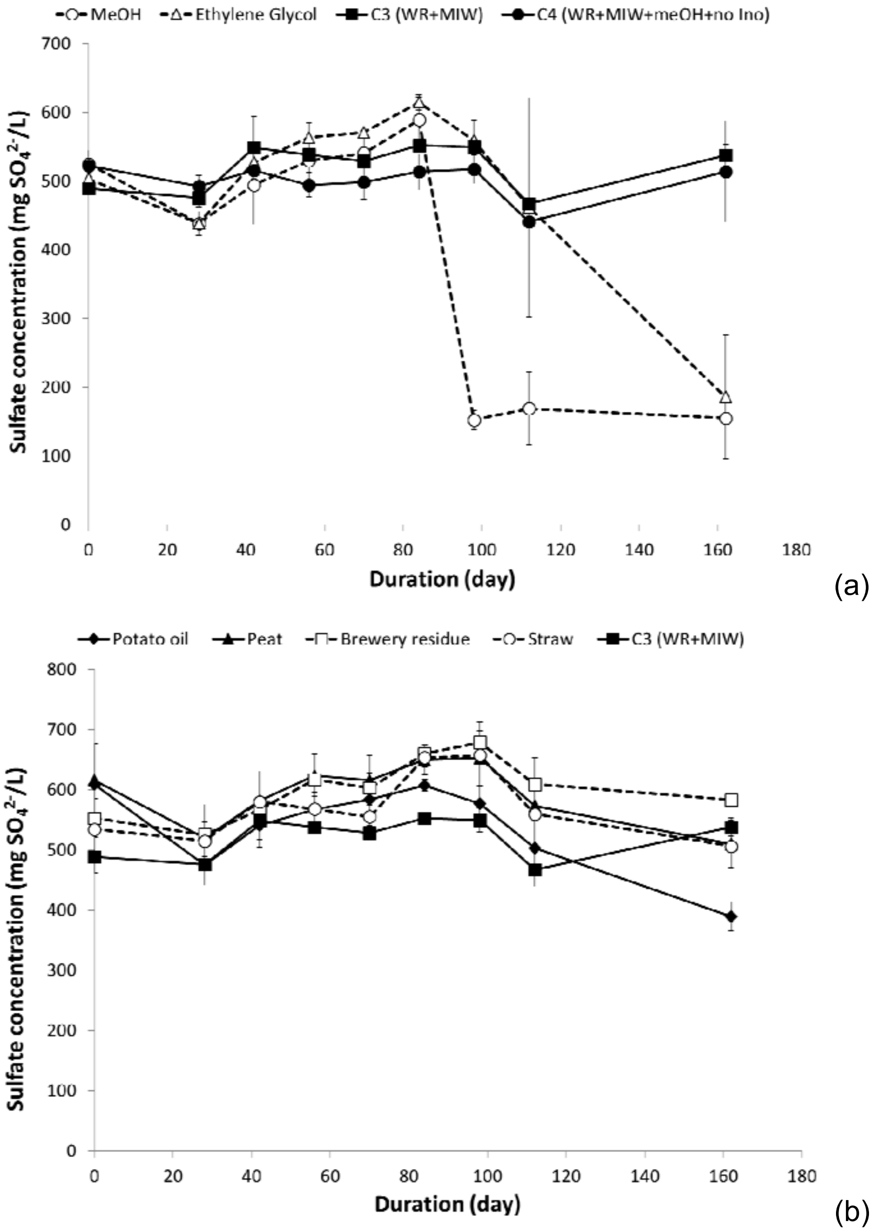

Figure 3-5 Evolution of the sulfates content measured in the bioreactors for the experiments performed in the presence of simple (a.) or complex (b.) sources of carbon during the experimental period

Steady diminution in sulfate concentration was observed in the control C1 (MIW), from 506 ± 20 mg/L at day 14 to 264 ± 38 mg/L at day 98. Figure 5 presents the evolution of sulfate concentrations in the bioreactors using simple (Fig. 5a) and complex (Fig. 5b) organic sources of carbon during the 162 days of experiments.

3.7.4.1 Sulfate consumption in the presence of simple organic carbon sources

With both simple organic carbon substrates, sulfate concentrations remained quite stable, between 420 and 580 mg/L during the first 84 days of experiments (Fig. 5a). After the 84th day, the concentration of sulfate dropped drastically for methanol (from 525 ± 20 mg/L to 151 ± 28 mg/L) when the bioreactors contained inoculum. In comparison, when methanol was added but without addition of inoculum (Control C4), sulfate concentration stayed above 440 ± 6 mg/L (which is the lowest concentration of sulfate for C4 measured). This suggested that the microorganisms in the inoculum are responsible for the decreased of sulfate concentrations and that SRB are actively producing sulfide. Sulfate concentration also decreased when ethylene glycol was used (from 503 ± 3 mg/L to 186 ± 90 mg/L) but at a slower rate than with methanol. Sulfate removal yields obtained with methanol and ethylene glycol were estimated at 71.2% and 36.9%, respectively. The reduction of sulfate by SRB was also suggested by the consumption of TOC during the same period for the experiments performed in the presence of methanol and ethylene glycol. On the other hand, when no inoculum had been added to methanol (C4), the sulfate concentration remained stable between 526 ± 21 mg/L (t = 0 d) and 440 ± 6 mg/L (t = 112 d). As WR had been found to be AMD generating and released up to 435 ± 93 mg SO_4^{2-}/L after 162 days (control C2: WR + DIW, not shown) and as the presence of native SRB is strongly suspected, it may be thought that native SRB consumed sulfate released by WR in the control C4. ORP values remained around 100 mV for both simple carbon sources until day 84 and decreased to -126 ± 13 mV for methanol and -109 ± 7 mV for ethylene glycol. Then, ORP values remained stable around those negative values until day 112 and increased up to 0 mV at day 162. According to these negative ORP values, all the conditions were favorable to the activity of SRB after 84 days of experiments which was confirmed by the reduction of sulfates at day 84 and the consumption of TOC. It may have taken a lengthier time for microbes to adapt to methanol and ethylene glycol as sources of carbon because those sources of carbon may have been toxic to some microbes at the beginning; requiring them to have an acclimation period (Tsukamoto 1999). Indeed, alcohols are toxic to microorganisms at high concentrations, presumably because they damage the cell membrane and inhibit glycolytic enzymes (Dürre *et al*. 1988; Weijma 2000). However, as alcohol toxicity towards bacteria lessens with a decrease in the length of the carbon chain, it can be speculated that toxicity will not occur at concentrations below 10 g/L (Weijma 2000) when using methanol or ethylene glycol. In the present study, the initial TOC content was around 251 ± 19 mg/L and 241 ± 5 mg/L for methanol and ethylene glycol, respectively; which is far below the toxicity limits defined by Weijma (2000). However, this could have been harder and longer for SRB to assimilate the carbon, especially at a temperature of 5°C compared to the optimal temperature of SRB growth (25-30°C) **(**Rees *et al*. 1995; Rozanova *et al*. 2001; Sievert and Kuever 2000)**.**

3.7.4.2 Sulfate consumption in the presence of complex organic carbon sources

According to the results presented in Fig. 5b., the concentrations of sulfate did not dramatically decreased with complex carbon sources as observed with simple carbon sources. Although a slight decrease can be observed after 84 days with the complex carbon sources. Again, this might reflect that either very low sulfate consumption or release and consumption occurring simultaneously. Noteworthy, with all the complex organic carbon sources, ORP values decreased during the first 14 days from around 100 mV for all substrates at t=0 to -141 ± 13 mV for peat, to -190 ± 69 mV for brewery residue, to -249 ± 29 mV for straw and to -273 ± 13 mV for potato oil. ORP values remained negative up to day 112; reflecting some bacterial activities; then it went up to about 0 mV between the 112th and 162th day. It can be thought that bacterial activity was lower as it started to have a lack of carbon between day 98 and day 162.

Considering the initial sulfate concentration at t=0 in each bioreactors and the final sulfate concentration after 162 days of experiments, the sulfate concentration in the bioreactors fed with potato oil decreased by 36%. In comparison, the diminution of sulfate concentration was limited to 13.8% and 5.3% when using peat and straw and a 5.4% sulfate augmentation was observed when using brewery residue as carbon source. As ORP decreased to negative values, reflecting some bacterial activities, before any sulfate consumption was found, it may be thought that complex organic carbon sources supported some microbial communities in charge to degrade the organic matter (Sheoran *et al*. 2010) and subsequently support SRB growth. In addition, those results are consistent with Coetser *et al.* (2006) as the best sulfate removal was obtained with potato oil which was the complex carbon source with the higher content in crude fat. On the other hand, peat and straw had a high content in crude fiber and lignin which does not benefit sulfate reduction.

3.7.5 Laboratory observations

Figure 6 presents a picture of each bioreactor, performed in triplicates, that was taken after 162 days of experiments. It can be noticed that even no important sulfate and TOC consumption have been detected in some bioreactors with complex sources of carbon, the black precipitate (sulfide precipitates) in the observed is yet another suggestion of the presence of SRB and production of sulfide. Indeed, attributes of the positive SRB growth are the black precipitates occurrence and hydrogen sulphide formation (Luptakova and Macingova 2012).

As expected, bioreactors which contained methanol, ethylene glycol and potato oil were completely covered with black sulfide precipitates while bioreactors using brewery residue, peat and straw showed less black precipitates.

Figure 3-6 Bioreactors after 162 days of experiments

3.8 Conclusion

After 162 days, a diminution of both TOC and sulfate concentrations was observed in all bioreactors. In some of them, these consumptions could be explained by the presence of SRB. The use of simple organic sources of carbon (methanol and ethylene glycol) and complex organic sources of carbon (potato oil, brewery residue, peat and straw) to support SRB growth at 5°C showed that methanol and ethylene glycol led to a diminution of sulfate concentrations of 71.2% and 36.9%, respectively and sulfate consumption was limited to 13.8% and 5.3% when using peat and straw, respectively. Brewery residue did not favor sulfate consumption as a 5.4% sulfate augmentation was observed using this complex carbon source.

Regarding metals concentrations, WR has been found AMD generating (Nielsen *et al*. 2016) and released Zn and Cd as observed in control C2 (WR + DIW) and C3 (WR + MIW). Before bacterial activity was observed by the carbon and sulfate consumption, the concentration of metals decreased. For this reason, it was not possible to correlate the diminution of sulfate concentrations with metal removal. Adsorption may be the cause of the quick decrease in Cd and Zn concentrations as it is well-known to be an effective way of treatment for waters contaminated with heavy metals (Fu and Wang 2011; Kulkarni and Kaware 2013; Janin and Harrington 2013).

In accordance to the literature, this study revealed that simple organic carbon sources, such as methanol or ethylene glycol, are effective to support SRB's growth at cold temperature (5°C). Their use is reliable and allows a better control of the amount of carbon injected in the PSRB. Nevertheless, at cold temperature, a long adaptation period (98-112 days) is required before the consumption started and the use of simple carbon sources may require more sophisticated designs for passive bioreactors which can lead to costs augmentation, especially for remote mine sites. On the other hand, complex organic carbon sources have also benefited SRB's growth. They have been degraded more gradually than simple organic carbon sources. Although they did not bring sulfate removal yields as high as with simple organic carbon sources, complex carbon sources may be more appropriate for the treatment of MIW from remote mine sites.

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4 CHAPITRE 4

Semi-passive in-situ pilot scale bioreactor successfully removed sulfate and metals from mine impacted water under subarctic climatic conditions

Bioréacteurs semi passifs faisant appel à des bactéries sulfato réductrices indigènes à l'echelle pilote et en climat froid

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Note : L'étudiant est le premier auteur de cet article. Toutes les manipulations, le design et l'installation des bioréacteurs pilotes sur le site minier de Keno Hill Silver distric au Yukon, la quasi-totalité des échantillonnages sur site, le traitement des données ainsi que la rédaction de l'article prêt à être soumis ont été effectués par l'étudiant sous la supervision des autres co-auteurs.

