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TRAITEMENT BIOLOGIQUE PASSIF DU DRAINAGE MINIER ACIDE: SOURCES DE CARBONE, MÉCANISMES D'ENLÈVEMENT DES MÉTAUX ET ÉCOTOXICITÉ

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TRAITEMENT BIOLOGIQUE PASSIF DU DRAINAGE MINIER ACIDE: SOURCES DE CARBONE, MÉCANISMES D'ENLÈVEMENT DES MÉTAUX ET ÉCOTOXICITÉ

présentée par : NECULITA Carmen Mihaela
en vue de l'obtention du diplôme de : Philosophiae Doctor
a été dûment acceptée par le jury d'examen constitué de:

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À ma mère

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AVANT-PROPOS

Les travaux de recherche réalisés durant cette thèse ont fait l'objet de plusieurs publications dans des revues avec comité de lecture et dans des comptes rendus de conférences, ainsi que des présentations dans des conférences et symposiums. L'ensemble des publications générées est détaillé ci-dessous.

Revues avec comité de lecture

Neculita C.M., Zagury G.J., & Bussière B. (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria - Critical review and research needs. *Journal of Environmental Quality*, *36*, 1-16.

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Neculita C.M., Zagury G.J. & Bussière B. (2007). Passive treatment of highly contaminated acid mine drainage in batch reactors. *Proceedings of the 16th International Symposium on Mine Planning and Equipment Selection (MPES 2007) and the 10th International Symposium on Environmental Issues and Waste Management in Energy and Mineral Production (SWEMP 2007), Bangkok, Thailand*, December 11-13, 2007.

Conférences

Neculita C.M., Zagury G.J., & Bussière B. (2008). Removal mechanisms and potential metal mobility in passive bioreactors for treatment of highly contaminated acid rock drainage. *Joint Conference of the 6th International Acid Sulfate Soil Conference and the Acid Rock Drainage Symposium, South China Agricultural University, Wushan, Tianhe District, Guangzhou, P.R. CHINA*, September 16-20, 2008 (accepté).

Neculita C.M., Zagury G.J., & Bussière B. (2008). Influence of hydraulic retention time on effectiveness of sulphate-reducing passive bioreactors for treatment of highly contaminated acid rock drainage. *Joint Conference of the 6th International Acid Sulfate Soil Conference and the Acid Rock Drainage Symposium, South China Agricultural University, Wushan, Tianhe District, Guangzhou, P.R. CHINA*, September 16-20, 2008 (accepté).

Vigneault B., Neculita C.M., Zagury G.J., Tish B., & Kwong J. (2008). Should metal bioavailability be considered in the evaluation of treatment and remediation methodologies for acid mine drainage? *The 5th SETAC World Congress, Sydney, Australia*, August 3-7, 2008 (accepté).

RÉSUMÉ

Le drainage minier acide (DMA), qui est caractérisé par un faible pH et des concentrations élevées en sulfates et en métaux dissous, est un problème préoccupant pour l'industrie minière. Au cours des dernières années, les bioréacteurs passifs sulfato-réducteurs se sont avéré très prometteurs comme biotechnologies de traitement du DMA. Ils offrent plusieurs avantages dont des taux élevés d'enlèvement des métaux à faible pH, des boues stables, des faibles coûts d'opération et une consommation minimale d'énergie.

Les bioréacteurs passifs sulfato-réducteurs ont prouvé leur efficacité à court terme (1-5 ans) pour traiter les eaux contaminées par le DMA. Cependant, l'efficacité à long terme peut être parfois limitée en raison du fait que le traitement est basé sur l'activité de la microflore anaérobie (y compris les bactéries sulfato-réductrices (BSR)) et qu'il dépend principalement de la composition du mélange réactif constituant le bioréacteur. La composante la plus importante du mélange réactif, qui contrôle la performance et l'efficacité du système à long terme, est la source de carbone organique.

Une caractérisation rigoureuse et méthodique est nécessaire afin de prédire la biodégradabilité des substrats organiques par les BSR. La performance des bioréacteurs de terrain peut être aussi limitée par la charge du DMA à traiter, par la toxicité des métaux, ainsi que par les propriétés hydrauliques du mélange réactif. La précipitation des sulfures est le mécanisme désiré d'enlèvement des métaux. Cependant, d'autres mécanismes incluant l'adsorption et la précipitation des métaux sous forme d'hydroxydes et des carbonates peuvent également se produire.

Les principaux objectifs de la thèse sont de quantifier la relation entre la composition des matériaux organiques naturels et leur potentiel de biodégradation en conditions sulfato-réductrices, d'évaluer le potentiel écotoxique de l'effluent traité, ainsi que

d'identifier/quantifier les mécanismes d'enlèvement des métaux d'un DMA très contaminé au moyen d'un bioréacteur passif sulfato-réducteur.

Dans le premier chapitre, plusieurs études menées dans le but de trouver le meilleur mélange de substrats organiques naturels pour le BSR sont présentées et les paramètres critiques de la conception et de l'exploitation du bioréacteur passif à long terme sont discutés. Les conclusions les plus importantes de cette revue critique de la littérature sur les bioréacteurs passifs sulfato-réducteurs indiquent qu'il reste encore des travaux à effectuer afin de mieux évaluer l'efficacité à long terme des mélanges réactifs et les mécanismes d'enlèvement des métaux. De plus, la spéciation des métaux, ainsi que l'évaluation de l'écotoxicité de l'effluent traité en provenance des bioréacteurs passifs sont à évaluer.

Dans la deuxième partie de cette étude, quatre matériaux organiques naturels (copeaux de bois d'érable, sciure de bois d'érable, fumier de volaille composté et compost de feuilles) ont été exhaustivement caractérisés afin d'évaluer leur capacité à servir de substrats et pour trouver le(s) paramètre(s) clef(s) qui relie la source de carbone organique à sa biodégradabilité. Trois mélanges réactifs constitués avec les quatre matériaux organiques naturels ont été comparés pour leur efficacité à traiter un DMA très contaminé (13-19 mg/L Mn, Cd, Ni et Zn; 1700 mg/L Fe; 5500 mg/L SO_4^{2-}; pH 5,5) dans des bioréacteurs de type batch à long terme (120-152 jours). Tous les mélanges ont été efficaces pour la réduction du sulfate et l'enlèvement des métaux (jusqu'à 91,8-99,8% pour les Fe, Ni, Cd, Zn et Mn). Des efficacités plus élevées ont été observées dans les réacteurs avec 30% (p/p) des déchets cellulosiques (copeaux et sciure de bois d'érable) où les concentrations en sulfate ont diminué de 5500 mg/L à < 1mg/L, comparativement aux réacteurs avec 2-3% de déchets cellulosiques où les concentrations finales en sulfate ont été de 2000-2750 mg/L. La caractérisation des matériaux organiques indique que des rapports C/N, Demande Chimique en Oxygène (DCO)/SO_4^{2-} et Carbone Organique Dissous (COD)/SO_4^{2-} plus élevés étaient associés à des meilleures conditions de sulfato-réduction et d'enlèvement des métaux. Les résultats

suggèrent que les rapports C/N et COD/SO_4^{2-} pris ensemble sont les paramètres «clefs» pour l'évaluation de la biodégradabilité des matériaux organiques naturels en conditions sulfato-réductrices.

Dans la troisième partie de l'étude, l'effet des deux temps de résidence hydraulique (TRH) de 7,3 jours et 10 jours sur l'efficacité à long terme des bioréacteurs de type colonne (3,5 L) a été évalué sur une durée de 11 mois pour le traitement d'un DMA très contaminé mais moins chargé en fer (10-15 mg/L Mn, Cd, Ni et Zn; 500 mg/L Fe; pH 2,9-5,7). L'évolution de la porosité et de la conductivité hydraulique du mélange réactif a aussi été suivie sur une durée de 15 mois. Les résultats indiquent que les bioréacteurs ont été efficaces aux deux TRH pour l'augmentation du pH et de l'alcalinité de l'eau contaminée et pour l'enlèvement du sulfate et des métaux (60-82% pour Fe et jusqu'à 99,9% pour Cd, Ni et Zn). Malgré le fait que la qualité de l'effluent traité est significativement meilleure à 10 jours de TRH par rapport à 7,3 jours de TRH, les résultats montrent qu'un TRH plus long a réduit la porosité et la perméabilité du mélange réactif, ce qui peut entraîner des problèmes hydrauliques et, éventuellement, une perte d'efficacité du système durant l'exploitation à long terme par rapport à un TRH plus court. Un compromis doit donc être fait lors de la conception d'un bioréacteur passif efficace à long terme, où on doit s'assurer que le système permet de respecter les normes au niveau de la qualité de l'effluent traité tout en limitant les problèmes hydrauliques dans le mélange réactif.

Dans la quatrième partie de l'étude, la toxicité aigüe et sous-létale a été testée sur l'effluent des bioréacteurs en colonne, aux deux différents TRH. Cette partie de l'étude est justifiée par le fait que les normes de rejets exigent que, mis à part les limites basées sur les paramètres physico-chimiques, l'effluent traité doit être non-toxique. L'effluent a été testé d'abord pour la toxicité aigüe (la daphnie *Daphnia magna* et la truite arc-en-ciel *Oncorhynchus mykiss*) et sous-létale (l'algue *Pseudokirchneriella subcapitata*, la daphnie *Ceriodaphnia dubia* et la plante *Lemna minor*). La toxicité aigüe a été observée pour *D. magna*. Une procédure d'identification de la source de toxicité a été ensuite

effectuée afin de trouver le(s) contaminant(s) toxique(s). Pour faciliter l'interprétation des résultats de toxicité, la spéciation des métaux dans l'effluent a également été déterminée à l'aide de l'ultrafiltration et de la modélisation thermodynamique à l'équilibre (VMINTEQ). À 10 jours de TRH, l'effluent n'a pas présenté de toxicité aigüe létale pour la truite arc-en-ciel mais une toxicité aigüe létale a été observée pour *D. magna*. La toxicité pour *D. magna* a été cependant éliminée par 2 h d'aération et la procédure d'identification de la source de toxicité a suggéré le Fe comme la principale cause du problème. Les résultats de la spéciation des métaux ont indiqué une instabilité des deux effluents durant l'aération, ce qui est concordant avec la toxicité causée par le Fe. Donc, les bioréacteurs de type colonne, exploités pendant plus de 9 mois ont été efficaces pour améliorer la qualité physico-chimique d'un DMA très contaminé à différents TRH. Néanmoins, l'étude sur la toxicité de l'effluent traité indique que la conception des systèmes passifs de traitement du DMA doit inclure suffisamment de TRH pour respecter les exigences de toxicité aigüe.

Dans la cinquième partie de cette étude, les bioréacteurs de type colonne ont été utilisés pour évaluer les mécanismes d'enlèvement des métaux, la stabilité des mélanges réactifs post-traitement à long terme, ainsi que la mobilité potentielle des métaux dans les mélanges réactifs après le traitement d'un DMA très contaminé. Plusieurs analyses physico-chimiques, microbiologiques et minéralogiques ont été effectuées sur les mélanges réactifs post-traitement prélevés des tranches du bas et du haut des quatre bioréacteurs, après 11 mois de suivi. Les concentrations en métaux ont été très élevées dans tous les mélanges réactifs, indépendamment du TRH, ce qui est en concordance avec la charge en métaux (Fe, Mn, Cd, Ni et Zn) du DMA et les concentrations faibles mesurées dans l'effluent traité. De plus, les concentrations de Fe (50,8-57,8 g/kg) et Mn (0,53-0,70 g/kg) ont été jusqu'à 2 fois plus élevées dans les tranches du bas, tandis que les concentrations de Cd (6,77-13,3 g/kg), Ni (1,80-5,19 g/kg) et Zn (2,53-13,2 g/kg) ont été jusqu'à 50 fois plus élevées dans le haut des bioréacteurs. Les extractions chimiques et les analyses élémentaires ont donné des résultats concordants, qui indiquent une fraction faible des métaux enlevés sous forme de sulfures (au plus 14%

des métaux récupérés des mélanges réactifs). Le Fe et le Mn ont été trouvés dans une forme chimique plus stable (fraction résiduelle de 42-74% pour Mn et 30-77% pour Fe) par rapport aux Cd, Ni ou Zn, qui semblent plus faiblement liés (fractions oxydante/réductrice) et qui ont une mobilité potentielle plus élevée. Les analyses minéralogiques montrent la présence de sulfures contenant du Fe, Cd, Ni et Zn, en plus des (oxy) hydroxydes et des carbonates. Les principaux mécanismes d'enlèvement des métaux sont donc l'adsorption et des mécanismes de liaison des métaux par la matière organique (pour le Cd, Ni et Zn) et la précipitation des (oxy) hydroxydes (pour le Fe et Mn).

Après 15 mois de suivi, les bioréacteurs n'ont pas perdu leur capacité d'enlèvement des métaux, qui sont stables dans le mélange réactif. De plus, l'augmentation de la mobilité potentielle des métaux dans les mélanges réactifs post-traitement pourrait être une alternative économiquement viable pour la récupération des métaux, qui devrait être évaluée dans le futur.

À la fin, l'intégration des résultats permet de faire ressortir les principales contributions de l'étude à l'avancement des connaissances et les conclusions, ainsi que des recommandations pour des futurs travaux.

ABSTRACT

Acid mine drainage (AMD), characterized by low pH and high concentrations of sulfate and heavy metals, is an important and widespread environmental problem related to the mining industry.

Sulfate-reducing passive bioreactors have received much attention lately as promising biotechnologies for AMD treatment. They offer advantages such as high metal removal at low pH, stable sludge, very low operation costs, and minimal energy consumption. Sulfate-reducing passive bioreactors have proved to be an effective technology for the treatment of AMD-contaminated waters over relatively short periods of time (1-5 years). However, long-term efficiency is sometimes limited because these types of bioreactors rely on the activity of anaerobic microflora [including sulfate-reducing bacteria (SRB)] which depends primarily on the reactive mixture composition. The most important component which controls performance and long-term treatment efficiency is the organic carbon source.

A rigorous and methodical characterization to predict the biodegradability of organic substrates by SRB still needs to be investigated. The performance of field bioreactors can also be limited by AMD load, metal toxicity, and by problems related to the hydraulic properties of the reactive mixture. Although sulfide precipitation is the desired mechanism of contaminant removal, many mechanisms including adsorption and precipitation of metal carbonates and hydroxides occur in passive bioreactors.

The main objectives of the thesis were to quantify the relation between the composition of a natural organic material and its potential for biodegradation under sulfate-reducing conditions, to evaluate the ecotoxicological potential of treated effluent, and to identify and quantify metal removal mechanisms by sulfate-reducing passive bioreactors from a highly contaminated AMD.

In the first Chapter, several studies conducted to find the best mixture of natural organic substrates for SRB are reviewed and critical parameters for design and long-term

operation are discussed. The main conclusion of this critical review of the available literature on sulfate-reducing passive bioreactors is that additional work needs to be done to properly assess the long-term efficiency of reactive mixtures and to assess metal removal mechanisms. Furthermore, metal speciation and ecotoxicological assessment of treated effluent from passive bioreactors have yet to be performed.

In the second Chapter of the present study, four natural organic materials (maple wood chips, maple sawdust, composted poultry manure, and leaf compost) were thoroughly characterized to assess their ability to serve as substrates and to find a parameter that links organic carbon sources with their biodegradability. Three reactive mixtures using the four organic materials were then comparatively evaluated for their performance to treat a highly contaminated AMD (13-19 mg/L Mn, Cd, Ni, and Zn; 1700 mg/L Fe; pH 5.5) in long-term (120-152 days) batch experiments. All three mixtures were successful for sulfate reduction as well as metal removal efficiency which reached 91.8-99.8% for Fe, Ni, Cd, Zn, and Mn. Higher efficiencies were observed in the reactors with 30% (w/w) cellulosic wastes (maple wood chips and sawdust) which decreased sulfate concentrations from 5500 mg/L to < 1mg/L, compared to reactors with 2-3% cellulosic wastes, where final sulfate concentrations were in the range 2000-2750 mg/L. Organic material characterization indicated that higher C/N ratios, Chemical Oxygen Demand (COD)/SO_4^{2-} ratios and Dissolved Organic Carbon (DOC)/SO_4^{2-} ratios were associated with better sulfate-reducing conditions and metal removal. Results suggested that C/N and DOC/SO_4^{2-} ratios considered together are key parameters to assess the biodegradability of natural organic materials under sulfate-reducing conditions.

In the third Chapter of the present study, the effect of two hydraulic retention times (HRTs) of 7.3 days and 10 days on the performance of 3.5L passive bioreactors was evaluated over an 11-month period for the treatment of a highly contaminated AMD (10-15 mg/L Mn, Cd, Ni, and Zn; 500 mg/L Fe; pH 2.9-5.7). Evolution of the porosity and hydraulic conductivity of the reactive mixture was also evaluated during the 15-month operation of two bioreactors. Results indicated that the bioreactors were effective at both HRTs for increasing the pH and alkalinity of the contaminated water and for

sulfate and metal removal (60-82% for Fe and up to 99.9% for Cd, Ni, and Zn). Although the quality of the treated effluent was significantly improved with the 10 days HRT compared to the 7.3 days HRT, results showed that the higher HRT reduced the porosity and the permeability of the reactive mixture which might lead to hydraulic related problems and, eventually, to limited effectiveness in long-term operation compared to a shorter HRT. A compromise must therefore be found for the design of a long-term effective passive bioreactor in order to respect the discharge limits in treated effluent and to limit the problems related to the hydraulic properties of the reactive mixture.

In Chapter 4 of the study, acute and sublethal toxicity was tested on effluent from column bioreactors filled with the mixture of four natural organic carbon sources and operated at the two different hydraulic retention times (HRTs) for treatment of the highly contaminated AMD. This part of the study was justified based on the fact that in addition to discharge limits based on physicochemical parameters, treated effluent is also required to be nontoxic. Effluent was first tested for acute (*Daphnia magna* and *Oncorhynchus mykiss*) and sublethal (*Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia*, and *Lemna minor*) toxicity. Acute toxicity was observed for *D. magna* and a toxicity identification evaluation (TIE) procedure was then performed to identify potential toxicant(s). Finally, metal speciation in the effluent was determined using ultrafiltration and geochemical modelling for the interpretation of the toxicity results. The 10 days HRT effluent was nonacutely lethal for rainbow trout but was acutely lethal for *D. magna*. The toxicity to *D. magna*, however, was removed by 2 h aeration and the TIE procedure suggested iron as a cause of toxicity. Sublethal toxicity of the 10 days HRT effluent was observed for all test species but it was reduced compared to the raw AMD and to a 7.3 days HRT effluent. Data on metal speciation indicated instability of both effluents during aeration and were consistent with the toxicity being caused by iron. Column bioreactors in operation for more than nine months efficiently improved the physicochemical quality of highly contaminated AMD at different HRTs. However,

the study indicated that design of passive treatment methods should include sufficient HRT and post-treatment aeration in order to meet acute toxicity requirements.

In Chapter 5, column bioreactors were used for studying mechanisms of metal removal, assessment of long-term stability of spent reactive mixtures, as well as potential metal mobility after treating highly contaminated acid mine drainage. Several physicochemical, microbiological, and mineralogical analyses were performed on spent reactive mixtures collected from four bioreactors, after operation for over an 11-month period. Consistent with the high metal concentrations in the AMD feed, and with low metal concentrations measured in the treated effluent, the physicochemical analyses indicated very high concentrations of metals (Fe, Mn, Cd, Ni, and Zn) in the top and bottom layers of the reactive mixtures from all columns. Moreover, the concentrations of Fe (50.8-57.8 g/kg) and Mn (0.53-0.70 g/kg) were up to twice as high in the bottom layers, whereas the concentrations of Cd (6.77-13.3 g/kg), Ni (1.80-5.19 g/kg), and Zn (2.53-13.2 g/kg) were up to 50-times higher in the top layers. Chemical extractions and elemental analysis gave consistent results, which indicated a low fraction of metals removed as sulphides (up to 14% of total metals recovered in spent reactive mixtures). Moreover, Fe and Mn were found in a more stable chemical form (residual fraction was 42-74% for Mn and 30-77% for Fe) relative to Cd, Ni or Zn, which seemed more weakly bound (oxidisable/reducible fractions) and showed higher potential mobility. Besides identifying (oxy)hydroxide and carbonate minerals, the mineralogical analyses identified metal sulphides containing Fe, Cd, Ni, and Zn. Metal removal mechanisms were, therefore, mainly adsorption and other binding mechanisms with organic matter (for Cd, Ni, and Zn), and precipitation as (oxy)hydroxide minerals (for Fe and Mn). After 15 months, the column bioreactors did not lose their capacity for removing metals from the AMD that are immobile during operation of the bioreactors. Moreover, metal mobility in spent reactive mixtures can be increased. This could be an economically viable alternative for metal recuperation, which should be evaluated in the future.

In the last Chapter, the main results and the conclusions of the study are presented, followed by few recommendations for future research.

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AMD - Acide Mine Drainage

SRB - Sulphate-Reducing Bacteria

PRB - Permeable Reactive Barrier

HRT - Hydraulic Retention Time

HRC - Hydrogen Release Compounds

COD - Chemical Oxygen Demand

DOC- Dissolved Organic Carbon

EAS - Easily Available Substances

TVS - Total Volatile Solids

TOC - Total Organic Carbon

TIC – Total Inorganic Carbon

MMER - Metal Mining Effluent Regulations

TIE - Toxicity Identification Evaluation

IC25 - 25 % Inhibition Concentration

IC50 - 50% Inhibition Concentration

LC50 - 50% Lethal Concentration

EC50- 50% Effect Concentration

XRD - X-Ray Diffraction

DSC-TGA - Differential Scanning Calorimetry and Thermogravimetric Analysis

SEM-EDS - Scanning Electron Microscopy with X-ray Energy Dispersion

SEM-BSE - Scanning Electron Microscopy in Backscattered Electrons

LISTE DES ANNEXES

(les annexes 3, 4, 6 et 7 sont fournies sur CD-Rom)

INTRODUCTION

Contexte du travail et problématique

L'exposition en surface des stériles et des résidus générés par l'industrie minière peut entraîner la contamination des eaux par la génération de drainage minier acide (DMA), qui est caractérisé par un faible pH et des concentrations élevées en sulfates et en métaux dissous. Le traitement du DMA est exigé afin de limiter les impacts sur l'environnement.