4.1 Résumé

Le drainage minier acide (DMA) est l'un des principaux défis environnementaux auxquels fait face l'industrie minière dans les climats nordiques. Les technologies actives conventionnelles utilisées dans le traitement des DMA sont coûteuses, tant au niveau de l'exploitation que des coûts d'investissement. De nos jours, il existe une demande croissante pour développer des traitements passifs fiables, rentables et durables. Les bioréacteurs passifs sulfato-réducteurs (BPSR) offrent une technologie verte et prometteuse reposant sur l'utilisation de bactéries sulfato-réductrices (BSR). Cette étude pilote a été menée sur le site historique de la mine Keno Hill Silver district dans le territoire du Yukon.

Deux bioréacteurs sulfato-réducteurs pilotes, de 200 L chacun, ont été étudiés sur une période de 14 mois dans le but de traiter un drainage minier neutre aux prises avec une contamination en Zn (860 µg/L de Zn) et en Cd (12 µg/L de Cd). Afin de cultiver des BSR natives, la mélasse a été injectée comme source de carbone. Le temps de rétention hydraulique dans les bioréacteurs était de deux semaines. La température, le pH, le potentiel d'oxydoréduction, la conductivité et les concentrations résiduelles en Zn, Cd, sulfates et carbone organique total (COT) ont été mesurés et une caractérisation génomique de la communauté microbienne a confirmé la présence de BSR potentielles. Les données recueillies au cours de cette expérience de 14 mois ont permis de mieux comprendre les mécanismes impliqués dans les bioréacteurs sulfato-réducteurs. Les résultats obtenus ont mis en évidence que les BSR, bien que touchées par des températures froides, peuvent être efficaces pour effectuer des procédés de réduction des sulfates et de précipiter ainsi les métaux sous forme de sulfures en climat froid. L’efficacité des abattements en Zn variait entre 11,5% en hiver et 95,5% en été. L’efficacité des abattements en Cd a varié entre 21,1% en hiver et 96,6% en été.

4.2 Abstract

Acid mine drainage (AMD) is one of the major environmental challenges facing the mining industry in northern climates. Conventional active technologies used in the treatment of AMD are expensive, in terms of both operating and capital costs, and there is increasing demand to develop reliable, cost-effective, and sustainable passive treatments. Passive sulfate-reducing bioreactors (PSRB) offer a promising and green technology that relies on the use of sulfate-reducing bacteria (SRB). This pilot scale study was conducted on the historical mine site of Keno Hill Silver Silver district, Yukon Territory.

Two pilot scale duplicates sulfate reducing bioreactors (200 L each) were monitored over a 14 months period in order to treat a neutral mine drainage struggled with Zn and Cd contamination (860 µg/L of Zn and 12 µg/L of Cd). In order to enhance the growth of native SRB, molasses has been injected as carbon source. Hydraulic residence time in bioreactors was fixed at 2 weeks. The temperature, pH, oxido-reduction potential, conductivity and residual concentrations of Zn, Cd, sulfates, and total organic carbon (TOC) were measured and a genomic characterization of the microbial community has been performed which confirmed the presence of potential sulfate reducing bacteria (SRB).

Data collected during this 14 months experiment have led to a better understanding of the mechanisms involved in sulfate reducing bioreactors. It has been shown that these SRB, although impacted by cold temperatures, can be effective in performing sulfate reduction processes and thus precipitating metals as sulfides in a cold climate. Zn removal efficiency varied between 11.5% in winter and 95.5% in summer whereas Cd removal efficiency varied between 21.1% in winter and 96.6% in summer.

4.3 Abbreviations

AMD	Acid mine drainage
AMOVA	Analysis of Molecular Variance
ARD	Acid rock drainage
DNA	Deoxyribonucleic acid
HRT	Hydraulic retention time
MIW	Mine impacted water
NMD	Neutral mine drainage
NMDS	Non-metric multidimensional scaling
ORP	Oxidation reduction potential
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PSRB	Passive sulfate-reducing bioreactors
SRB	Sulfate-reducing bacteria
TOC	Total organic carbon
WR	Waste rock

4.4 Introduction

The oxidation of sulfide minerals leads to the production of sulfuric acid-rich solutions that contain high concentrations of toxic metals (Baker and Banfield, 2003). Both operational and abandoned mining activities led to oxidizing environment where biological and chemical reactions generate sulfuric acid and mobilize heavy metals associated with the ores, waste rocks, and/or tailings from mining operations. Natural water seepages like infiltration of snowmelt during spring time and rainfalls into waste rocks or tailings can also bring to the contamination of downstream aquatic environments and ground waters (Willow and Cohen, 2003). Mine drainages are usually presented as acidic but waters affected by mining and mineral processing can also be neutral. According to Nordstrom *et al.,* (2015), neutral mine drainage (NMD) refers to drainage waters which contain dissolved constituents, principally sulfates and dissolved metals derived from sulfide oxidation with pH values ranging between 6 to 9. Consistent with Calugaru (2014), NMD may occur under different conditions. The most common is when a portion of the drainage contains a sufficient amount of minerals capable of consuming acid and maintaining the pH near neutrality. In this case, geochemical reactions (eg, precipitation) present in the drainage do not significantly affect the content of certain soluble metals, which remain at concentrations high enough to have an impact on the environment. Nordstrom *et al.* (2015) also pointed out that mine drainages can be neutral when the acid consumption associated with carbonate-derived neutralization capacity is sufficient to maintain neutral pH conditions.

Active treatment systems are currently recognized as Best Available Technologies (Hatch, 2014) and widely used for treatment of MIW. However passive treatment systems (PTS) are increasingly proposed and utilized for mine closure. PTS rely on natural chemical and biological processes and require little or no maintenance and energy once in operation (Younger *et al.,* 2002; Johnson and Hallberg 2005). For the removal of metals from mine impacted water, bioremediation techniques using sulfate-reducing bacteria have been widely studied (Mayes *et al.,* 2011; Hashim *et al.,* 2011; Johnson and Hallberg 2005; Neculita *et al.,* 2007; Ness *et al.,* 2014; Nordin 2010). Once sulfate-reducing optimal conditions are established, sulfide precipitation becomes the predominant mechanism of metal removal from AMD or NMD (Song, 2003).

As metal sulfides are highly insoluble and less bio-available compared with other metal species, such as carbonates or hydroxides, sulfide precipitation by SRB is an interesting and promising technology for metal removal (Wildeman and Updegraff, 1997; Sheoran *et al.,* 2010). Some passive treatments require active management to sustain desired conditions and processes, rendering them semi-passive (Martin *et al.,* 2009).

Many PSRB projects have been conducted from the laboratory to the pilot scale (Dvorak *et al.,* 1992; Glombitza, 2001; Wang *et al.,* 2000; Touze *et al.,* 2007; Degens, 2012; Song *et al.,* 2012; Lefticariu *et al.,* 2015). As small industrial system, pilot experiments generate information about the behavior of the system for use in design of larger facilities. Pilot scale projects provide valuable data for the design of full-scale plant.

Gandy and Jarvis (2012) investigated the performance of a compost-based PSRB for the remediation of a discharge from an abandoned metal mine (2.0–2.5 mg/L of zinc with circumneutral pH) at a laboratory-scale column and a pilot-scale system and compared the two experiments. These authors noticed a decrease in sulfate concentration within both systems, although it was unclear whether bacterial sulfate reduction was the dominant mechanism for metal removal. The laboratory-scale column was most effective at removing zinc, with approximately 96% of the influent zinc attenuated within the system, while the pilot scale system removed, on average, 84% of the influent zinc. According to Gandy and Jarvis (2012), the poorer performance of the pilot-scale reactor was possibly due to preferential flow, as indicated by a greater reduction in hydraulic residence time than in the laboratory-scale system. It seemed that temperature also plays an important role in the attenuation of zinc within such systems, possibly linked to reduced microbial activity during periods of low temperature.

This *in situ* pilot scale project was intended to provide data about sulfate reducing bioreactors efficiency for the treatment of mine water in Keno, YT in collaboration with Alexco Resource Corp. This company owns the historic Keno Hill Silver District, located in Canada's Yukon Territory and is in charge of the remediation and maintenance of the mining site. One of the Keno Hill mine sites: the Silver King Mine Site is struggled with heavy metals contamination in its groundwater. Indeed, residual veins from mine exploitation leach Cd and Zn. In order to treat this underground mine impacted water (MIW), Alexco

Resource Corp. wanted to implement an *in situ* passive biochemical treatment to achieve permanent closure and reach the discharge limits imposed by the Water Use License (QZ12-057 delivered on January, 30th 2013). As most of the old mine tunnels are flooded, the system is mildly anaerobic and initial sulfate concentration is sufficient to support sulfate reduction by native sulfate-reducing bacteria (SRB). Alexco Resource Corp. dug a 110 m borehole to pump MIW and started to inject molasses as carbon source in the groundwater pool in order to support bacterial growth, especially potential native SRB. The presence of native SRB in the Keno Hill mine site has been reported by Londry (2013) who conducted a technical assessment of the potential microbial roles in metal removal for the bioremediation project Galkeno 900 conducted by Alexco Resources Corp. using microbial molecular (DNA) techniques. It showed SRB were present within sediments collected in several creeks located in the United Keno Hill Mine area. The presence of native SRB in the Silver King MIW had been reported in a previous study conducted by Nielsen *et al.* (2016) and this pilot scale study intended to provide a genomic characterization of the microbial community from the Silver King MIW and from the pilot bioreactors.

Laboratory experiments have been previously conducted to demonstrate the feasibility of this process and the aim of the present study was to assess the efficiency of sulfate reducing bioreactors at a pilot scale.

4.5 Material and Methods

4.5.1 Experimental Design

This in situ pilot bioreactors study is intended to replicate Alexco's underground water treatment. To do so two duplicate pilot scale bioreactors have been installed above ground on the Silver King mine site. The bioreactors were built using 200 L polyethylene drums. On the top of each bioreactor, a 30 PSI pressure relief valve (No. 174A, WATTS, Canada) was installed to prevent any damages due to hydrogen sulfide (H_2S) gas production (Tsuchida *et al.,* 2011). The outlet pipe was also installed in the top cover of the bioreactor with a 2" polypropylene bulkhead fitting in order to reduce the air space in the bioreactor and to keep the system as anoxic as possible. Water coming out of the bioreactors was collected in two open-top 200 L polyethylene drums (outlet 1 and outlet 2). A temperature probe (UZ-35626-50, Oakton, Australia) was installed on the top of the bioreactors through a drilled hole and sealed with silicone. Each pilot bioreactor was filled with 40 L of waste rocks which corresponded to 20% (v/v).

A Masterflex peristaltic pump (Masterflex L/S Series) with a pump head (Easy-Load II L/S, model 77202-60) was used to pump MIW from the 1000 L tank to the bottom of the bioreactors, so that the flow goes up. The pipe was connected to the bottom of bioreactors with a 2" polypropylene bulkhead fitting. The flow rate was calculated to have a 2 weeks hydraulic retention time (HRT) in the bioreactors to reflect the HRT in the historic tunnels underground estimated by Alexco Resource Corp through tracer monitoring.

Another Masterflex peristaltic pump (Masterflex L/S Series) with a pump head (model 7519-75) was used to pump the carbon source solution from a 10 L glass bottle and to inject it into the bottom of the bioreactors through a Masterflex polyethylene T-connector. A timer (08645-15, Digital Programmable Plug-In Timer Controller; 115 VAC, Cole-Parmer, Canada) was used to start and shut down the Masterflex peristaltic pump for 51.6 minutes every day at 6 mL/min. Molasses have been chosen as the carbon source for this experiment. The molasses solution was prepared biweekly in the laboratory (Yukon College, Whitehorse, YT, Canada) from Molasses (Crosby's 100% Natural Fancy Molasses; Crosby Molasses Company Limited, St. John, NB, Canada). In order to prevent the molasses from settling down in the glass bottle during operation of the bioreactors, the bottle was constantly stirred with a stirrer (Thermolyne Cimarec 3, Thermo Fisher Scientific, USA).