Les bioréacteurs passifs sulfato-réducteurs, avec une conception relativement simple et peu couteuse, ont prouvé leur efficacité à court terme (3-5 ans) comme technologie alternative aux usines traditionnelles de traitement du drainage minier acide (DMA) pour les sites abandonnés ou fermés avec des conditions extrêmes pendant l'hiver (Gusek *et al.*, 1999; Reisinger *et al.*, 2000; Reisman *et al.*, 2003; Kuyucak *et al.*, 2006). Les bioréacteurs passifs utilisent la capacité des bactéries sulfato-réductrices (BSR) d'augmenter le pH et l'alcalinité de l'eau, ainsi que d'immobiliser les métaux par précipitation sous forme de sulfures, en présence d'une source de carbone organique disponible et d'un environnement géochimique favorable (ex. pH > 5, Eh < -100 mV etc.). L'efficacité du bioréacteur passif durant l'exploitation à long terme peut toutefois être limitée par certains facteurs, tels que:

Disponibilité du carbone organique pour les bactéries anaérobies

La sélection rigoureuse de la source de carbone organique est importante afin d'assurer la performance et l'efficacité du traitement à long terme (Waybrant *et al.*, 1998; Cocos *et al.*, 2002; Zagury *et al.*, 2006). Cependant, les paramètres reliant la composition d'un substrat organique à sa biodégradabilité par les bactéries sulfato-réductrices (BSR) doivent encore à ce jour être identifiés.

Qualité et débit du DMA et évolution des paramètres hydrauliques

Généralement, les bioréacteurs sont utilisés pour le traitement du DMA légèrement contaminé et pour de faibles débits. L'optimisation du temps de résidence hydraulique (TRH) est basée sur la qualité du DMA à traiter et l'effluent traité (Younger *et al.*, 2002). De plus, la maximisation du TRH est limitée par les dimensions finales du bioréacteur, qui doivent tenir compte de la surface disponible. Cependant, il n'y a pas d'information disponible sur l'évolution comparative des paramètres hydrauliques (porosité et perméabilité) à différents TRH pour une même qualité du DMA.

Réglementation sur la qualité de l'effluent traité

À part les critères de rejets basés sur la qualité physico-chimique, la non-toxicité de l'effluent minier traité est très importante dans le contexte d'une tendance mondiale à introduire dans les réglementations des critères de rejet basés sur la toxicité. Au Canada, le Règlement sur les Effluents des Mines de Métaux (REMM) (Environnement Canada, 2002) exige que toutes les mines canadiennes produisent un effluent qui n'est pas toxique pour la truite arc-en-ciel. De plus, dans certaines provinces canadiennes, la toxicité aigue non-létale pour les daphnies (*Daphnia magna*) est également exigée. Lorsque l'effluent est toxique, l'identification du contaminant source de toxicité à l'aide d'une procédure spécifique est aussi exigée, ainsi que des mesures pour la réduction/élimination de la toxicité (ESG, 2002). Enfin, si le contaminant source de toxicité est un métal, l'étude de la partition/spéciation des métaux peut aider à identifier la source de toxicité (Mount *et al.*, 1997). Toutefois, il y a très peu d'information disponible au sujet de la toxicité des effluents de mines traités au moyen de biotechnologies comme les réacteurs passifs sulfato-réducteurs (Riesen *et al.*, 2005).

Mécanismes d'enlèvement et forme chimique des métaux

Quelques travaux ont déjà été publiés sur l'évaluation des mécanismes d'enlèvement des métaux et la stabilité des mélanges réactifs, ainsi que sur la forme chimique des

métaux et leur mobilité potentielle (Machemer et Wildeman, 1992; Jong et Parry, 2004b; Gibert *et al.*, 2005b). Cependant, les bioréacteurs remplis de mélanges complexes, constitués de plusieurs sources de carbone organique et qui sont utilisés pour le traitement d'un DMA très contaminé n'ont pas été étudiés. Les extractions chimiques (analyses destructives) et les analyses minéralogiques (non-destructives) sont généralement utilisées. La procédure d'extraction séquentielle et la détermination simultanée des métaux solubles et des sulfures volatiles en milieu acide aident également à mieux identifier la forme chimique des métaux, ainsi qu'à une évaluation globale des mécanismes de leur enlèvement dans le mélange réactif. De pus, les analyses minéralogiques permettent l'étude de la morphologie et de la forme chimique des précipités métalliques dans l'échantillon solide et la confirmation des suppositions basées sur les résultats des extractions chimiques (Neculita *et al.*, 2006).

Une revue exhaustive de la littérature pertinente sur le sujet est présentée dans le chapitre I de la présente thèse. Cette revue critique fait ressortir les besoins de recherche sur les principaux volets touchés par la thèse incluant: les travaux qu'il reste à effectuer afin de trouver les meilleures sources de carbone organique pour les BSR, la compréhension des mécanismes d'enlèvement des métaux et l'écotoxicité de l'effluent traité au moyen d'un bioréacteur passif sulfato-réducteur.

Hypothèses

Dans ce contexte, les hypothèses de recherche de ce projet sont les suivantes:

1. *La caractérisation physico-chimique et microbiologique des matériaux organiques naturels, couplée à leur potentiel de promouvoir la sulfato-réduction à long terme peut permettre de trouver les paramètres clefs reliant la composition chimique d'un substrat à sa biodégradation, afin de constituer un mélange réactif plus efficace.*

2. *L'impact écotoxique du DMA et de l'effluent traité est expliqué par la forme chimique des métaux dans le mélange réactif et dans l'effluent traité.*

3. *Le temps de résidence hydraulique (TRH) est un paramètre crucial pour l'efficacité du traitement et les propriétés physico-chimiques et hydrauliques du mélange sont influencées par le TRH.*

4. *L'identification et la quantification des mécanismes d'enlèvement des métaux dans un bioréacteur passif, couplées avec l'évaluation de la spéciation et le fractionnement des métaux dans le mélange réactif et dans l'effluent traité peuvent aider à la conception d'un réacteur plus efficace à long terme.*

Objectifs

Les principaux objectifs de cette thèse sont:

1. Caractériser du point de vue chimique et microbiologique quatre matériaux naturels organiques (copeaux de bois d'érable, sciure de bois d'érable, fumier de volaille composté et compost de feuilles) susceptibles d'être utilisés dans les bioréacteurs passifs.

2. Identifier les paramètres de caractérisation les plus importants pour promouvoir la sulfato-réduction et l'enlèvement des métaux.

3. Évaluer l'efficacité de trois mélanges réactifs pour le traitement d'un DMA très contaminé, afin de choisir le plus efficace.

4. Évaluer l'efficacité et la stabilité en conditions dynamiques des bioréacteurs remplis du mélange réactif le plus efficace et exploités à deux temps de rétention hydraulique (TRH) différents.

5. Évaluer l'évolution des paramètres hydrauliques et statuer sur l'influence du TRH sur l'efficacité d'un traitement.

6. Évaluer l'impact écotoxique, la partition (par filtration et ultrafiltration) et la spéciation des métaux dans le DMA et dans l'effluent traité.

7. Corréler l'impact écotoxique à la spéciation et la partition des métaux dans l'effluent.

8. Évaluer la minéralogie et le fractionnement des métaux dans le mélange réactif collecté à la fin du traitement en conditions dynamiques afin d'évaluer les mécanismes d'enlèvement des métaux et leur mobilité potentielle.

Organisation de la thèse

La présente thèse est articulée en 6 chapitres, dont les premiers 5 sont présentés sous la forme d'articles scientifiques publiés, acceptés ou soumis.

Le chapitre 1 présente une revue critique de littérature sur le traitement du drainage minier acide (DMA) au moyen des bioréacteurs passifs sulfato-réducteurs.

Le chapitre 2 traite des trois premiers objectifs du projet, soient:

- la caractérisation physico-chimique et microbiologique de quatre matériaux naturels organiques (copeaux et sciure de bois d'érable, fumier de volaille composté et compost de feuilles) susceptibles d'être utilisés dans les bioréacteurs passifs
- l'identification des paramètres de caractérisation les plus importants
- l'évaluation de l'efficacité de trois mélanges réactifs pour le traitement d'un DMA très contaminé, afin de choisir le plus efficace.

Les chapitres 3, 4 et 5 répondent aux objectifs 4 à 8. Ils présentent la discussion des résultats obtenus durant les tests en bioréacteurs type colonne soient:

- l'évaluation de l'efficacité et la stabilité en conditions dynamiques des bioréacteurs remplis du mélange réactif le plus efficace et exploités avec deux temps de rétention hydraulique (TRH) différents (chapitre 3)
- l'évolution des paramètres hydrauliques et l'influence du TRH sur l'efficacité d'un traitement (chapitre 3)
- l'évaluation de l'impact écotoxique, la partition et la spéciation des métaux dans le DMA et dans l'effluent traité (chapitre 4)
- la corrélation entre l'impact écotoxique et la spéciation et la partition des métaux dans l'effluent (chapitre 4)
- l'évaluation de la minéralogie et du fractionnement des métaux dans le mélange réactif post-traitement en conditions dynamiques afin d'évaluer les mécanismes d'enlèvement des métaux et leur mobilité potentielle (chapitre 5).

Dans le chapitre 6, une intégration des principaux résultats obtenus et les conclusions issues de l'ensemble du travail sont présentées. À la fin de la thèse, des recommandations sont suggérées.

Présentation des manuscrits

Article #1

Titre du manuscrit

Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs

Auteur et co-auteurs

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Résumé

La revue de littérature sur le traitement du DMA au moyen d'un bioréacteur passif sulfato-réducteur est présentée sous la forme d'un article, qui a été publié dans la revue *Journal of Environmental Quality* (2007), 36, 1-16.

Au début de l'article, sont présentés les processus de génération du drainage minier acide, la classification des systèmes passifs de traitement du DMA, ainsi que les nouvelles tendances dans la législation minière des États-Unis et du Canada sur la qualité de l'effluent traité et l'objectif du travail. Quatre volets importants du bioréacteur passif sont ensuite discutés d'un point de vue critique:

(1) les principes, les caractéristiques et les mécanismes du traitement,

(2) les facteurs d'influence sur l'efficacité,

(3) les performances à court et à long terme et

(4) des conclusions et des besoins de recherche.

L'état actuel de connaissances sur ce type de traitement est présenté en détail, à partir des 129 publications les plus pertinentes sur le sujet. Une attention particulière est accordée aux sources de carbone organique disponibles pour les BSR, aux mécanismes d'enlèvement des sulfates et des métaux, ainsi qu'aux problèmes qui peuvent affecter l'efficacité du bioréacteur passif durant son exploitation à long terme. L'évaluation du potentiel toxique d'un effluent traité au moyen d'un bioréacteur est le sujet sur lequel il y a très peu de travaux publiés à présent, malgré le fait que la nouvelle législation minière du Canada et des États-Unis exige que l'effluent traité ne soit pas toxique.