Equations 4-1 and 4-2 present the reactions of the reduction of sulfate into sulfide and the precipitation of metal as sulfide that occur in an PSRB.

Equation 4.1 $\mathbf{SO_4^{2-} + 2\ C\ (organic) + 2\ H_2O + SRB <=> H_2S_{(aq)} + 2\ HCO_3^-}$

Equation 4.2 $\mathbf{M^{2+} + H_2S <=> MS_{(s)} + 2\ H^+}$

As described in Equation 4-1, 2 moles of carbon are required to reduce one mole of sulfate. Molasses has been chosen as the complex organic carbon source for this study. Based on the average sulfate concentration calculated during Alexco's MIW characterization, 8.9 mmol/L of carbon were required to allow sulfate reduction by SRB.

To prevent the bioreactors from freezing, the entire setting was installed in a 8 x 12 ft insulated shed located on site besides the borehole (Figure 1-6) and was heated at 5°C in winter time to represent the average temperature of the underground water (Alexco's data).

4.5.2 Water collection

Alexco Resource Corp. dug a 110 m deep borehole at the location called Silver King Mine site. The borehole is thought to connect to one of the vein historically mined and to a network of old flooded tunnels. A submersed pump was installed in the borehole by Alexco Resource Corp. and was constantly pumping (4 - 16 L /s) from the borehole, up to the surface and into pipes leading to the lime treatment plant (Gault and Harrington, 2016). Every two weeks, water from the submersed pump was used to fill up a 1000 L tank. This tank was emptied each time before the refill.

4.5.3 Waste rocks characterization

The waste rocks (WR) used in this experiment were collected at the Keno Hill mine site in June 2014 from two different waste rock piles (WR1 and WR2) that were built when the Silver King tunnels were excavated (personal discussion with Alexco Resources, 2014). Approximately 20 L of WR1 from pile 1 and 20 L of WR2 from pile 2 were added to the bioreactors in order to replicate the underground conditions and provide a bacterial surface growth. The particle size distribution and chemical composition, expressed as oxides, of the waste rocks used in this study are described in a previous publication (Nielsen *et al.,* 2016). The potential for acid generation from the waste rock samples was evaluated initially using a Sobek test and the results showed a “low” risk for ARD generation (Nielsen *et al.,* 2016).

4.5.4 Pilot Bioreactor Monitoring

During this 13 months experiment, various parameters were followed in the pilot bioreactors to estimate the native SRB activity and consequently, the removal of metals (Cd and Zn).

Samples were collected weekly from the 200 L outlet drums 1 & 2. According to the 2 weeks HRT calculation, approximately 100 L was collected in each outlet drum every week, this volume of water was stirred with a PVC pipe for complete homogenization and the sample was taken. Temperature was measured by connecting a thermometer (Acorn Temp 5, Oakton, Australia) to the probes installed on the bioreactors’ top. An extra probe was used to measure the influent MIW’s temperature in the 1000 L feed tank. pH and conductivity were measured in both of the outlet drums and in the 1000 L MIW influent tank. Samples for the analysis of sulfate were collected using a 50-mL high-density polyethylene plastic bottle, sent to the laboratory in Whitehorse (YT, Canada) and stored in a freezer (-17°C) until analysis. The percentage of sulfate consumption was calculated by subtracting the measured sulfate concentration in each bioreactor from the sulfate concentration measured weekly in the MIW divided by the sulfate concentration in MIW. The temperature presented is the monthly average of the temperatures of the water measured weekly inside each bioreactor. Samples for total organic carbon (TOC) determination were collected in a 50-mL capacity bottle and acidified with H_2SO_4 (2%, v/v) and sent to the laboratory and stored in a fridge (4°C) until analysis. For the determination of the dissolved Zn and Cd contents, 50-mL sample was collected and stored at 4°C after acidification using HNO_3 (2%, v/v).

During December 2015, loss of power shut down the heating system in the shed housing the bioreactors. One of BR1’s pipes froze and broke and BR1 was partially emptied. No sampling for BR1 was completed during this period.

4.5.5 Biological sample collection

The term “sampling bags” refers to passive samplers used for the collection of microorganisms that attach to the surfaces of the sample bags and the rocks within them (i.e. biofilm). Approximately 20 sampling bags made with light cotton cheese cloth filled with few grams of the two waste rocks types, originating from both piles. Samlping bags were installed in the middle of each bioreactor, suspended with fish line for bacterial sample collection and in the MIW after being pumped from the Silver King Mine’s borehole.

Once per month, a passive sampler was collected in order to have a bacterial genomic characterization. The bioreactors’ caps were opened to sample one of the sampling bag from BR1 and one of the passive sampler from BR2. Then, passive samplers were frozen into liquid nitrogen in a thermoflask (container 2122, Thermo scientific, USA) transported from field to the laboratories in the same thermoflask and stored in a -80°C freezer before a DNA extraction was performed. The oxidation-reduction potential (ORP) was determined in each pilot bioreactor after the passive sampler sampling, while the caps were opened. This parameter was quantified without mixing the water to reduce the variability of the ORP measurement.

After the microbes sampling and ORP measurements, bioreactors’ caps were sealed with silicone to keep the system as anoxic as possible.

4.5.6 DNA extraction and sequencing

DNA was extracted from biofilms on the passive samplers including those attached to both rocks and bag material taken from the bioreactors at different time points (Table 4-1) using the fast DNA^{TM} spin kit for soil according to the manufacturer's specifications (MP bio Solon, OH, USA). Amplicons for the v4 variable region of the 16S rRNA gene were produced from target DNA by polymerase chain reaction (PCR) with primers: 515f 5’ GTGCCAGCMGCCGCGGTAA 3’, 806r 5’ GGACTACHVGGGTWTCTAAT 3’ using methods described in Caporaso (2012) 24. Amplicons were sequenced using Illumina MiSeq technology by microbiome INSIGHTS (Vancouver, BC, Canada).

DNA extraction has been done at UBC laboratories in May 2016 but the pilot bioreactors were monitored up to August 2016. That is why are only presented the genomic characterization from July 2015 to May 2016, whereas the bioreactors have been monitored until August 2016.

Table 4-1 **Bags samples from BR1, BR2 and MIW for genomic characterization with temperatures and pH (averages per month)**

Date	Description	T °C	pH
July 1, 2015	BR1	17.6 ± 2.0	6.7 ± 0.3
	BR1	17.6 ± 2.0	6.7 ± 0.3
	BR2	17.6 ± 2.0	6.9 ± 0.1
	BR2	17.6 ± 2.0	6.9 ± 0.1
August 1, 2015	BR1	14.9 ± 1.7	6.0 ± 0.1
	BR1	14.9 ± 1.7	6.0 ± 0.1
	BR2	14.0 ± 1.6	6.9
	BR2	14.0 ± 1.6	6.9
September 1, 2015	BR1	6.8 ± 0.2	6.8 ± 0.1
	BR1	6.8 ± 0.2	6.8 ± 0.1
October 1, 2015	MIW	3.1	6.5
	MIW	3.1	6.5
	BR 1	4.1	6.8
	BR 1	4.1	6.8
	BR2	4.0	6.6
	BR2	4.0	6.6
November 1, 2015	MIW	1.7 ± 0.1	6.6 ± 0.5
	MIW	1.7 ± 0.1	6.6 ± 0.5
	BR1	6.1 ± 2.7	7.3 ± 0.6
	BR1	6.1 ± 2.7	7.3 ± 0.6
	BR2	3.8 ± 0.5	7.1 ± 0.6
	BR2	3.8 ± 0.5	7.1 ± 0.6
	Rocks from BR2	3.8 ± 0.5	7.1 ± 0.6
December 1, 2015	BR1	5.0 ± 1.6	6.4 ± 0.1
	BR1	5.0 ± 1.6	6.4 ± 0.1
	BR2	3.4 ± 1.3	6.6 ± 0.1
	BR2	3.4 ± 1.3	6.6 ± 0.1
January 1, 2016	MIW	2.4	7.0
	MIW	2.4	7.0
	BR2	8.0	7.0
	BR2	8.0	7.0
February 1, 2016	MIW	2.5 ± 0.4	7.1
	MIW	2.5 ± 0.4	7.1
	BR1	5.5 ± 0.9	7.1
	BR1	5.5 ± 0.9	7.1
	BR2	5.6 ± 0.9	7.1
	BR2	5.6 ± 0.9	7.1
March 1, 2016	BR2	10.7 ± 6.2	7.2 ± 0.1
April 1, 2016	MIW	3.2 ± 1.2	7.1 ± 0.8
	MIW	3.2 ± 1.2	7.1 ± 0.8
	BR1	8.5 ± 3.5	7.3 ± 0.8
	BR1	8.5 ± 3.5	7.3 ± 0.8
	BR2	10.0 ± 5.0	7.2
	BR2	10.0 ± 5.0	7.2
May 1, 2016	MIW	2.6	6.6
	MIW	2.6	6.6
	BR1	12.3	7.6
	BR1	12.3	7.6
	BR2	12.4	6.9
	BR2	12.4	6.9

4.5.6.1 Bioinformatics

All pre-processing and sequence quality control steps were performed using USEARCH (v 9.0.2132, 32 bit for Linux) according to the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_pipeline.html; date accessed 1 Oct 2016) (Edgar, 2013). Briefly, paired end reads were combined, as suggested by the uparse pipeline and unpaired reads or reads with a maximum expected error probability > 1 were removed from the analysis. As suggested by Huse *et al.* (2010), the remaining sequences were pre-clustered using the furthest neighbor algorithm with 1% sequence dissimilarity as the threshold to prevent over estimation of operational taxonomic units (OTUs) richness, using the cluster smallmem command. OTUs were assigned using the UPARSE greedy algorithm for an OTU definition of 97% sequence similarity. Although this step also removes chimeras, a dedicated chimera removal step was additionally used post OTU clustering using the UCHIME 2 algorithm with SILVA gold dataset as reference (Edgar, 2016). Global singleton OTUs (those that are represented by a single sequence in the entire dataset) were removed due to their unknown nature. The bacterial OTUs were classified to phylum, class, family, and/or genus level using the Ribosomal Database Project bysian classifier (train set 14) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). Following classification, sequences not classified as bacteria were removed from the dataset. Raw sequence files were submitted to the National Center for Biotechnology Information Sequence Read Archive under SRA Accession.

4.5.6.2 Community analysis

All samples were subsampled to the same total number of reads (3000) per sample using the MOTHUR function subsample to eliminate biases of alpha diversity estimations (Gihringet *et al.,* 2012; Schloss *et al.,* 2009). Observed number of OTUs as well as expected number of OTUs were calculated in MOTHUR. Expected number of OTUs was estimated using the nonparametric Chao 1 (Chao and Shen, 2003). Library coverage was estimated by calculating the ratio of observed to expected OTU numbers (Annexe 1). Coverage for all samples was above 50% of the richness which indicates adequate sequencing depth.

Differences between the compositions of the communities (beta diversity) in each of the samples were performed in R (version 3.3.1) using the metaMDS function from the package with weighted Bray-Curtis dissimilarity index (Oksanen *et al.,* 2015; Bray and Curtis, 2015). In order to prevent biases of the beta diversity estimation due to highly abundant OTUs, the samples were log transformed prior to the generation of the distance matrix (Costea *et al.,* 2014). Non-metric multidimensional scaling (NMDS) ordination was used to visualize the distances between the communities based on the matrix generated with the metaMDS. K-means clustering was used to assess the number of clusters to which samples group to and Analysis of Molecular Variance (AMOVA) was used to test for the statistical significance of the grouping (Excoffier *et al.,* 1992; Martin, 2002). The function envfit from the package vegan was used to test which genus level groups significantly correlate with the grouping of the samples based on the NMDS. Indicator species analysis was done in MOTHUR using the indicator command to determine which OTUs significantly explain the grouping of the samples.

4.5.7 Analytical Methods

The dissolved metals present in the MIW, the effluents emerging from the bioreactors and controls were analyzed by an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) apparatus (Varian, Vista-AX CCO, Palto Alto, CA, USA). Detection limits were, 1.4 µg/L for Cd and 1.5 µg/L for Zn. Quantification were 4.8 µg/L for Cd and 5.0 µg/L for Zn. Sulphate concentrations were determined spectroscopically, using a SmartChem® 170 Analyzer (Unity Scientific, USA) following Yukon Research Centre protocols, which were developed from the SmartChem® 170 Analyzer user manual. The instrument was calibrated using Accuspec sulphate standards (SCP Sciences, Quebec). QC standards at lower and upper detection limit concentrations of 10 mg and 80 mg, respectively, were analyzed per 10-15 samples. The total organic carbon content of each substrate was determined using a Primacs TOC Analyzer (Skalar, Netherlands) following Yukon Research Centre protocols developed from the Primacs user manual. Briefly, substrate materials were dried, milled, and sieved to 412 µm, prior to analysis. The instrument was calibrated using oxalic acid dihydrate standards (ACS reagent, Sigma-Aldrich Canada), as described in the Primacs user manual.

pH measurements were performed using a pH meter (PCD 650, Oakton, Australia) (pH electrode, Oakton, Brisbane, Australia) equipped with a double junction Ag/AgCl electrode (Cole Parmer Canada, Montréal, QC, Canada). Calibration of the pH meter was performed daily using certified buffer solutions (buffer solution pH = 10.00; pH 7.00; pH 4.00, Fisher Scientific, Montréal, QC, Canada). Oxidation-reduction potential measurements were performed using a double junction electrode ORP (ORP Electrode tested. 59001-77, Cole Parmer, Montréal, QC, Canada). Calibration of the ORP meter was performed using a certified solution (ORP Standard, Thermo Fisher Scientific, Waltham, MA, USA). The pH and ORP probes were rinsed with DIW between each sample.

4.6 Results

The mine drainage from Silver King mine site was neutral with an average pH of 7.3 ± 0.5 (Alexco's characterization, Table 2-4). During the bioreactor experiments, the mine influenced water feed remained neutral throughout the study period from July 2015 to August 2016 (Table 4-1).

4.6.1 Sulfate reduction in pilot bioreactors

Figure 4-1 shows the percentage of sulfate consumption during the 14 months experiment along with the temperature inside both bioreactors. Although MIW characteristics may vary over such a long period of time, sulfate concentration remained fairly stable with an average of 418 ± 131 mg/L over the 14 months experiment, which is close to Alexco's MIW characterization with a sulfate average of 426 ± 182 mg/L (Table 2-4).

Figure 4-1 a. shows that in BR1, the highest sulfate consumption yields were observed in summer time with 76.6% in August 2015 and 91.9% of sulfate removal in August 2016, when the internal temperature of the BR1 was around 17.5 ± 2.0°C in August 2015 and 16.0°C in August 2016. It can be noticed that when the temperature in BR1 went down, the sulfate removals decreased. Especially during fall/winter time, between October 2015 and February 2015, when the temperature is between 4.1 ± 0.0°C and 5.3 ± 1.0°C inside the bioreactors, the sulfate consumption was between 0.0% and 37.5%, respectively. The duplicate bioreactor BR2 (Figure 4-1 b) showed similar trend as BR1, with the highest sulfate removal observed in summer time with 85% in July 2015 when the temperature was 17.6 ± 1.9°C and

84.1% in August 2015 when the temperature was 15.8 ± 0.2°C. As with BR1, sulfate consumption in BR2 decreased with the temperature during winter time.

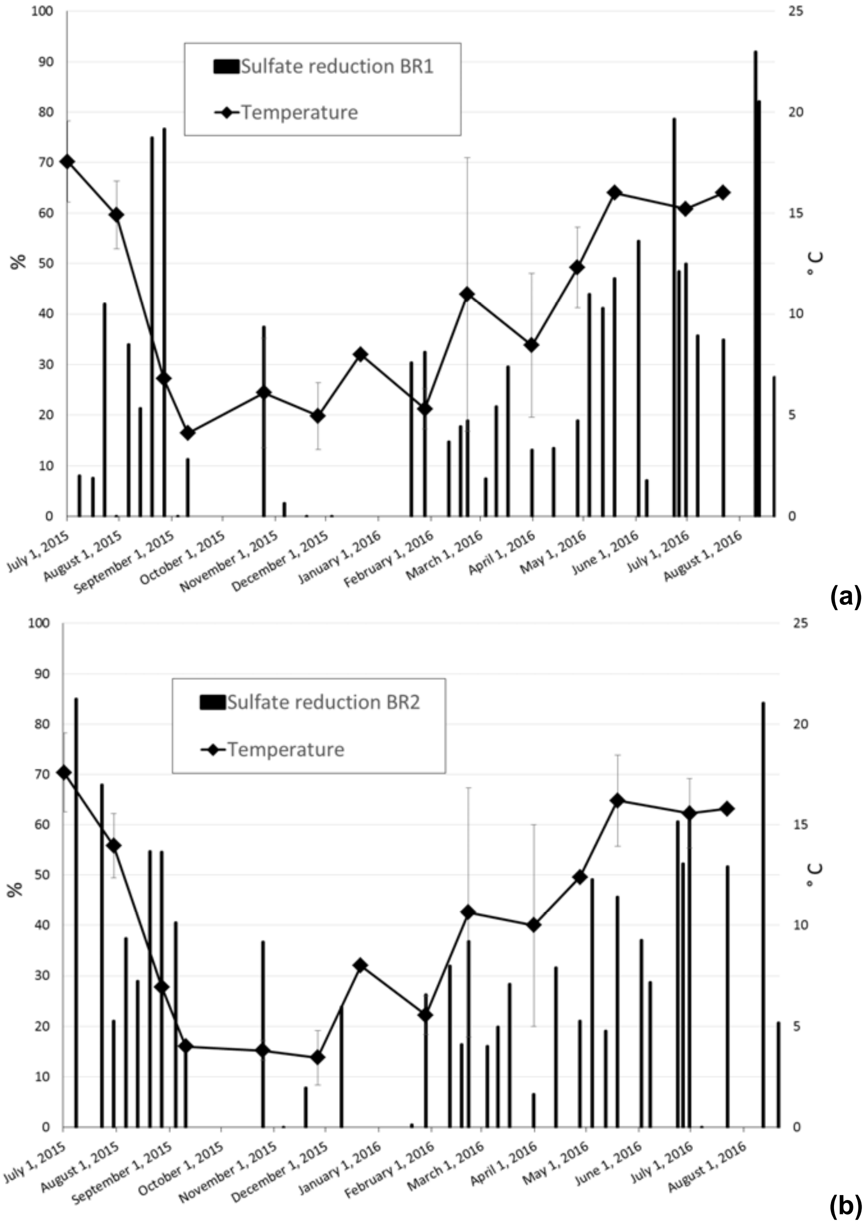

Figure 4-1 **Sulfate reduction and temperature in BR1 (a) and in BR2 (b) during the 14 month experiment**

4.6.2 Carbon consumption

Figure 4-2 presents carbon concentration in BR1 and BR2 and MIW during the 14 month experiment. Not surprisingly, first carbon samples from July, 8th 2015 were very closed to the calculated carbon concentration with 7.0 mmol/L in BR1 and 9.3 mmol/L in BR2. But it is interesting to notice that carbon consumption started very quickly as the carbon concentration dropped to 1.4 mmol/L in BR1 and 2.3 mmol/L in BR2 within the first two weeks of operation (on July, 23rd 2015) which corresponded to 83.9% and 73.8% of carbon consumption, respectively. During summer 2015, between July 8th and September 10th, carbon consumption was up to 96.8% in BR1 and 96.6% in BR2 with lowest residual carbon concentrations of 0.3 mmol/L in both BR1 and BR2.

Then, during fall and winter time, residual carbon concentration increased in both bioreactors to reach 4.6 mmol/L in BR2 in November 2015 and 3.8 mmol/L in BR1 in February 2016, which corresponded to 48.3% and 57.3% of carbon consumption, respectively.

During springtime 2016, when temperature went up to 11.0 ± 6.7°C in March in BR1 and 10.5 ± 6.1°C in BR2, residual carbon concentration decreased to 0.3 mmol/L and 0.4 mmol/L which represented 96.3% and 95.9% of carbon consumption in BR1 and BR2, respectively. From May 2016 to June 2016, carbon concentration in the MIW increased from 0.1 mmol/L in March 2016 to 2.3 mmol/L in July 2016, so did the carbon concentration feeding into the two bioreactors.

Despite increasing carbon concentration feeding into the bioreactors during summer 2016 and increasing residual concentration collected in the effluent of the bioreactors at 1.6 mmol/L in BR1 and BR2, the carbon removal efficiency remained high with 82.1% and 82.0 % of carbon consumption in BR1 and BR2, respectively, in July 2016. The carbon consumption observed during the summer 2016 was closed to the consumption observed in summer 2015.

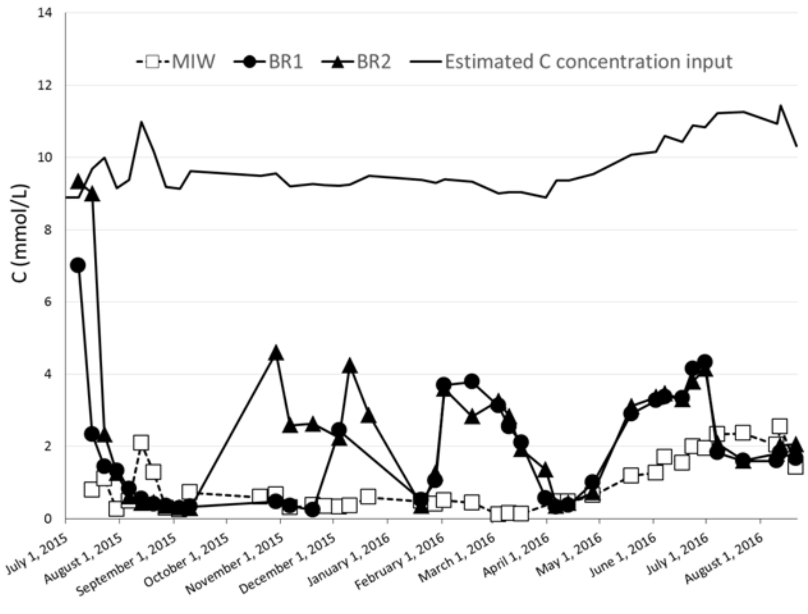

Figure 4-2 **Carbon concentration in MIW, BR1 and BR2 and calculated carbon concentration in the input during the 14 months experiment**

4.6.3 Zinc and Cadmium removals

4.6.3.1 Zinc and Cadmium in MIW

Figure 4-3 presents Zn and Cd concentrations measured in MIW and both bioreactors. At the beginning of the experiment, Zn and Cd concentrations in the MIW were closed to the concentrations expected based on the average of the 5-years water quality monitoring program conducted by Alexco and reported in Table 2-4. However, as expected, the conditions underground changed during the course of the 14 months experiments with discontinuous pumping of the water from the borehole, changing water levels and changing precipitations above ground along the seasons. In July 2015, Zn concentration was 640 µg/L and remained relatively stable until early September 2015. Then, Zn concentration started to decrease from 915 µg/L (September, 10th 2015) to 270 µg/L (February, 2nd 2016). Then, Zn concentration remained between 208 µg/L (May, 19th 2016) and 531 µg/L (August, 12th 2016). Cd concentration was also closed from Alexco's characterization with 10.5 µg/L in July 2015, then it went up to 14.6 µg/L mid-

August 2015 before it continuously decreased to 1.30 µg/L in January 2016. Cd concentrations remained below 4.1 µg/L during winter time and suddenly increased up to 12.6 µg/L during summer 2016.

Calcium was also monitored in the MIW but is not shown. Total Ca concentration in MIW remained stable at 162 ± 6 mg/L during the 14 months experiment. At this concentration, the risk of forming gypsum (Fajtl *et al.,* 2002) or ettringite is low and is unlikely to affect the overall process (Fajtl *et al.,* 2002).