Cette revue critique de littérature fait ressortir les besoins de recherche sur les principaux volets touchés par la présente thèse dont: les travaux à effectuer afin de trouver les meilleurs sources alternatives de carbone organique pour les bactéries sulfato-réductrices, la compréhension des mécanismes d'enlèvement des métaux et l'écotoxicité de l'effluent traité au moyen d'un bioréacteur passif sulfato-réducteur.

Article #2

Titre du manuscrit

Biological treatment of highly contaminated acid mine drainage in batch reactors: long-term treatment and reactive mixture characterization

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Résumé

Le deuxième chapitre est présenté sous la forme d'un article, publié dans la revue *Journal of Hazardous Materials* (2008).

L'article traite d'abord la caractérisation physico-chimique et microbiologique de quatre matériaux organiques naturels (copeaux de bois d'érable, sciure de bois d'érable, fumier de volaille composté et compost de feuilles) susceptibles d'être utilisés dans la constitution des mélanges réactifs pour les bioréacteurs passifs sulfato-réducteurs. Par la suite sont présentés les résultats des essais en bioréacteurs de type batch réalisés avec trois mélanges réactifs constitués avec quatre matériaux pour le traitement d'un DMA très contaminé, pour une durée de 120-152 jours. Deux des trois mélanges réactifs testés ont déjà prouvé leur efficacité pour le traitement du DMA en bioréacteurs batch (40-70 jours), tandis que le troisième mélange a été développé pour les besoins du présent projet.

Les objectifs du travail ont été de trouver les paramètres physico-chimiques «clefs» permettant de relier la composition d'un matériel organique naturel à sa biodégradabilité en conditions sulphato-réductrices et de sélectionner le mélange réactif le plus efficace afin de le tester par la suite dans des bioréacteurs de type colonne.

L'interprétation des résultats de caractérisation a permis de proposer les rapports C/N et Carbone Organique Dissous (COD)/SO_4^{2-} considérés ensemble comme paramètres «clefs» qui permettent de relier la composition d'un matériel organique naturel à sa biodégradabilité en conditions sulfato-réductrices.

Les résultats ont aussi montré que le mélange réactif nouvellement développé est le plus performant pour l'augmentation du pH et de l'alcalinité et pour l'enlèvement des sulfates et des métaux. En effet, ce mélange est caractérisé par des rapports C/N et COD/SO_4^{2-} plus élevés comparativement aux deux autres mélanges testés. C'est donc le mélange qui a été choisi pour les tests dans des bioréacteurs type colonne.

Article #3

Titre du manuscrit

Effectiveness of sulphate-reducing passive bioreactors for treating highly contaminated acid mine drainage: I. Effect of hydraulic retention time

Auteur et co-auteurs

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Résumé

Le troisième chapitre est présenté sous la forme d'un article, soumis le 6 mars 2008 afin d'être publié dans la revue *Applied Geochemistry*.

L'article traite en détail le montage et le suivi (11-15 mois) de six bioréacteurs de type colonne, en triplicatas pour deux temps de résidence hydraulique (TRH) de 7,3 jours et 10 jours. Les réacteurs on été remplis du mélange réactif le plus efficace, sélectionné suite aux tests en batch et alimentés avec un DMA très contaminé. Durant le suivi, la qualité de l'effluent traité a été analysée sur une base hebdomadaire. En parallèle, l'évolution des paramètres hydrauliques (la conductivité hydraulique saturée k_{sat} et la porosité) du mélange réactif a été évaluée de façon sporadique. L'objectif de cette partie de l'étude est d'évaluer l'influence du TRH sur la qualité de l'effluent traité, de même que sur l'évolution des paramètres hydrauliques.

Les analyses de la qualité de l'effluent traité ont montré que les bioréacteurs sont efficaces pour le traitement du DMA pour les deux TRH testés. De plus, une qualité significativement meilleure a été obtenue à 10 jours de TRH comparativement à 7,3 jours de TRH. Cependant, l'analyse de l'évolution des paramètres hydrauliques indique qu'à un TRH plus long (10 jours), la valeur de k_{sat} a diminué d'une manière importante, ce qui a limité l'écoulement gravitaire dans ces colonnes. Par contre, dans les colonnes avec un TRH de 7,3 jours, la valeur de k_{sat} a diminué moins au début des tests et a resté presque constante par la suite.

En conclusion, les résultats montrent qu'un compromis doit être fait pour une conception optimale d'un bioréacteur passif entre les conditions pour respecter les normes de rejet de l'effluent traité et les paramètres hydrauliques du système de traitement.

Article #4

Titre du manuscrit

Toxicity and metal speciation in acid mine drainage treated by passive bioreactors

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Résumé

Le quatrième chapitre est présenté sous la forme d'un article, sous presse dans la revue *Environmental Toxicology and Chemistry* (2008), 27(8).

Le travail traite de l'évaluation de l'efficacité des bioréacteurs passifs sulfato-réducteurs pour le traitement d'un DMA très contaminé en termes d'enlèvement de la toxicité, de la source de toxicité, le cas échéant, ainsi que de la corrélation qui existe entre la partition/ spéciation des métaux et la toxicité de l'effluent traité.

Des tests de toxicité aquatique ont été réalisés sur une durée de 7 semaines, avec les organismes exigés par la nouvelle législation minière canadienne (voir chapitre I de la thèse). Les essais ont été réalisés sur le DMA et les deux effluents traités en provenance des bioréacteurs type colonne, exploités pendant plus de 7 mois à des TRH de 7,3 jours et 10 jours. L'identification de la source de toxicité en utilisant une procédure spécifique, ainsi que la partition (par filtration et ultrafiltration) des métaux dans l'effluent ont également été réalisées. Des simulations ont été effectuées avec les logiciels VMINTEQ et WHAM, afin d'évaluer la spéciation des métaux dans les échantillons qui ont été testés pour leur toxicité.

Les bioréacteurs ont été efficaces pour la réduction de la toxicité du DMA. De plus, un TRH hydraulique plus long (10 jours) a donné une meilleure qualité de l'effluent et une moindre toxicité comparativement avec un TRH plus court (7,3 jours).

Les trois conclusions les plus importantes du présent chapitre sont les suivantes:
(1) l'effluent des colonnes exploitées à un TRH de 10 jours satisfait l'exigence de la législation canadienne sur la qualité de l'effluent traité, en termes d'absence de toxicité aigüe sur la truite arc-en-ciel, ainsi que sur les daphnies (*Daphnia magna*); cependant, une aération préalable de l'effluent pendant 2 heures est nécessaire avant le test sur les daphnies;
(2) la source de toxicité sublétale de deux effluents est identifiée comme étant le fer;
(3) la spéciation et le partitionnement des métaux sont corrélés à la toxicité.

Article #5

Titre du manuscrit

Effectiveness of sulphate-reducing passive bioreactors for treating highly contaminated acid mine drainage: II. Metal removal mechanisms and potential mobility

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Résumé

Le cinquième chapitre est présenté sous la forme d'un article, soumis le 6 mars 2008 afin d'être publié dans la revue *Applied Geochemistry*.

L'article traite les résultats des analyses physico-chimiques, microbiologiques et minéralogiques qui ont été réalisées sur les mélanges réactifs récupérés dans quatre bioréacteurs de type colonnes, après 11 mois d'exploitation. L'objectif de cette partie de l'étude était d'obtenir de l'information sur les mélanges réactifs après le traitement d'un DMA très contaminé afin d'identifier/quantifier les mécanismes d'enlèvement des métaux, la stabilité des mélanges réactifs à long-terme, ainsi que la mobilité potentielle des métaux.

Les concentrations des métaux ont été élevées dans tous les mélanges réactifs, indépendamment du TRH, ce qui est en concordance avec la charge en métaux (Fe, Mn, Cd, Ni et Zn) du DMA et les concentrations faibles mesurées dans l'effluent traité. Les extractions chimiques et les analyses élémentaires donnent des résultats en concordance, qui indiquent une fraction faible des métaux enlevés sous forme de sulfures (au plus 14% des métaux récupérés dans les mélanges réactifs). Les analyses minéralogiques ont identifié la présence probable de sulfures métalliques contenant du Fe, Cd, Ni et Zn, en plus des (oxy) hydroxydes et des carbonates. Donc, les mécanismes principaux d'enlèvement des métaux ont été l'adsorption et d'autres mécanismes de liaison des métaux avec la matière organique (pour le Cd, Ni et Zn) et la précipitation des (oxy) hydroxydes (pour le Fe et Mn).

Après 15 mois d'exploitation, les bioréacteurs n'ont pas perdu leur capacité d'enlèvement des métaux du DMA. Ces derniers restent stables dans le mélange réactif. Une augmentation de la mobilité potentielle des métaux dans des mélanges réactifs post-traitement est possible. La récupération des métaux par la lixiviation de ces mélanges réactifs pourrait être une alternative viable techniquement et économiquement. Cette option devrait être évaluée dans le futur.

CHAPITRE I

ARTICLE #1: PASSIVE TREATMENT OF ACID MINE DRAINAGE IN BIOREACTORS USING SULFATE-REDUCING BACTERIA: CRITICAL REVIEW AND RESEARCH NEEDS

1.1 Abstract

Acid mine drainage (AMD), characterized by low pH and high concentrations of sulfate and heavy metals, is an important and widespread environmental problem related to the mining industry. Sulfate-reducing passive bioreactors have received much attention lately as promising biotechnologies for AMD treatment. They offer advantages such as high metal removal at low pH, stable sludge, very low operation costs, and minimal energy consumption. Sulfide precipitation is the desired mechanism of contaminant removal; however, many mechanisms including adsorption and precipitation of metal carbonates and hydroxides occur in passive bioreactors. The efficiency of sulfate-reducing passive bioreactors is sometimes limited because they rely on the activity of an anaerobic microflora [including sulfate-reducing bacteria (SRB)] which is controlled primarily by the reactive mixture composition. The most important mixture component is the organic carbon source. The performance of field bioreactors can also be limited by AMD load and metal toxicity. Several studies conducted to find the best mixture of natural organic substrates for SRB are reviewed. Moreover, critical parameters for design and long-term operation are discussed. Additional work needs to be done to properly assess the long-term efficiency of reactive mixtures and the metal removal mechanisms. Furthermore, metal speciation and ecotoxicological assessment of treated effluent from on-site passive bioreactors have yet to be performed.

Keywords: Acid mine drainage; Passive bioreactors; Sulfate-reducing bacteria; Natural organic carbon sources; Metal toxicity

1.2 Introduction

Mine wastes represent a source of potential environmental risk, particularly when the wastes contain sulfide minerals that can oxidize and generate acid mine drainage (AMD) (Aubertin and Bussière, 2001; Blowes *et al.*, 2003; Tabak and Govind, 2003; Willow and Cohen, 2003). The main characteristics of AMD are low pH and high concentrations of dissolved heavy metals and sulfates (Tsukamoto *et al.*, 2004). Acid mine drainage is considered the most important and widespread mining industry related pollution problem around the world (Tsukamoto and Miller, 1999). For example, massive sulfide tailings from ore having a pyrite content higher than 95% generated one of the worst AMD contaminations, with hydrogen ion concentrations (H+) as high as 103.6 mol L^{-1}, total dissolved metal concentrations as high as 200 000 mg L^{-1}, and sulfate concentrations as high as 760 000 mg L^{-1} (Nordstrom *et al.*, 2000).