4.6.3.2 Zinc and Cadmium in Bioreactors effluents

Despite the fact significant variations in Zn and Cd concentrations in MIW were observed, Zn and Cd removals were studied based on weekly MIW Zn and Cd concentrations.

4.6.3.2.1 Zinc removal

In July 2015, at the beginning of this pilot scale experiment, Zn concentration in MIW was 640 µg/L (Figure 4-3 a.). In the first sample of effluent collected, Zn concentrations in treated water from the bioreactors were low with 72.7 µg/L for BR1 and 87.3 µg/L for BR2.

During summer 2015, from July 8th 2015 to September 10th 2015, when Zn concentrations in MIW was 587 ± 157 µg/L, Zn concentrations in treated water were lower with 95.3 ± 61.0 µg/L in BR1 and 123 ± 47 µg/L in BR2. That corresponds to Zn removal of 82.7 ± 13.9% in BR1 and 78.5 ± 9.3% in BR2 on average during summer (July to September included) when the temperature was mild (Figure 4-1).

During fall and winter time, from October 29th 2015 to February 22nd 2016, Zn concentration decreased significantly in MIW to 270 µg/L (on February 2nd 2016) while residual Zn concentrations in the bioreactors effluent increased hence. The Zn removal averages decreased down to 36.6 ± 15.5% in BR1 and 40.6 ± 19.9% in BR2. On January 28th 2016, both bioreactors were at their lowest Zn removal efficiency with 28.2% for BR1 and 13.6% for BR2, with initial Zn concentration in the MIW of 303 µg/L and residual Zn concentration in the bioreactors effluent of 217 µg/L in BR1 and 261 µg/L in BR2.

During springtime 2016, between March 4th 2016 and April 6th 2016, Zn concentration in MIW remained low and around 331 ± 20 µg/L. In the same period, Zn concentrations in treated water were reduced to 132 ± 50 µg/L in BR1 and 145 ± 11 µg/L in BR2, which represents 59.9 ± 15.5% and 56.0 ± 3.2% of Zn removal, respectively, over the period March 4th to April 6th. From springtime 2016 to summer 2016, Zn removal increased continuously to reach 92.8% in BR1 and 85.7% in BR2 mid-July 2016.

4.6.3.2.2 Cadmium removal

Figure 4-3 b. presents Cd concentrations in MIW and in the bioreactors. As observed with Zn removal, Cd concentration in MIW was 10.5 µg/L at the beginning of the experiment, which was closed to Alexco's 5-years water quality average (Table 2-4).

During summer 2015, when temperatures were above 4.1°C, Cd concentration in treated water remained very low, under 2.60 µg/L in BR1 and under 1.50 µg/L in BR2, which represented 89.9 ± 12.4% for BR1 and 91.1 ± 6.0% in BR2 of Cd removal efficiency. During fall-winter time, Cd concentration in MIW decreased significantly and Cd removal in bioreactors reached minimal removal yields of 51.0 ± 21.8% in BR1 and 50.9 ± 13.2% in winter time. As seen with Zn concentrations, Cd removal yields increased during springtime 2016. Thus, from March 10th 2016 to May 19th 2016, Cd removal was around 68.0 ± 7.7% in BR1 and 61.5 ± 17.0% in BR2.

During summer 2016, even if Cd concentration in MIW increased sharply (Figure 4-3 b.) from 0.90 µg/L on April 13th 2016 to 12.6 µg/L on August 12th 2016, Cd concentration in treated water remained stable and low with averages of 0.90 ± 0.60 µg/L and 1.40 ± 1.10 µg/L in BR1 and BR2, respectively. Those results are very closed to those obtained in summer 2015 and represented 88.7 ± 7.9% of Cd removal in BR1 and 83.3 ± 12.5% in BR2.

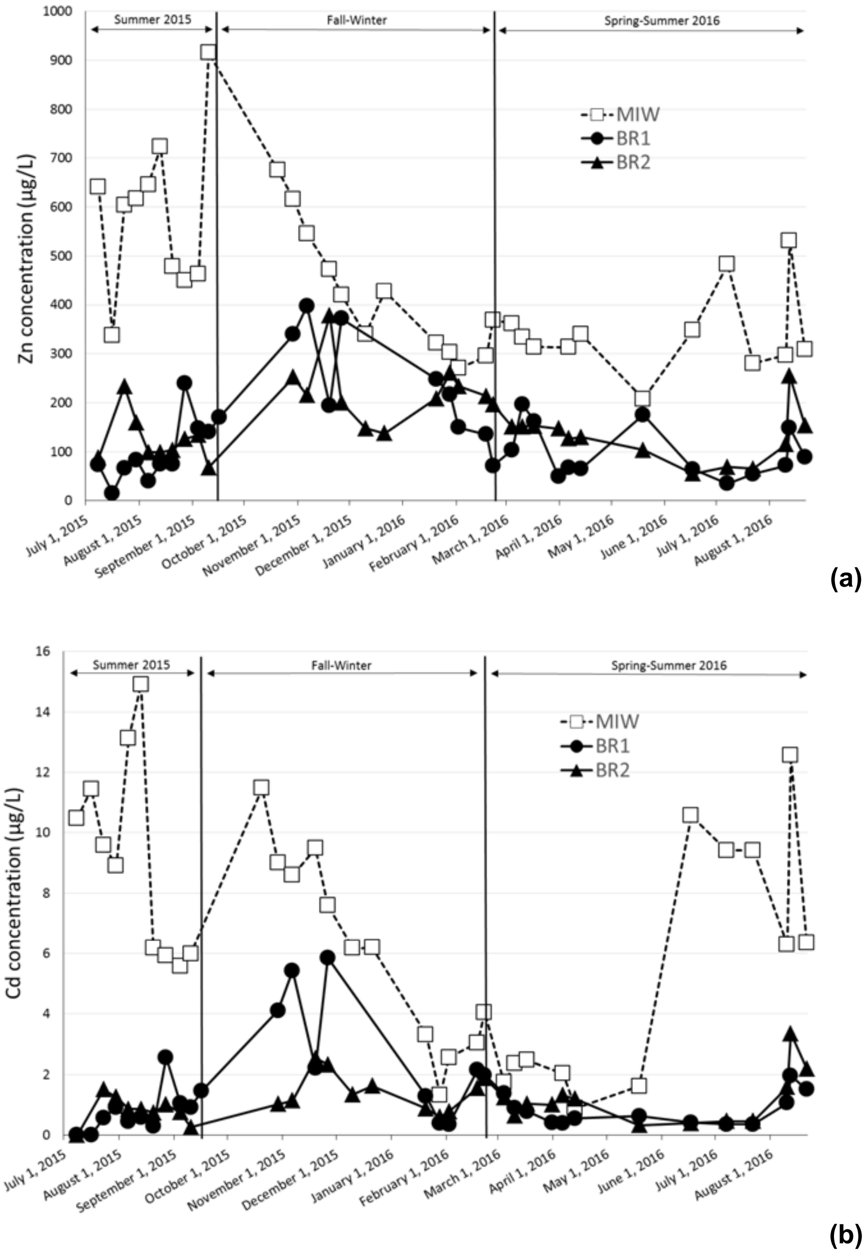

Figure 4-3 Zn (a) and Cd (b) concentrations in MIW, BR1 and BR2 during the 14 months experiment

4.6.4 Characterization of the bacterial community in the bioreactors and their mine impacted water feed

The microbial community is a key component of the function of these bioreactors and the genomic study presented here identified and tracked taxonomic groups on a monthly basis from the bioreactors operated at the Keno Hill Mine District. Bacteria found in the MIW are the source population for the communities inhabiting the bioreactors. Thus, it is not surprising that the Bioreactors and MIW samples shared similar class level taxonomic groups (Figure 4-4). Nevertheless, there were some differences both in the presence and the relative abundance of these groups between the two sample types. The differences were mainly in the abundance of members of the classes *Bacilli* and *Betaproteobacteria,* which were more abundant in the MIW samples and members of the classes *Clostridia* and *Deltaproteobacteria* which were more abundant in the Bioreactors samples (Figure 4-4).

These results agreed with Lefticariu *et al.* (2015)'s findings, which showed high relative abundance of members of the classes *Clostridia* and *Deltaproteobacteria* in a 460 days study of a bioreactor treating AMD. As both of the classes *Clostridia* and *Deltaproteobacteria* contains members capable of sulfur reduction (Campbell and Postgate, 1965; Druschel *et al.,* 2004; Lefticariu *et al.,* 2015; Meyer and Kuever, 2007; Pruden *et al.,* 2007; Sallam and Steinbüchel, 2009), the classification of dominant OTUs (relative abundance > 1%) from these classes to identify potential sulfur reducers has been examined (Figure 4-5). Two of the dominant OTUs belonging to the *Deltaproteobacteria* class (OTU 33 and OTU 186) classified as members of genera capable of sulfur reduction (unclassified genus of the family *Desulfobacteraceae* and member of the genus *Desulfobulbus*, respectively) (Figure 4-5 a.). Both were present in the bioreactors samples and not in the MIW samples. *Desulfocapsa*, another potential sulfur reducer, was also present only in bioreactors samples but did not have abundance > 1% in all bioreactors samples (Figure 4-5 a.).

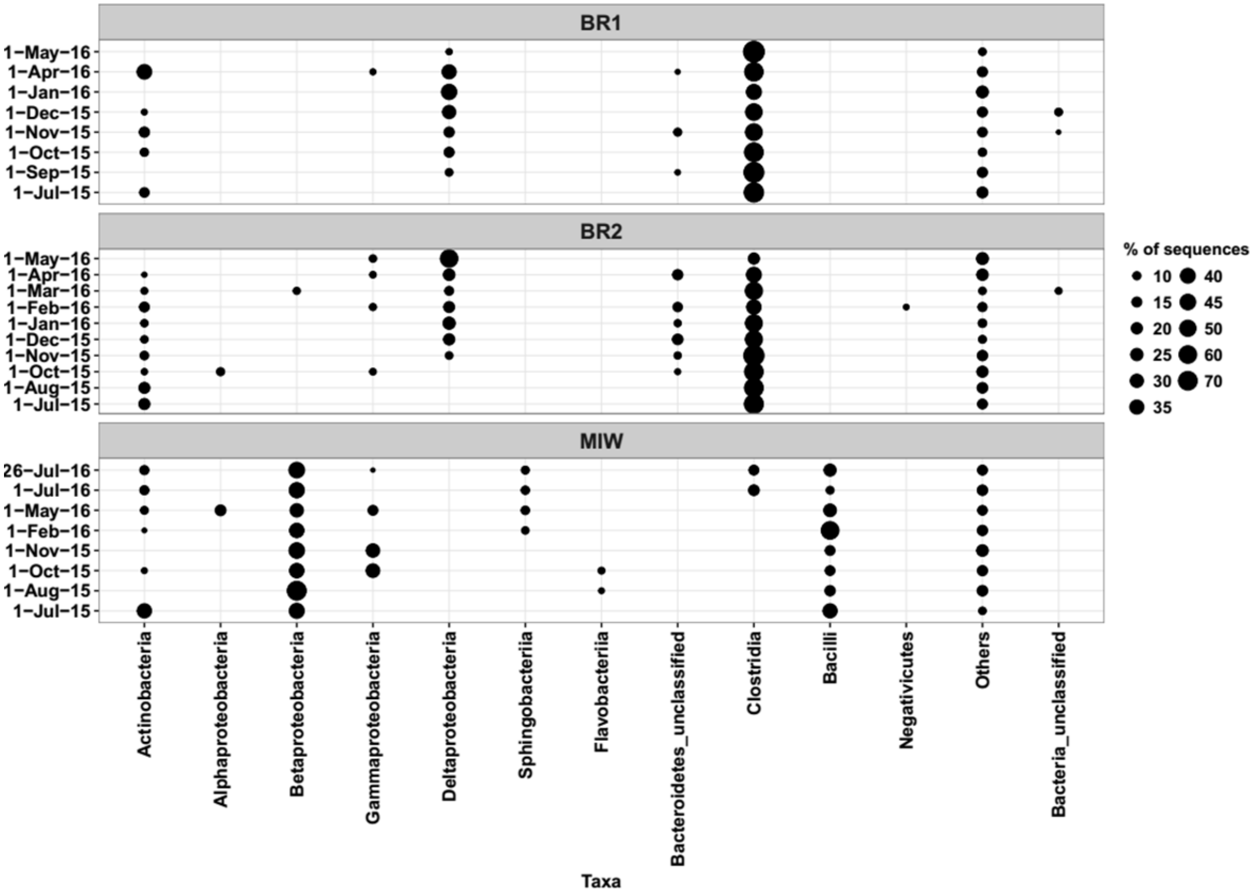

Figure 4-4 **Relative abundance of class level taxonomic groups within the bioreactors (BR1, BR2) and mine impacted water (MIW). Classes with relative abundance < 5% of the total number of sequences per sample were grouped in the others category**

There were several OTUs belonging to many genera within the class *Clostridia* that occurred exclusively or in greater relative abundance in bioreactors samples but not in the MIW samples (Figure 4-5 b.). Of those, both OTUs classified as members of the genus *Clostridium* (*Clostridium* sensu stricto) and those classified as members of the genus *Desulfosporosinus* are potential sulfur reducers (Figure 4-5 b.). *Desulfosporosinus* sp. are spore-forming and therefore more resilient to extreme environments. Several species were found to be active at low pH and in metal-contaminated environments. In some cases, neutralization of acidic mine wastes was attributed to the presence of *Desulfosporosinus*. Thus, these results strongly support that the bioreactor is an environment conducive to the colonization of sulfate reducing bacteria.

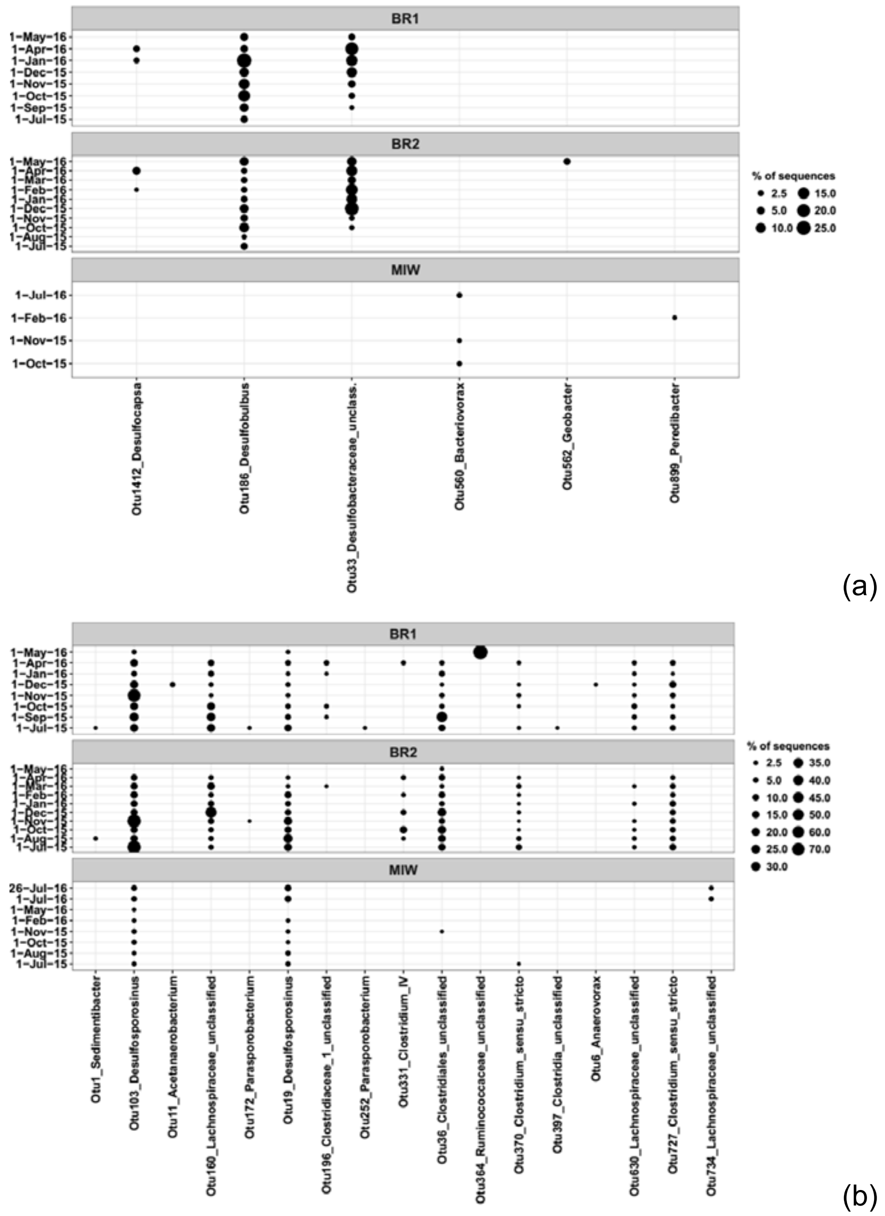

Figure 4-5 Relative abundance of OTUs belonging to class Deltaproteobacteria (a) and Clostridia (b). Only OTUs with relative abundance >1% of the total number of sequences per sample are presented.

Examining the composition of the communities from the bioreactors and MIW at a finer scale (at the total OTU level), there were striking differences between the two sample types. This is apparent by examining the grouping of samples based on the NMDS ordination plot, where two clusters are noticeable, one for MIW and one for bioreactor samples, respectively (Figure 4-6). As seen in Figure 4-6, there are no distinct differences in structure between samples from BR1 and BR2 as they do not cluster separately. Also no discernable trends in microbial structure shifts between the different months were observed. Similarity between the microbial community structures of BR1 and BR2 is attributed to their similar operating conditions and since they received the same influent water. Consistency in microbial structure over time suggests relatively stable environmental conditions under which the system was operated (Table 4-1).

Supervised k-means clustering confirmed that two was the minimal number of clusters required to explain the grouping of the communities, and the MIW and bioreactor samples were split into these two groups. The microbial population structures of these bioreactor and MIW groups were statistically significantly different based on AMOVA ($Fs_{(1,56)}=45.13$, $p<0.001$, Figure 4-6).

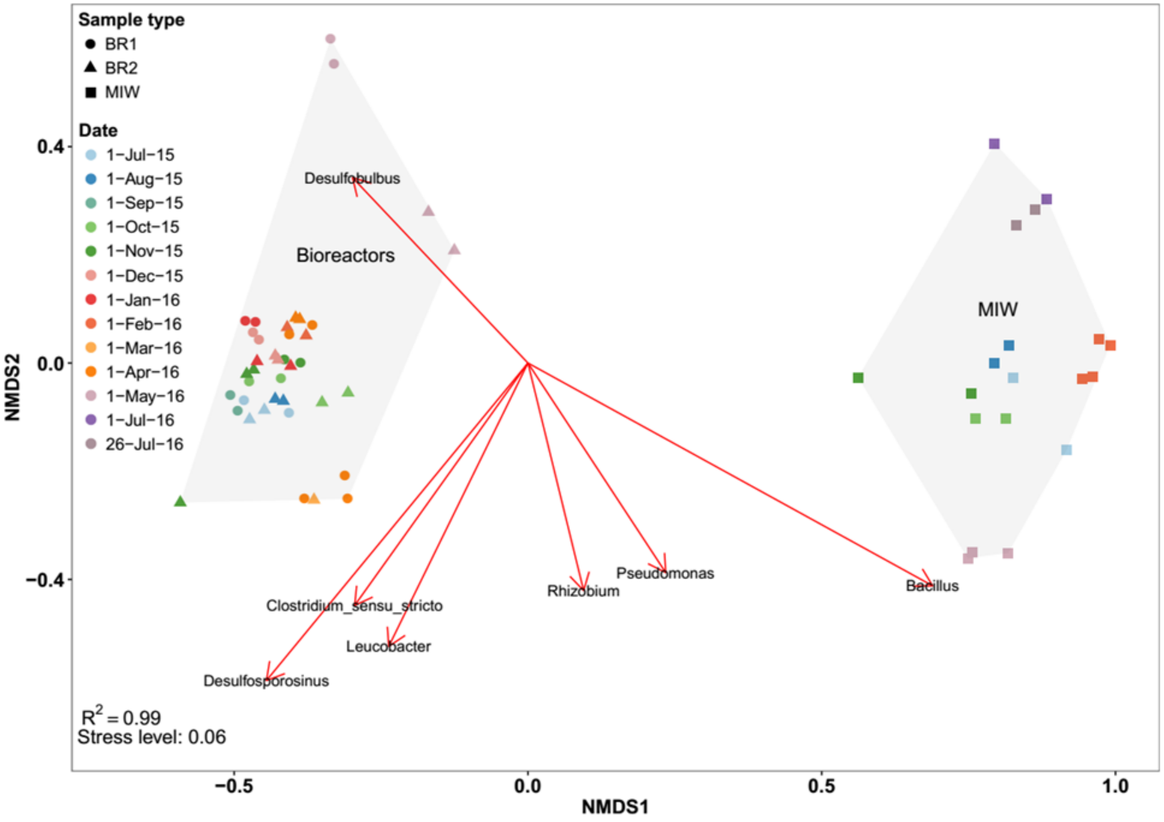

Figure 4-6 NMDS based on weighted Bray-Curtis distances on log transformed OTU read count. MIW = mine impacted water, BR1 and BR2 = bioreactors. Gray polygons represent statistically significant groups based on AMOVA ($p < 0.01$). Arrows indicate genera that significantly correlate with the representation of the communities in multidimensional space ($p < 0.05$). Length of the arrows scaled by strength of correlation. Arrows point in direction they contribute to the way communities are positioned in the ordination plot.

Several genera significantly correlated with the separation of the two clusters (Figure 4-6). Mainly the genera *Desulfobulbus* of the class *Deltaproteobacteria*, *Clostridium* and, *Desulfosporosinus* of the class *Clostridia*, and *Leucobacter* of the class *Actinobacteria*, significantly correlated with the bioreactors while the genus *Bacillus* of the class *Bacilli* significantly correlated with the MIW. Of the four genera that positively correlated with the bioreactors, the first three contain members that are potential sulfur reducers.

Looking at specific OTUs that correlated significantly with the separation to the two groups (indicator OTUs), it is possible to see again that OTUs belonging to sulfate reducers are among those that positively correlate with the bioreactors groups and not with the MIW group (Annexe 2).

The ordination plot also showed that while belonging to the same group, the April and May samples from the bioreactors and the May and July samples from the MIW were most distant from other samples of their respective groups (Figure 4-6). Therefore, the samples from MIW and bioreactors groups were analyzed by sample type to identify which genera within these particular samples correlated positively with the difference in community composition (Annexe 3). The genera which positively correlated with the difference between the later samples and the rest were not the same for the MIW and bioreactors. For MIW samples, low abundance genera (indicated by the “others” category on Annexe 3) along with members of the genus *Rhizobium* were positively correlated with this difference. In the BR samples, it was members of the genera *Leucobacter* and *Clostridium*. This suggests that the change in the later samples from the bioreactors was not dependent on the change in the later samples of the MIW community and might stem from a factor not measured in this study.