Numerous approaches have been used to prevent AMD generation or to treat, control, and mitigate its effects. Several technologies, for example, have been developed to stop weathering processes by controlling the waste deposits and reducing the transfer of oxygen and water to the waste [Mine Environment Neutral Drainage (MEND) Report, 2001]. However, these prevention techniques do not represent the scope of the present review.

1.2.1 Acid mine drainage formation

The processes by which AMD is generated are currently quite well understood. Acid mine drainage is generated through a combination of chemical and biological processes by which pyrite is converted to sulfates and iron oxyhydroxides. However, the detailed mechanisms still need to be clarified (Usher *et al.*, 2004). AMD is generated when sulfides, in particular pyrite and pyrrhotite, are exposed to water and oxygen. The AMD generation is further amplified when the reactions are catalyzed by aerobic bacteria such as *Acidithiobacillus* (formerly *Thiobacillus) ferrooxidans* (Zagury *et al.*, 1997; Brown *et al.*, 2002). Factors such as bacterial activity, pH, sulfide mineral surface area,

crystallography, type of sulfide minerals, temperature, and oxygen concentration control the rates of AMD generation (Berghorn and Hunzeker, 2001). Several reactions are involved in the weathering of pyrite (Stumm and Morgan, 1981). The process is initiated at neutral pH by the release of ferrous iron (Fe^{2+}) into solution by pyrite oxidation, according to the following reaction:

(1) $FeS_{2(s)} + 7/2O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$

The next step occurs at lower pH values (<4):

(2) $Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2\ H_2O$

Iron-oxidizing bacteria, many of which tend to be most active at pH 2.0 to pH 4.0, can increase the rate of Fe^{2+} oxidation by factors greater than 10^6 (Brown *et al.*, 2002).
Ferric iron is not soluble in water if the pH is higher than 2.3 to 3.5, depending on total iron concentration. Therefore, it precipitates as oxyhydroxide releasing H^+ and lowering the pH:

(3) $Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$

As the pH decreases, the cycle reinitiates because ferric iron remains in solution and is reduced by pyrite, which generates additional ferrous iron and acidity (a self-perpetuating process), until either ferric iron or pyrite is depleted:

(4) $FeS_{2(s)} + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2\ SO_4^{2-} + 16H^+$

The rate of pyrite oxidation by Fe^{3+} is much higher than oxidation by O_2. Furthermore, this oxidation of 1 mole of pyrite releases 16 moles of H^+ (reaction 4) compared to 2 moles of H^+ in reaction 1. For these reasons, the oxidation of Fe^{2+} to Fe^{3+} (reaction 2) is often referred to as the "rate determining step" in the acid-generating process.
The overall reaction is given by adding reactions (1) through (3):

(5) $FeS_{2(s)} + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_{3(s)} + 2\ SO_4^{2-} + 4H^+$

These reactions progressively increase water acidity, resulting in mobilization of metals from mine wastes.

To avoid significant environmental impacts, waters contaminated by AMD must be collected and treated to remove metals and to increase the pH before being discharged into the environment. Traditionally, AMD treatment was performed with alkali to neutralize the acidity, increase water pH, and precipitate metals as hydroxides and carbonates (Ritcey, 1989; Santos *et al.*, 2004). Unfortunately, dumping of limestone in streams succeeds only as long as the water is anoxic. When the neutralized water is exposed to the atmosphere, ferrous iron oxidizes, hydrolyses, precipitates, coats the limestone, and slows its rate of dissolution. The effect may therefore be limited (Gazea *et al.*, 1996).

Other technologies to treat AMD, such as ion exchange, reverse osmosis, electrodialysis and electrolytic recovery are also available but are expensive and not commonly used (Prasad *et al.*, 1999). The solubility product of most metal hydroxides is higher than that of metal sulfides (Gazea *et al.*, 1996). Therefore, stabilization of metals is preferred in the form of sulfides. In the past 20 yr, research has focused on passive biological methods for AMD treatment because of their numerous advantages. They produce a high degree of metal removal at low pH (pH 3 to 6), denser, less voluminous and more stable sludge compared to sludge obtained during AMD chemical treatment. Moreover, they allow lower operation costs, and minimal energy consumption (Gazea *et al.*, 1996; Willow and Cohen, 2003). Nevertheless, treatment performance and long term efficiency still need to be improved (Beaulieu *et al.*, 1999, 2000; Tsukamoto *et al.*, 2004; Johnson and Hallberg, 2005a; Kalin *et al.*, 2006).

1.2.2 Classification of passive treatment systems

Passive treatment technologies have received much attention lately and the literature offers extensive studies related to these systems (Gazea *et al.*, 1996; Ziemkiewicz *et al.*, 2003). Several classifications have been proposed on the basis of different criteria such as: (1) aerobic or anaerobic processes, (2) complexity and requirements for maintenance, and (3) dominant chemical or biological processes occurring during treatment.

One classification separates aerobic passive treatment systems such as aerobic wetlands, open limestone channels, diversion wells, oxic limestone drains, and pyrolusite treatment beds from anaerobic systems, namely compost and/or anaerobic wetlands, anoxic limestone drains, and vertical flow reactors (Berghorn and Hunzeker, 2001). In another classification, "passive" systems such as aerobic wetlands and compost reactors/ wetlands are separated from "active" systems such as sulfidogenic bioreactors and accelerated iron oxidation with immobilized biomass (Johnson and Hallberg, 2002). In the authors' opinion, the most appropriate classification to date (Figure 1.1) separates the chemical passive treatment from the biological passive treatment, which involves sulfate-reducing bacteria (SRB). Permeable reactive barriers (PRBs) for groundwater treatment can be classified either as biological or as chemical passive treatment systems (Brown *et al.*, 2002).

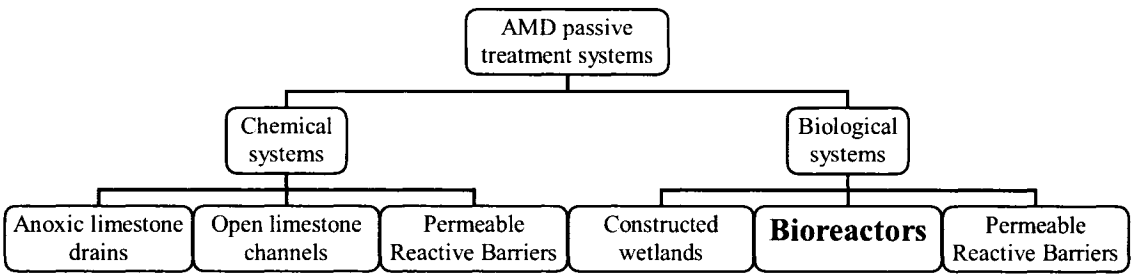

Figure 1.1 Classification of AMD passive treatment systems

The net distinction between active and passive bioreactors is not clear-cut, since a certain degree of upkeep and maintenance is required even for passive bioreactors (Brown *et al.*, 2002). For the present study, however, "passive bioreactor" means a reactor using a simple flow-through design, with an AMD feed over a solid reactive mixture acting as a source of carbon for SRB and as a physical support for microbial attachment and metal sulfide precipitation. The reactive mixtures used and the mechanisms of sulfate and metal removal are very similar in passive bioreactors and permeable reactive barriers (PRBs) using SRB. Comprehensive reviews describing

laboratory and full-scale experiments using PRBs are available in the literature (Blowes *et al.*, 2000; Gibert *et al.*, 2002). Therefore, PRBs' performance will not be specifically assessed and discussed in the present review paper.

1.2.3 New trends in US and Canadian mining legislation

North American mining legislation requires effluent toxicity assessment. Therefore, the performance of passive bioreactors will also need to be evaluated in terms of ecotoxicity reduction. In Canada, the Metal Mining Effluent Regulations (MMER) (Environment Canada, 2002) under the authority of the Fisheries Act requires that all mines with an effluent flow higher than 50 m^3/day conduct an Environmental Effects Monitoring (EEM) program. The regulation prescribes limits for the discharge of deleterious substances, and a requirement for effluent to be non-acutely lethal to rainbow trout and *Daphnia magna*. Since 2003, the metal mines are also required to conduct, two to four times per year, a series of four freshwater sublethal tests to determine if the effluent has the potential to affect fish, invertebrates, algae and plants. In marine ecosystems, three sublethal toxicity tests (fish, invertebrate, and algae) are also required. The sublethal toxicity tests to be used are the following: invertebrate *Ceriodaphnia dubia* reproduction test, algal growth inhibition test using *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), fathead minnow *Pimephales promelas* or rainbow trout *Oncorhynchus mykiss* embryo development inhibition test, and macrophyte *Lemna minor* growth inhibition test.

Similarly, in the US, all point source discharges from mining operations are to be authorized under a National Pollutant Discharge Elimination System (NPDES) permit, as described in the Clean Water Act [Clean Water Act (CWA), 1977]. In 2002, the USEPA set new guidelines establishing test procedures for the analysis of pollutants in order to add a series of standardized acute and short-term chronic whole effluent toxicity (WET) tests to the list of approved methods under the CWA (US EPA, 2002). The methods measure the toxicity of effluents to freshwater, marine, and estuarine organisms. The ratified WET methods are the following: a survival and reproduction

test of the invertebrate *Ceriodaphnia dubia*, a growth inhibition test of algae *Selenastrum capricornutum*, a larval survival and growth inhibition test of sheepshead minnow *Cyprinodon variegatus* and inland silverside *Menidia beryllina*, and a survival, growth inhibition, and fecundity test of crustacean *Mysidopsis bahia*.

1.2.4 Objective of the critical review

Biological passive treatment of AMD has been the focus of numerous studies, but in fact much remains to be learned regarding fundamental interactions within these complex biological reactors. To the authors' knowledge, there has been so far no integrated review dealing with critical factors for the design and long-term operation of passive on-site bioreactors for AMD treatment. However, several papers are available on physicochemical and biological processes occurring within wetlands (Gazea *et al.*, 1996; Wildeman and Updegraff, 1997; Sheoran and Sheoran, 2006). These papers address potential problems related to constructed wetlands - these systems allow little or no system control, are subject to seasonal and other variations, do not allow accurate assessment of their sizing and performance because of insufficient data available, may be ineffective when used in isolation, and may be subject to catastrophic system failure (Johnson and Hallberg, 2002). Comprehensive evaluations on the performance of passive systems excluding on-site bioreactors are also available (Ziemkiewicz *et al.*, 2003). Moreover, other papers have discussed the advantages which sulfur cycle bacteria offer for sulfate-rich wastewater treatment in wetlands and in active bioreactors (Hulshoff Pol *et al.*, 2001; Lens *et al.*, 1998, 2002). Recently, a review paper assessed the applicability, suitability, efficiency, and cost-effectiveness of various AMD treatment schemes based on available monitoring data from the UK. However, in this work, special emphasis was given to the use of wetlands as a passive biotechnology (Brown *et al.*, 2002). Finally, a book describing passive mine water remediation with a special emphasis on field studies in the UK is also available (Younger *et al.*, 2002).