4.7 Discussion

The monitoring of pilot scale PSRB brought new challenges and issues, especially in cold climate. The conditions underground changed during the course of the 14 months experiments and initial Zn and Cd concentrations in MIW varied significantly. These variations in Zn and Cd MIW concentrations could be explained by a groundwater dilution/concentration effect. Alexco pumped water from the Silver King borehole and water was reinjected to create a recirculation loop within the flooded mine workings. During spring 2015 where there was high recharge rate due to freshet, the pump in use could not adequately control the water level in the underground mine tunnels and this led to the complete flooding of the mine workings and overflow of water was observed according to Gault and Harrington (2016). This event might have caused a dilution effect from November 2015 to February 2016 leading to the decrease of Zn and Cd concentrations as shown in Figure 4-3. The pump was replaced in November 2015 by a more powerful unit and better groundwater level management was achieved, keeping the water level lower. On April 2016, the effects of freshet and the associated increased recharge rate were first observed as the groundwater level started to increase sharply. In response to this, the recirculation of water to the Silver King pit was suspended and the dewatering well pumping rate was increased on May 2016. The increased pumping rate slowed the water level rise in the mine workings and managed to maintain the groundwater level between May and mid-July 2016 (Gault and Harrington, 2016). This groundwater level being maintained might have probably caused a concentration effect on Zn and Cd concentrations in the same period as shown in Figure 4-3. Degens (2012) implemented pilot-scale PSRB to treat groundwater struggled with high concentrations of metals. This author also had issues with the groundwater's flow rate that seasonally varied from less than 3 kL/day in late autumn to more than 5 kL/ day in late winter. One of the consequences of working with real groundwater is that precise hydraulic retention rate is hard to measure and must be estimated (Degens, 2012).

This pilot scale project intended to know if passive, or semi-passive sulfate reducing bioreactor would be efficient- suitable technology to handle Zn and Cd removal in the MIW from Silver King mine site. The presence of native SRB in the mine site was expected as it has been reported in previous studies of the

Keno Hill mine site (Londry, 2013; Nielsen *et al.,* 2016) but the genomic characterization performed in this study brought to light potential SRB which make the process possible.

The best Zn and Cd removal yields were found to be during summers 2016 and 2017, when temperatures were above 10°C, to reach 95.5% of Zn removal in BR1 (July 2015) and 96.3% of Cd removal in BR2 (July 2016). Zn and Cd removal yields went down during fall-winter time. The trends with sulfate reduction and carbon consumption were similar, with high sulfate reduction and carbon consumption during summer time and lower sulfate reduction and carbon consumption during winter time. Despite changes in sulfate reduction, microbial characterization showed a relatively stable community structure and relatively constant abundance of SRB (Figures 4-5 and 4-6).

Thus, it would appear that while temperature played a key role in controlling sulfate reduction, it had little impact on the structure of the bacterial community in the bioreactors. This would imply that the changes to the sulfate reduction came from a physiological adaptation of community members rather than a change in the membership and composition of the community. Temperature dependence of sulfate reduction by SRB in bioreactors has been previously documented in literature. A study by Van den Brand *et al.* (2014) observed the effect of temperature on acetate and propionate consumption by SRB in saline wastewater in batch bioreactors monitored at 10°C and 20°C. While the population of SRB was similar under both conditions, the authors reported that a decrease in temperature led to a substantial decrease in sulfate reduction rate. Furthermore, Touze *et al.* (2007) studied a 200 dm^3 pilot scale PSRB to treat MIW and also found the average sulfate reduction was correlated with temperature (between 5 and 17°C). While both studies reported that sulfate reduction still occurred in temperatures lower than 10°C, better efficiencies were obtained at higher temperatures.

ORP measurements (not presented) showed that in February 2016, when temperatures were 5.3 ± 1.0°C in BR1 and 5.5 ± 0.9°C in BR2, ORP was around -28 mV and -46 mV in BR1 and BR2, respectively. When temperatures went up during spring-summer and were between 12.3°C (May 2016 in BR1) and 15.6 ± 1.7°C (July 2016 in BR2), ORP decreased to -163 ± 57 mV for BR1 and -199 ± 74 mV. Optimal ORP values for SRB to catabolize sulfates and release sulfides is in the range of -100 to -300 mV (Gloyna 1972; Gibert *et al.,* 2002; Harerimana *et al.,* 2010). Hence decreased ORP results observed in summer may be attributed to an increase in SRB's activity. In addition, when the microbial samples were collected inside the two bioreactors, a strong hydrogen sulfide smell and black precipitates were observed. These observations are attributes of sulfate reduction according to Luptakova and Macingova (2012). Cd removal by sulfate reduction process have been assessed by Wang *et al.* (2001) who demonstrated that the sulfide secreted by some bacteria could be used to precipitate Cd in a complex of Cd and sulfur, most likely Cd sulfide (CdS). Those authors were able to remove 99.8% of Cd with an initial 50 µM concentration in solution.

The decrease of carbon consumption observed during fall-winter compared to carbon consumption in summer 2015 and 2016 might be an indicator of the decline in SRB's activity, as suggested by the observed decrease in sulfate consumption (Figure 4-1) and was possibly due to low temperatures. Genomic characterization showed a complex but stable bacterial community. Thus, even if SRB were still alive, they were potentially less efficient to reduce sulfate and consume carbon during fall/winter time. As the carbon consumption was still significant in fall and winter time, some microbial community members likely utilized carbon but they may not be SRB or SRB may have consumed carbon for other metabolic activities that were not sulfate reduction. Van den Brand *et al.* (2014) observed that the decrease in sulfate reduction rate as a result of temperature decrease from 20 to 10°C was similar for acetate and propionate, used as carbon sources, consumption. Indeed, both acetate and propionate consumption rates by SRB were 1.9 times lower at 10°C than at 20°C.

The large and stable microbial community, characterized in this study, benefited the system. Indeed, when using complex carbon sources, a more complex community of microbes are required to do both, carbon hydrolysis and sulfate reduction (Sheoran *et al.,* 2010).

It had been noticed that carbon concentration varied in the MIW during the 14 months experiment. In December 2015 and between February 2016 and July 2016, the increase in carbon concentration in MIW can be explained by the fact Alexco injected carbon sources (molasses and methanol) in the MIW through a borehole. These injections were planned as part of Alexco's *in situ* trial process (Gault and Harrington, 2016).

Carbon consumption results paralleled Gandy and Jarvis (2012)'s findings. Indeed, those authors found that environmental conditions, in particular temperature, play an important role in zinc removal. They monitored a pilot-scale system during the initial 8 months and found that while effluent temperature varied between 2°C and 12°C, the area-adjusted zinc removal rate ranged from 0.10 to 0.82 $g/m^2/day$. They observed that the decrease in Zn removal was caused by reduced SRB activity during cold period when the conditions within the reactive substrate were not reducing enough to support the development and sustainability of SRB. Gould *et al.,* (2012) studied the effect of carbon addition on the performance of columns bioreactors operating at various temperatures to treat water with Zn contamination (100 mg/L). They found that, while using a mixture of pulp mill biosolids, sand and limestone, Zn removal decreased to 60% at 4°C compared to 99% at 20°C. Then, they added carbon sources (lactate/acetate) to the bioreactors monitored at 4°C and noticed that the bioreactors achieved 95% of Zn removal in 170 days. Those authors concluded that low temperature operation of a PSRB can be enhanced by the addition of a labile carbon source. Although no SRB taxa were detected in the Silver King Mine's groundwater (Figures 4-4 and 4-5), they may have been very rare in MIW and grew rapidly in the anoxic conditions with the molasses addition in the bioreactors.

It must be noticed that if sulfate reduction by native SRB seemed to play an important role on metal removal, other processes such as co-precitation or sorption also control Zn and Cd precipitation. Correlations were made between sulfate consumption and Zn or Cd removals with linear regressions (not shown). For BR1, R^2 were less than 0.22 for sulfate/Zn and less than 0.18 for sulfate/Cd and for BR2, R^2 were less than 0.33 for sulfate/Zn and less than 0.05 for sulfate/Cd. This means that even if sulfate consumption is an indicator of sulfate reduction and sulfide precipitation, it was not linearly correlated with the efficiency of Cd or Zn removal in this study.

4.8 Conclusion

This pilot semi passive sulfate reducing bioreactor study showed Yukon native SRB could grow and be used to treat a neutral mine drainage struggled with Zn and Cd contamination in cold climate. During this 14 months experiment, Zn and Cd concentrations in bioreactors outlets were always under the discharge limits imposed by the water use license. Conditions in bioreactors allowed the implementation of a stable all year round SRB community which benefited this mine impacted water treatment. Metals and sulfate removals can be used as indicators of this PSRB's performance but genomic characterization indicated that potential SRB were present in the 2 pilot bioreactors. The microbial population significantly affected the efficiency of these bioreactors in the remediation of NMD. However, this passive treatment system is temperature limited as temperature played a key role in the bioreactor's efficiency. Indeed, this 14 months study showed that native SRB from the Keno Hill Silver district, although impacted by cold temperatures, can be effective in performing sulfate reduction processes and thus precipitating metals in sulfides form in a cold climate. Zn removal efficiency varied between 11.5% in winter and 95.5% in summer. Cd removal efficiency varied between 21.1% in winter and 96.6% in summer. The differences between microbial population in MIW and in bioreactors suggests that, in cold climate, bioreactors provided relatively stable environmental conditions and were conducive to the colonization of SRB.

4.9 Acknowledgments

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ANNEXE I

DNA recovery, Expected number of OTUs estimated using the nonparametric Chao 1 and Coverage estimated by calculating the ratio of observed to expected OTU numbers

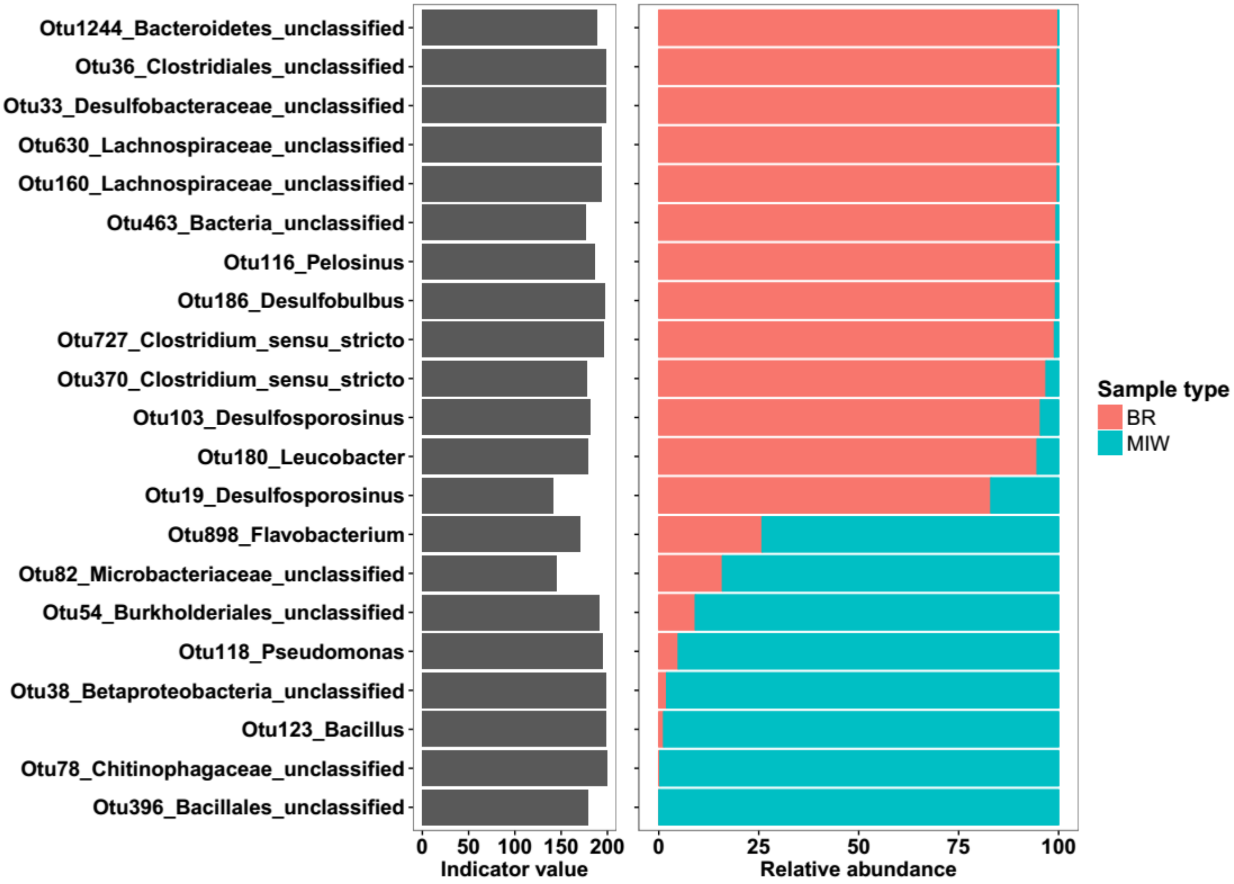
Sample	DNA quantity(ng/µL)	Observed OTUs	Chao1	Coverage
BR1 old	4.80	79.5	143.0	0.56
BR1 old	6.68	74.1	122.9	0.60
BR1	8.78	102.5	147.7	0.69
BR1	8.74	104.1	162.3	0.64
BR2	8.74	93.1	135.2	0.69
BR2	8.20	98.9	140.3	0.70
BR1	1.84	83.3	153.2	0.54
BR1	2.60	82.2	160.5	0.51
BR2	6.82	88.7	127.2	0.70
BR2	7.98	97.8	156.5	0.62
BR1	7.30	108.9	170.4	0.64
BR1	8.58	127.0	202.6	0.63
MIW	3.76	92.5	169.7	0.55
MIW	6.20	103.4	191.3	0.54
BR 1	8.18	88.4	132.0	0.67
BR 1	8.28	100.1	152.8	0.66
BR2	8.42	121.9	179.2	0.68
BR2	8.08	120.8	188.1	0.64
MIW	1.05	92.2	149.4	0.62
MIW	3.88	98.3	183.6	0.54
BR1	8.38	107.9	157.4	0.69
BR1	8.34	101.5	144.1	0.70
BR2	5.48	105.1	154.9	0.68
BR2	8.84	109.9	174.6	0.63
BR1	8.00	110.5	165.9	0.67
BR1	11.2	136.8	206.1	0.66
Rocks BR2	10.4	105.3	178.2	0.59
BR2	4.46	93.3	126.3	0.74
BR2	4.72	96.0	145.0	0.66
MIW	6.68	104.6	139.7	0.75
MIW	7.62	114.5	167.2	0.68
BR2	5.32	107.5	154.5	0.70
BR2	5.52	108.1	153.1	0.71
BR2	3.62	93.9	146.4	0.64
BR2	4.28	99.3	150.3	0.66

BR2	1.52	79.0	110.8	0.71
MIW	1.04	103.3	134.8	0.77
BR1	2.42	58.8	92.6	0.63
BR1	2.40	53.4	87.8	0.61
MIW	3.68	106.2	166.6	0.64
MIW	2.62	108.3	184.2	0.59
BR1	1.41	59.0	85.5	0.69
MIW	8.90	88.1	157.5	0.56
MIW	9.22	82.5	137.4	0.60
BR2	9.44	100.1	165.7	0.60
BR2	9.08	113.2	189.3	0.60
BR1	3.76	64.7	91.8	0.71
BR1	5.04	66.6	88.4	0.75
BR2	8.96	125.7	183.2	0.69
BR2	7.46	126.3	194.1	0.65
MIW	5.18	148.1	227.1	0.65
MIW	7.72	155.2	224.6	0.69
BR2	1.09	103.0	114.2	0.90
BR2	2.38	128.1	156.3	0.82
MIW	n.a	141.9	255.6	0.56
MIW	n.a	130.8	235.0	0.56
MIW	n.a	109.5	205.7	0.53
MIW	n.a	102.9	176.0	0.58

n.a : non applicable

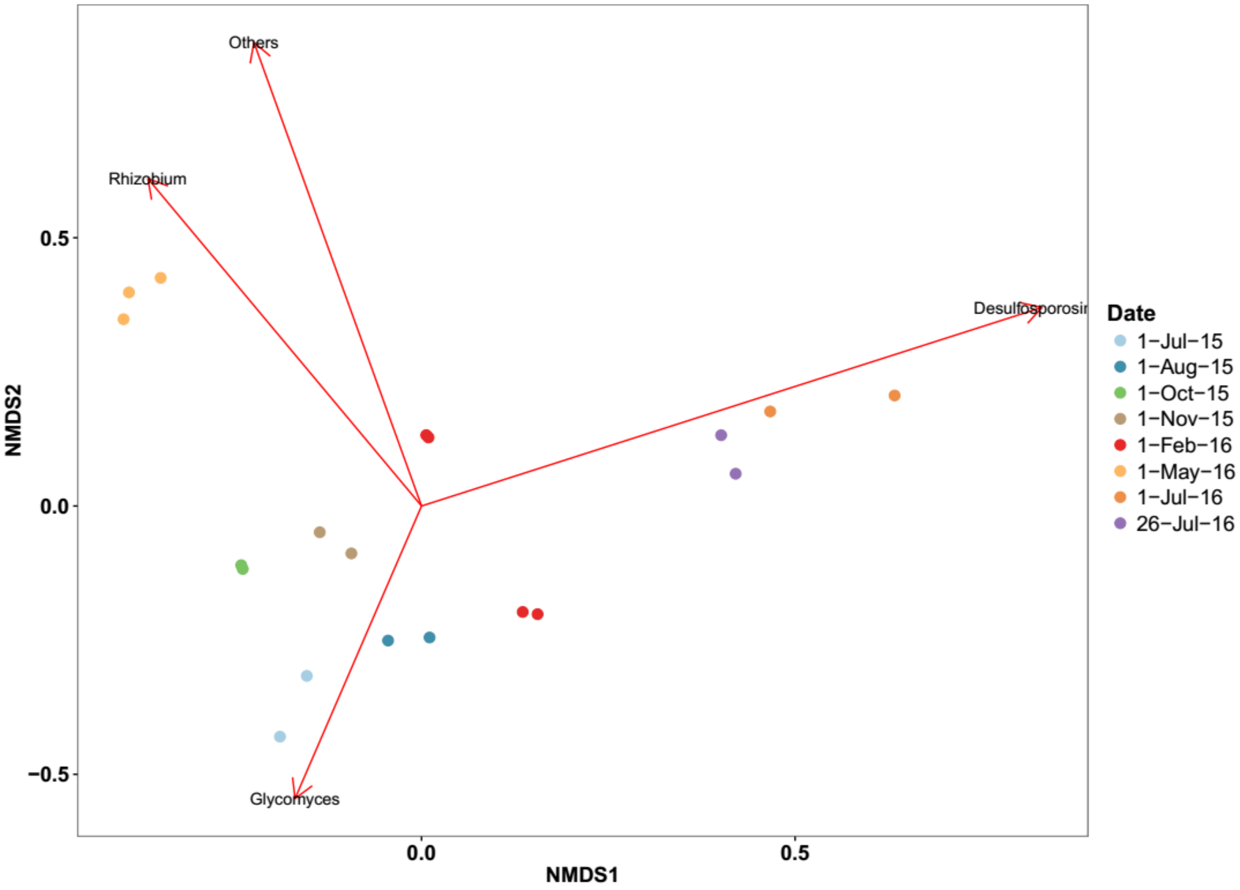
ANNEXE II

Statistically significant indicator OTUs (p < 0.05, relative abundance >1%) for the separation of the communities to two groups, bioreactors (BR) and mine impacted water (MIW). Relative abundance in this case indicates what proportion of the sequences belonged to each of the groups.

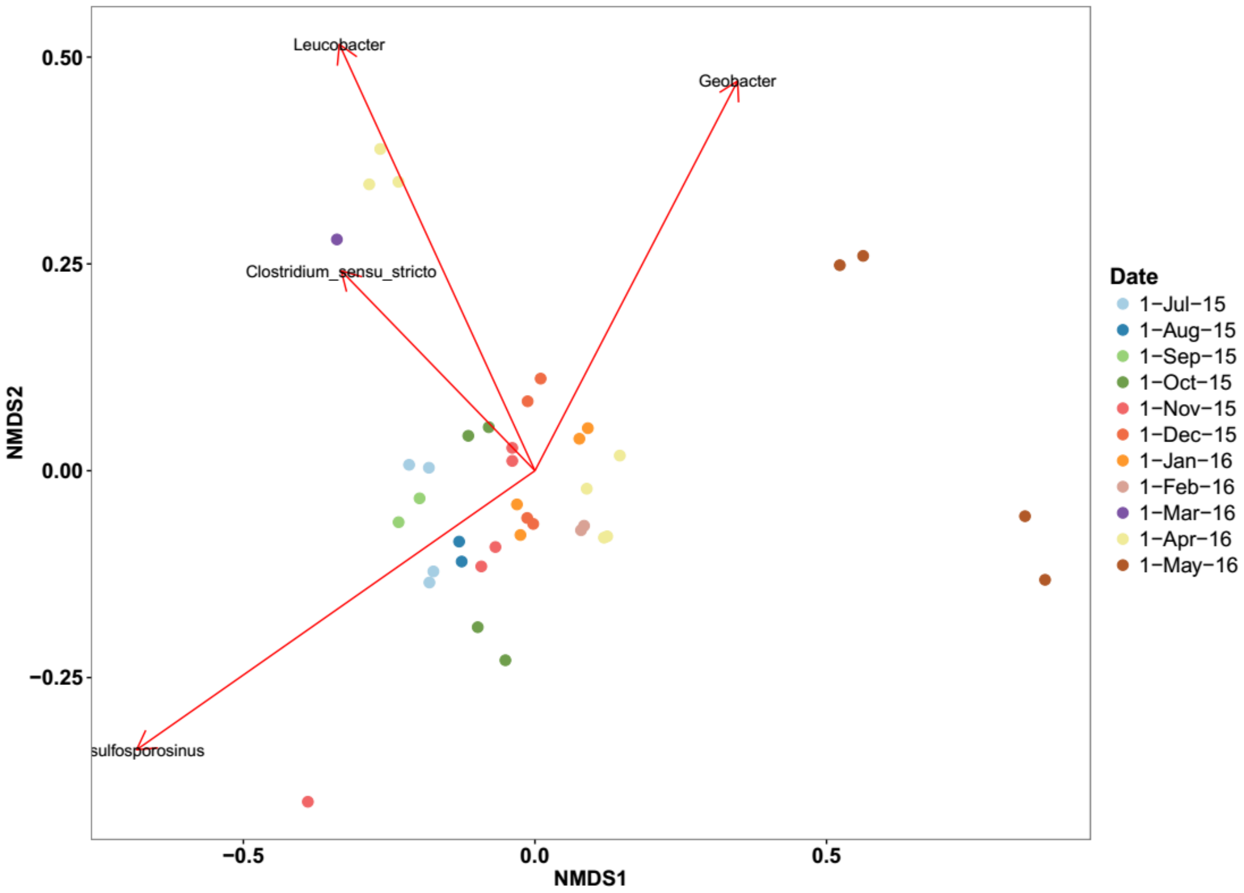


ANNEXE III

NMDS of MIW samples (a) and BR samples (b). Arrows indicate genera that significantly correlate with the representation of the communities in multidimensional space ($p < 0.05$). Length of the arrows scaled by strength of correlation. Genera with abundance < 1% were grouped in the others category



(a)


Leucobacter
Geobacter
Clostridium_sensu_stricto
sulfosporosinus
NMDS2
0.50
0.25
0.00
−0.25
NMDS1
−0.5
0.0
0.5
Date
1−Jul−15
1−Aug−15
1−Sep−15
1−Oct−15
1−Nov−15
1−Dec−15
1−Jan−16
1−Feb−16
1−Mar−16
1−Apr−16
1−May−16

(b)

ANNEXE IV

Formes chimiques sous lesquelles les métaux sont présents dans l'effluent minier selon le logiciel MINEQL+ (Version 4.6)

Métaux	Concentration (mol/L)	Distribution (%)
Cadmium		
$Cd(HS)_4^{2-}$	3,46e^{-09}	97,2
$Cd(HS)^{3-}$	9,12e^{-11}	2,6
TOTAL Cd^{2+}	3,56e^{-09}	100
Zinc		
$Zn(HS)^{3-}$	6,42e^{-05}	12
Sphalérite	0,000466	87,3