The present article intends to be an integrated critical review of current knowledge about passive treatment of AMD in on-site bioreactors. Critical parameters for design

and long-term operation such as the composition of reactive mixtures are reviewed. Special attention is accorded to natural organic carbon sources which offer long-term availability, and improved efficiency of the passive bioreactors; an assessment of their potential biodegradability is also provided. Considering the new trends in North American legislation, non-previously treated aspects in available review papers such as organic carbon sources, sulfides and dissolved metal toxicity to SRB and effluent ecotoxicity are also discussed. Finally, some unexplored research needs and perspectives are suggested.

1.3 Passive bioreactors: principle, characteristics, and mechanisms

1.3.1 Sulfate reduction principles

Sulfate-reducing bacteria are either heterotrophic or authotrophic anaerobes, capable of reducing sulfate to sulfide by a dissimulator, bioenergetic metabolism when provided with a suitable organic carbon source (Postgate, 1984). Substrate (electron donor) oxidation is coupled to sulfate (terminal electron acceptor) reduction. The resulting energy is used by SRB for growth and development. The reaction is generally expressed as (Widdel, 1988):

(6) $2\ CH_2O + SO_4^{2-} \rightarrow 2\ HCO_3^- + H_2S$

where CH_2O represents a simple organic carbon source. The dissolved inorganic carbon neutralizes the pH and favors the precipitation of metal carbonate minerals. The soluble sulfides (H_2S, HS^-, and S^{2-}) react with metals to form metal sulfide precipitates:

(7) $H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+$

where M is a cationic metal such as Cd, Fe, Ni, Cu, or Zn.

Further information on the principles of sulfate reduction can be found in Widdel (1988) and in Hao *et al.* (1996).

1.3.2 Organic carbon sources

AMD generally contains relatively low concentrations of dissolved organic carbon (<10 mg L^{-1}) (Kolmert and Johnson, 2001). Therefore, the most critical limiting factor for the microbial activity is the availability of carbon from an additional organic source (Gibert *et al.*, 2004; Zagury *et al.*, 2006). The challenge for having an efficient on-site bioreactor is to select a suitable organic substrate to make the process efficient and economically feasible. Selection of the organic carbon source is usually made on the basis of availability and costs of the added electron donor per unit of reduced sulfate. The remaining contaminants in the treated water must be present in low concentrations or easy to remove (Hulshoff Pol *et al.*, 2001).

1.3.2.1 Simple organic carbon sources

Sulfate-reducing bacteria use the easily degradable fraction of organic matter such as low molecular weight compounds with simple structures (e.g., methanol, ethanol, lactate) (Dvorak *et al.*, 1992; Nagpal *et al.*, 2000a; Tsukamoto *et al.*, 2004), polylactic acid (Edenborn, 2004), simple carbohydrate monomers (e.g., glucose or sucrose) (Mizuno *et al.*, 1998), and whey (Christensen *et al.*, 1996). In terms of energy and biomass produced, lactate is a superior electron donor compared to others such as ethanol, acetic acid, propionate and acetate (Nagpal *et al.*, 2000b). In terms of moles of bicarbonate produced per mole of substrate consumed, the lactate-utilizing processes are superior to ethanol-utilizing processes (3 vs. 2, respectively) since they are better at neutralizing the acidity in the treated effluent (Kaksonen *et al.*, 2004a). The main drawback is that only certain species of SRB (*Desulfotomaculum*) are capable of oxidizing lactate and ethanol to CO_2, while others (*Desulfovibrio*) can partially oxidize the C2-C4 organic carbon molecules to acetate, and very few can use acetate alone (*Desulfotomaculum acetoxidans*) (Nagpal *et al.*, 2000b).

1.3.2.2 Complex organic carbon sources

Alternatively, less expensive organic carbon sources such as waste material from the agricultural and food processing industry have been assessed for their potential to sustain sulfate-reduction. The alternative organic carbon sources may be selected between two groups of materials: cellulosic wastes and organic wastes (Kuyucak and St-Germain, 1994). Generally, cellulosic wastes include sawdust, hay, alfalfa, and wood chips, whereas organic wastes include cattle manure, cow manure, horse manure, poultry manure, sheep manure, rabbit manure, granular or sewage sludge, peat, pulp mill, molasses, and compost (see Table 1.1). There is a general consensus that these substrates alone do not significantly promote the activity of SRB (Christensen *et al.*, 1996; Waybrant *et al.*, 1998, 2002; Cocos *et al.*, 2002; Gibert *et al.*, 2003; Zagury *et al.*, 2006).

Higher sulfate reduction rates have been obtained with reactive mixtures containing more than one organic carbon source (Waybrant *et al.*, 1998, 2002; Cocos *et al.*, 2002; Zagury *et al.*, 2006). Generally, these mixtures contain relatively biodegradable sources (poultry manure, cow manure or sludge) and more recalcitrant ones (sawdust, hay, alfalfa or wood chips). In fact, the comparison of different studies dealing with the same substrate or different organic substrates is very difficult because of different durations for each study. For example, studies have been performed over 14 d (Jong and Parry, 2003), 70 d (Tassé and Germain, 2002; Zagury *et al.*, 2006), 23 mo (Drury, 1999), or 32 mo (Zaluski *et al.*, 2003). In very short-term experiments, the aging of the material and the clogging of the matrix are not addressed (Jong and Parry, 2003; Zagury *et al.*, 2006). Higher proportions of coniferous bark and/or sawdust have been associated with sluggish sulfate reduction rates in short-time experiments (Tassé and Germain, 2002), whereas a mixture containing a high content of sawdust (40% sawdust, 10% wood chips, 10% alfalfa hay, 10% cow manure, 29 % limestone, and 1% cement kiln dust) gave the best efficiency in a long-term field study (Reisman *et al.*, 2003).

Furthermore, contradictory conclusions emerge from studies performed with the same organic carbon source but using different proportions in the reactive mixture. In an attempt to find the best reactive mixture for use in permeable reactive walls, Waybrant *et al.* (1998) concluded that sheep manure (100%) did not produce the reducing conditions necessary for bacterial activity and excluded this organic source from their batch assays.

In contrast, Gibert *et al.* (2004) clearly indicated sheep manure (15% of reactive mixture) as the most successful organic material for creating reducing conditions and sustaining active sulfidogenesis (sulfate removal >99%) in a batch experiment. Similarly, in the experimental study of Amos and Younger (2003), cattle manure (100%) was rejected at an early stage due to low permeability, whereas a mixture of cow manure (80%) and cut straw (20%) was successfully used over 32 mo by Zaluski *et al.* (2003).

Gibert *et al.* (2004) report that the findings of Cocos *et al.* (2002) (who found limited degradability of lignin-cellulosic substrates in a 41-d batch test) and those of Waybrant *et al.* (1998) (who concluded that a cellulosic material alone could sustain satisfactory bacterial activity in a 125-d column test) are contradictory. In fact, the conclusion of Waybrant *et al.* (1998) was that after the acclimation period (20 to 65 d), sulfate reduction rates were higher in the reactive mixtures that contained a variety of organic carbon sources. In the short-term study of Cocos *et al.* (2002), a higher proportion of poultry manure was essential for promoting higher sulfate reduction rates. Further, the observation made by Waybrant *et al.* (1998) was that the cellulose entailed a slightly lower sulfate reduction rate compared to other substrates tested (sewage sludge, leaf mulch, wood chips, sheep manure, and sawdust) alone or in mixture. This is in agreement with the results of Chang *et al.* (2000) who observed similar performance at later stages of experiments (after 20 wk) in bioreactors using several sources of waste materials containing cellulose. In this later study, the cellulose was the main component used during 35 wk of operation.

Table 1.1 Characteristics of some passive bioreactors reported in the literature

Reactor scale	Total volume	Organic matter source	pH		SO_4^{2-} (mg L^{-1})		References
	L		Influent	Effluent	Influent	Effluent	
Field bioreactor	765600	Mixture of softwood dust, hay, and cattle manure	4 – 5.5	5 - 6	175 - 250	200 - 275	Johnson and Hallberg (2005b)
Field bioreactor	92000 - 108000	Mixture of cow manure and cut straw	3.3 - 7.5	6.5 – 7.5	70 – 229	not reported	Zaluski *et al.* (2003)
Pilot-scale bioreactor	20000	Cattle manure, sawdust, hay, and alfalfa	3.5 - 7.5	6.5 - 7.5	< 60	< 40	Reisinger *et al.* (2000)
Pilot-scale bioreactor	200 – 4500	Spent mushroom compost (mixture of manure, hay, straw, corn cobs, and wood chips)	3.2 - 6.2	6.4 - 7.1	1002 - 2997	831 - 2387	Dvorak *et al.* (1992)
Pilot-scale bioreactor	3900	Methanol	2.9-3.2	6.9	1900 – 2100	832	Glombitza (2001)
Pilot-scale bioreactor	570	Rice stalks, cow manure, and limestone	3.6	6.2	not reported	not reported	Cheong *et al.* (1998)
Bench-scale bioreactor	200	Mixture of shredded wood chips, sawdust, alfalfa hay, and cow manure	3 - 3.5	5.5 – 7	3000 - 3500	2500 - 4500	Reisman *et al.* (2003)
Laboratory bioreactor	45	Mixture of cow manure, sawdust, and whey	2.5 - 3.5	6.5	< 1000	< 300	Drury (1999)
Laboratory bioreactor	25-29	Livestock manure	2.7 - 6.2	6.3 - 7.1	1000	922 - 970	Willow and Cohen (2003)
Laboratory bioreactor	17	Alfalfa, hay, timothy hay, and cereal straw	3.5	6.5	1010	420 - 960	Béchard *et al.* (1994)
Laboratory bioreactor	4.8	Lactate	4.52	7.2	2280-2315	< 400	Jong and Parry (2003)
Column	9	Mixture of wood chips, leaf compost, and poultry manure	3.8 – 4	7	1500	1220	Beaulieu *et al.* (2000)
Column	4.7 - 7.8	Spent manure revitalized with methanol/ethanol	2.5 - 4.2	5.4 – 7.3	900	400 - 500	Tsukamoto *et al.* (2004)
Column	0.25	Spent mushroom compost, oak chips, spent oak, sludge, and organic-rich soil	6.8	7 - 8.5	2580	200 - 650	Chang *et al.* (2000)
Column Batch	Column: 0.12 Batch: 0.5	Compost, oak leaf, poultry manure, and sheep manure	2.4	6 - 7.5	540 (column) 1040 (batch)	<850(column) < 200(batch)	Gibert *et al.* (2004)
Batch	31	Whey	3.0 - 4.4	3.5 – 6.0	857 - 936	715 - 5390	Christensen *et al.* (1996)
Batch	25	Bedded cattle manure and mixture of cattle slurry screenings, and green waste compost	4.2	5.9 - 6.3	14752	not reported	Amos and Younger (2003)
Batch	2	Single source or mixture of maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure, conifer sawdust	4	6.5 - 8.5	4244	163 - 5575	Zagury *et al.* (2006)
Batch	1	Several barks and wood chips	1.6	5 - 6	2500	750 - 1250	Tassé and Germain (2002)
Batch	0.5 - 1	Single source or mixtures of sewage sludge, leaf mulch, wood chips, sheep manure, sawdust, and cellulose	2.5 - 6	6.5 - 7	1200 - 4800	< 35	Waybrant *et al.* (1998)
Batch	0.5	Mixture of leaf compost, poultry manure and wood chips	5.5 - 6	7.9	2000 - 3200	< 90	Cocos *et al.* (2002)

The efficiency of cellulosic substrates for the biological treatment of AMD has been confirmed by several studies (Tuttle *et al.*, 1969a, 1969b; Waybrant *et al.*, 1998; Chang *et al.*, 2000; Tassé et Germain, 2002; Johnson and Hallberg, 2005b), while other studies suggested that cellulosic wastes alone entailed carbon-limiting conditions (Béchard *et al.*, 1994) or did not sustain SRB growth (Kuyucak and St-Germain, 1994). With sawdust as the sole nutrient source, a mixed bacterial culture containing cellulose-degrading bacteria and SRB was capable of reducing sulfate at pH 3.0, whereas pure cultures of SRB did not reduce sulfate below pH 5.5 (Tuttle *et al.*, 1969b). These results stress the importance of a well established microflora in the presence of mixtures of cellulosic and other complex natural organic carbon sources.

1.3.3 Microflora

Sulfate-reducing bacteria cannot directly oxidize complex organic carbon compounds such as carbohydrates, proteins, lipids, cellulose, and hemicellulose polymers (Postgate, 1984). When such organic carbon sources are provided, synergism between three groups of microorganisms (acidogens, methanogens and sulfate reducers) is essential to provide short-chain organic carbon compounds for SRB (Tuttle *et al.*, 1969a, 1969b; Kuyucak and St-Germain, 1994). Hydrogen produced by acidogenic bacteria during anaerobic digestion can be utilized by all three groups of bacteria and competition may occur (Raskin *et al.*, 1996; Mizuno *et al.*, 1998). The study of changes in Gibbs free energies for hydrogen-consuming reactions under standard and steady-state conditions showed that SRB have a thermodynamic advantage over methane-producing bacteria and homoacetogenic bacteria (Mizuno *et al.*, 1998). Therefore, SRB would outcompete methane producing bacteria if sulfate is provided as a final electron acceptor.

A recent study indicated that compost-based sulphate-reducing bioreactors are dominated by non-sulfate-reducing bacteria (Hallberg and Johnson, 2005a). Three to four metabolic groups involved in AMD treatment processes were found, using cellulosic or organic substrates as a solid support and cellulolytic microorganisms, fermenters and respirers, methanogens, and SRB (Béchard *et al.*, 1994; Logan *et al.*,

2005) as organic carbon source for microbial growth. Recently, cellulolytic and fermenters communities were found limited at no time by organic carbon availability during column experiments conducted for 100 d. However, SRB showed great limitation through all but the early establishment phase (Logan *et al.*, 2005). In a passive field bioreactor containing a mixture of 95% softwood and 5% hay, a 10-month shut down period before its monitoring appeared as a long enough lag time to allow key microbial populations to increase in numbers and activities in the absence of AMD throughput (Johnson and Hallberg, 2005b). Availability of nutrient sources for cellulolytic and fermenters is vital to the long-term sustainability of passive treatment systems (Lynd *et al.*, 2002). Anaerobic degradation of complex organic carbon compounds to simpler molecules by cellulolytic and fermenters microbes may limit the rate at which substrates become available to SRB (Logan *et al.*, 2005). Sulfate reduction seems controlled by cellulose degradation and therefore, future research for exploring means by which to enhance cellulose hydrolysis is needed. More work must be conducted to understand and differentiate the fundamental biochemical and microbiological reactions that occur in anaerobic bioreactors with complex organic substrates. This might be a key step for the successful implementation of SRB-based AMD remediation systems.

1.3.4 Tests for assessing the biodegradability of complex organic substrates

Organic carbon available for bacteria is contained in the dissolved organic matter (DOM), which consists of a rapidly degradable fraction (e.g., simple organic compounds), the polysaccharides fraction that is degraded more slowly, and the recalcitrant fraction that remains in solution up to 180 d (Marschner and Kalbitz, 2003).

For the quantification of DOM biodegradability, the duration of incubation and the measure of biodegradation are crucial parameters (Marschner and Kalbitz, 2003). Chemical composition of an organic substrate controls its biodegradability pattern (Gibert *et al.*, 2004; Zagury *et al.*, 2006). Results of biodegradation studies strongly depend on the experimental methodology; for example the duration of incubation and

the method of quantification [monitoring of dissolved organic carbon (DOC) concentration vs. CO_2 efflux from the sample during incubation] (Marschner and Kalbitz, 2003). Some researchers attempted to predict the degradability of complex organic substrates by assessing their chemical composition (protein and carbonate, cellulose, hemicellulose, and lignin content, solvent and water-extractable organic matter, and easily extractable fractions) (Prasad *et al.*, 1999; Chang *et al.*, 2000; Gibert *et al.*, 2004; Zagury *et al.*, 2006). The conclusion is that none of these chemical extractions alone is sufficient to accurately predict the degradability of organic materials. Therefore, a standardized method is still warranted to predict the ability of organic substrates to promote sulfate reduction and metal removal.

Several models were developed to describe the influence of organic materials degradation on sulfate reduction rates in passive bioreactors and permeable reactive barriers (Drury, 2000; Benner *et al.*, 2002; Mayer *et al.*, 2002; Amos *et al.*, 2004). They all potentially oversimplify sulfate reduction because they do not consider bacterial growth and decay (Hemsi *et al.*, 2005). On the contrary, the biochemical model developed by Hemsi *et al.* (2005) coupled sulfate reduction kinetics to organic materials decomposition and biochemical processes. The simulated results showed the importance of kinetics used to describe the decomposition of solid organic materials.

1.3.5 Configurations of organic substrates and depletion of organic carbon

In many passive bioreactors, the organic matter matrix also serves as a support for microbial attachment and metal precipitation (Tsukamoto *et al.*, 2004). The most effective design is typically when the substrate is sandwiched between pipes set in inert gravel in the top and bottom of the basin (URS Report, 2003).

The lifetime of such bioreactors is limited by the amount of reducing equivalents readily available to SRB affecting the extent of microbial activity and treatment efficiency (Gibert *et al.*, 2004; Tsukamoto *et al.*, 2004). Other configurations include bioreactors filled with a combination of organic matter, crushed limestone, and cobbles placed in

two to four discrete chambers (Zaluski *et al.*, 2003), and site-specific passive systems that incorporate anaerobic and aerobic cells and limestone and rock filters (Johnson and Hallberg, 2005b; Kuyucak *et al.*, 2006).

Evaluation of substrate longevity based on fixed amounts of organic carbon and limestone and the relative consumption rates observed is, however, useless due to high variability in kinetics of sulfate reduction during the treatment (Reisman *et al.*, 2003). A proposed solution to extend the long-term performance of a bioreactor was the addition of an alternative organic source to a depleted matrix.

Simple organic compounds such as methanol (Tsukamoto *et al.*, 2004), sucrose (Béchard *et al.*, 1994; Lloyd *et al.*, 2004), lactate (Tsukamoto and Miller, 1999; Tsukamoto *et al.*, 2004), and acetate (Gibert *et al.*, 2004) were successfully tested. Another solution was to bioactivate the bacterial consortia with an easily available organic source (e.g., lactate) and then to replace it with a less expensive source such as ethanol (Kaksonen *et al.*, 2003) or a mixture of wood chips, leaf compost, and poultry manure (Beaulieu *et al.*, 2000). In the former study, Kaksonen *et al.* (2003) reported partial degradation of ethanol to acetate that increased residual organic carbon in the effluent. In the latter study, a longer lag period was necessary for SRB to get acclimated to the more complex organic carbon sources.

New formulations of suitable organic carbon sources, such as patented mixtures of organic materials (methonak, molasses, methanol, and wood chips) commercialized by ARCADIS treatment systems or hydrogen release compounds (HRC) by REGENESIS technologies are also available. Commercialized reactive mixtures can be used for *in-situ* treatment of AMD contaminated waters in pit lakes, smelter ponds, and flooded and underground workings (e.g., Gilt Edge Mine, Anchor Hill Pit Lake, Hollister Mine, and Sweetwater Mine). Hydrogen release compounds are an electron donor organic material designed to produce controlled release of lactic acid when hydrated. Hydrogen release compounds can be directly injected in the contamination source area or used in PRB applications.

1.4 Metal removal mechanisms

According to the literature, the main mechanisms of metal removal in bioreactors are precipitation in the form of sulfides (Pb^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Fe^{2+}, Zn^{2+}), hydroxides (Fe^{3+}, Cr^{3+}, and Al^{3+}), and carbonates (Fe^{2+}, Mn^{2+}). Sorption mechanisms such as adsorption, surface precipitation, and polymerization on inorganic support, solid organic matter, bacteria, and metal precipitates also occur. Besides biologically mediated processes, AMD quality is improved by filtration of the suspended and colloid materials (Wildeman and Updergraff, 1997).

The metal removal mechanisms change during the life of a passive bioreactor. Upon start up of a passive bioreactor, the adsorption of dissolved metals onto organic sites in the substrate material will be an important process (Machemer and Wildeman, 1992; Gibert *et al.*, 2005a). In the pH range 4 to 7, SRB retain metals via biosorption due to the neutral and/or deprotonated state of binding ligands on cell walls. The biosorption by SRB is metabolism-independent (sorption onto the cell wall) or metabolism-related (transport, internal compartmentalization, and extracellular precipitation by metabolites) (Chen *et al.*, 2000). Factors such as availability of nutrients during growth, age and physiological state of bacterial cells, environmental conditions (pH, ionic strength, and temperature), presence of competitive ions, and concentration of the biomass can influence biosorption (El Bayoumy *et al.*, 1997; Chen *et al.*, 2000; Utgikar *et al.*, 2000; Santos *et al.*, 2004). Because of several factors of influence, the experimental results are not always in agreement. At pH 3.0, a biomass content > 6 g L^{-1} increased the efficiency of metal removal, favoring sedimentation of the iron precipitate and rates of filtration (Santos *et al.*, 2004). In another study conducted at pH 7.0, biosorption capacity was constant regardless of the experimental conditions (e.g., stirring and biomass type) (El Bayoumy *et al.*, 1997). These differing results may be due to a more or less active microbial population in the biomass used as well as to a possible competition between metals. Competition among Fe, Cu, Zn, and Mn for organic adsorption sites was confirmed by laboratory tests, and field-tests in wetlands (Machemer and Wildeman, 1992; Utgikar *et al.*, 2000). In the study of Chen *et al.* (2000), biosorption on

Desulfovibrio desulfuricans was strongly pH-dependent. For Cu (II) and Zn(II), biosorption increased within a pH range of 4.0 to 6.6. At pH below 3.0, metal biosorption was insignificant due to strong affinity of protons onto metal binding sites on biomass cell walls, while at pH > 5.0 for Cu(II) or 6.6 for Zn(II), the desorption or precipitation contribution increased significantly compared to adsorption. Functional groups capable of metal sorption such as carboxylic and phenolic groups are deprotonated at high pH and presumably available for binding dissolved metals (Dudal and Gérard, 2004). Therefore, at the slightly acidic to neutral pH of on-site sulfate-reducing bioreactors, adsorption of dissolved metals on the substrate material is an important metal removal mechanism. Over time, however, the adsorption sites become saturated. This saturation may take from 3 to 8 wk (Waybrant *et al.*, 1998; Willow and Cohen, 2003), to 4 to 8 mo (Zaluski *et al.*, 2003).

Once sulfate-reducing conditions are established, sulfide precipitation becomes the predominant mechanism of metal removal from AMD (Machemer and Wildeman, 1992; Béchard *et al.*, 1994; Song *et al.*, 2001). Sulfide precipitation is the desired mechanism of contaminant removal because metal sulfides are highly insoluble and less bioavailable compared to other metal species (Wildeman and Updergraff, 1997). Sulfate reduction in passive bioreactors is confirmed by lower concentrations of sulfates in the effluent than in the influent waters, and the presence of free sulfides (depending on metal concentrations and water pH) and lower redox potentials in the effluent waters (Johnson and Hallberg, 2005b). Metals can also be removed by coprecipitation with (or adsorption onto) Fe and Mn oxides and bacterially produced metal sulfides (Jong and Parry, 2004a; Watson *et al.*, 1995).

Proper studies of metal removal mechanisms should be based on data obtained from the effluent water chemistry and from the solid phase analysis of the bioreactor mixture. Geochemical modeling of water chemistry data in bioreactors can be performed with thermodynamic chemical equilibrium models such as WATEQ4F (Amos and Younger, 2003) and VMINTEQ (Waybrant *et al.*, 1998, 2002; Zagury *et al.*, 2006). These models give useful insights into the chemical equilibrium reactions potentially controlling the

concentrations of dissolved metals. Published results generally suggest that early decreases in metal concentration can be attributed to adsorption or precipitation of (oxy)hydroxides and carbonates. However, thermodynamic modeling results should be interpreted with caution since these models do not take into account the bacterial activity that entails the precipitation of metal sulfides (Zagury *et al.*, 2006).

Solid phase analysis is also an important step for elucidating the metal removal processes (Machemer *et al.*, 1993). Chemical analyses such as extraction procedures complemented with determination of acid volatile sulfides and simultaneously extracted metals are efficient tools to assess metal fractionation (Jong and Parry, 2004b, 2005).

Moreover, mineralogical analyses can help to identify the chemical form of metals in the solid phase. In some studies, the number of techniques for collecting mineralogical data has been limited by the poor cristallinity of the precipitates and the relatively low concentrations of metal sulfides (Song, 2003; Gibert *et al.*, 2005b). Among these methods, scanning electron microscopy has been the most successful technique, whereas X-ray diffraction or Mossbauer analyses have been less effective in detecting amorphous metal sulfides (Machemer *et al.*, 1993). Additional work is needed to accurately assess the various metal removal mechanisms occurring in passive bioreactors.

1.5 Factors of influence on SRB-based reactors efficiency

In passive bioreactors the activity of SRB is the rate-limiting step and bacteria have specific requirements that must be fulfilled. The design of a passive bioreactor requires a near-neutral pH, a source of sulfate, a source of organic carbon, a reducing environment, a solid support for microbial attachment and development, and a way to physically retain metal sulfide precipitates (Dvorak *et al.*, 1992; Lyew and Sheppard, 1997). At the start-up phase, the critical parameters are pH and reducing conditions, flow rate, AMD composition, nutrients, and temperature (Kuyucak and St-Germain,

1994). During treatment, the most limiting factors for the growth of SRB are sulfate concentrations and the type of organic carbon source (Garcia *et al.*, 2001).

1.5.1 Effect of pH, Eh, and temperature

In order for SRB to thrive, they require a pH in the range of 5 to 8 (Willow and Cohen, 2003). Outside this range, the rate of microbial sulfate reduction generally declines and the metal removal capacity is reduced. Low pH (< 5) normally inhibits sulfate reduction and increases the solubility of metal sulfides (Dvorak *et al.*, 1992). In any case, the presence of SRB has been detected in natural waters with pH < 3 (Gyure *et al.*, 1990; Kolmert and Johnson, 2001; Koschorreck *et al.*, 2003). Isolated strains were mostly acidophilic SRB, which are more efficient than the neutrophilic ones for remediating acidic waste waters (Kolmert and Johnson, 2001). The SRB were capable of sulfate reduction in a column bioreactor operated under acidic conditions with lactate as organic carbon source (Elliott *et al.*, 1998). At pH 3.25, 38.3% of influent sulfate was removed and pH of the medium rose to 5.82, whereas at pH 3.0 sulfate removal fell to 14.4% and sulfide production dropped below detection. Nevertheless, viable SRB were recovered from the bioreactor after operation at pH 3.0 for 3 wk (Elliott *et al.*, 1998). Efficient AMD treatment was also achieved at a pH as low as 2.5 (Tsukamoto *et al.*, 2004).

However, the existence of truly acidophilic SRB is currently not clear. Reduction of sulfate to sulfide has been demonstrated as occurring in extremely acidic environments, but attempts at isolating pure culture of acidophilic (acid-tolerant) SRB failed (Gyure *et al.*, 1990; Johnson, 1998). Nevertheless, a pH of 5.5 or higher is preferred for efficient treatment of AMD in an on-site passive bioreactor (URS Report, 2003).

For optimal performance, SRB need an anaerobic medium and an anoxic and reduced microenvironment with a redox potential (Eh) lower than –100mV (Postgate *et al.*, 1984). However, sulfate reduction was often observed in passive field bioreactors at positive Eh values (Reisman *et al.*, 2003; Zaluski *et al.*, 2003). Eh measurements of

aqueous samples collected at the outlets of the bioreactors might not reflect the real values present in pockets of organic matter where SRB live (Zaluski *et al.*, 2003). Their survival in these adverse conditions may also be explained by the formation of favourable anoxic microenvironments in the reactive mixtures (Lyew and Sheppard, 1999). Batch and column laboratory bioreactors successfully treated AMD at Eh values of -100 to –200 mV or lower during 23 d (Cocos *et al.*, 2002), 30 d (Beaulieu *et al.*, 2000), or 150 d (Gibert *et al.*, 2004). In passive field bioreactors, Eh values as low as –200 mV were maintained for periods ranging from 2 mo to more than 2 yr (Cheong *et al.*, 1998; Reisinger *et al.*, 2000).

In passive bioreactors, the operating temperature affects bacterial growth, kinetics of organic substrate decomposition, as well as hydrogen sulfide solubility. Generally, SRB can tolerate temperatures from below -5°C to 75°C (Postgate, 1984). Column experiments showed that the efficiency of AMD treatment was not significantly reduced at temperatures as low as 6°C (Tsukamoto *et al.*, 2004). Passive on-site bioreactors successfully operated for 32 mo at temperatures between 2°C and 16°C (Zaluski *et al.*, 2003) or over 2 yr at near-freezing temperatures (1 to 8°C) (Reisinger *et al.*, 2003; Kuyucak *et al.*, 2006). Low temperatures particularly affect the ability of SRB to acclimate, but once acclimated at higher temperature, SRB are not that affected by low temperature (Kuyucak and St-Germain, 1994; Tsukamoto *et al.*, 2004). In field bioreactors started during the winter, a 4-mo lag phase was observed for SRB to be established. However, winter freezing of a well-established SRB population had little or no effect on their activity (Zaluski *et al.*, 2003; Kuyucak *et al.*, 2006). The methanogens, which are found when bioreactors are supplied with complex organic carbon sources, are mainly mesophilic microorganisms. Therefore, they are more sensitive to low temperatures than SRB (Kuyucak and St-Germain, 1994).

1.5.2 Effect of solid support, hydraulic retention time (HRT), and hydraulic conductivity

SRB require a solid support (sand and/or gravel), onto which they can establish microenvironments for their survival in the presence of extreme conditions such as low pH or high oxygen concentrations (Lyew and Sheppard, 1997). Higher sulfate reduction rates are achieved if SRB have access to a porous surface, compared to suspended bacteria (Glombitza, 2001). A medium with large pore spaces, low surface area, and a large void volume is generally preferred because it minimizes the plugging of the bioreactor (Tsukamoto *et al.*, 2004). In terms of efficiency, better treatment occurs with greater surface area. Surface area and pore size need to be balanced in field-bioreactors (Tsukamoto *et al.*, 2004).

Effects of hydraulic retention time (HRT) on efficiency of bioreactors have been widely studied (Dvorak *et al.*, 1992; Al-Ani, 1994; Béchard *et al.*, 1994; Rockhold *et al.*, 2002; Kaksonen *et al.*, 2004b). The variability of hydraulic properties of porous media used in reactive mixtures may result in HRTs specific to each bioreactor. It is usually accepted that precipitation of metal sulfides occurs in at least 3 to 5 d (URS Report, 2003; Kuyucak *et al.*, 2006). A shorter HRT may not allow adequate time for SRB activity to neutralize acidity and precipitate metals or may result in biomass being washed out of the bioreactor. A longer HRT may imply depletion of either the available organic mater source or the sulfate source for SRB (Dvorak *et al.*, 1992). In a semicontinuous anaerobic laboratory bioreactor, more sulfates were reduced to sulfides with a 3-day HRT compared to a 1-day HRT, regardless of the organic carbon/sulfate ratio (Al-Ani, 1994).

During treatment, bacteria induce changes in the mixture properties due to accumulation of biomass and to generation of metabolic byproducts. The characteristics of the accumulated biomass are dependent on the type of bacteria, the substrate and loading rate, and the flow rate (Rockhold *et al.*, 2002). Bacterial activity might cause a decreased surface tension, decreased porosity and permeability, and pore clogging. The

hydraulic conductivity of the substrate material is also an important variable because this will affect the HRT (Bolis *et al.*, 1992; Benner *et al.*, 2001, 2002). Several studies were conducted to evaluate the effect of microbial growth and biomass accumulation on porosity and permeability of saturated porous media. A sand-packed column reactor using sewage bacteria and methanol as a substrate, showed a decrease in the saturated hydraulic conductivity (K_s) of 3 orders of magnitude following biomass accumulation (Taylor and Jaffe, 1990). A relatively small variation in hydraulic conductivity could entail important differences in residence times, and might result in decreased efficiency (Benner *et al.*, 2002). Efficient compost substrate passive bioreactors have a hydraulic conductivity about 1 x 10^{-4} cm s^{-1} (URS Report, 2003). Recently, sawdust has been increasingly used in reactive mixtures, due to a significantly higher permeability of around 10^{-2} cm s^{-1} to 10^{-3} cm s^{-1}. When sawdust is used, however, there is an increased vulnerability for mixture compaction. Presoaking the substrate before AMD treatment can help provide a more stable hydraulic conductivity and a more consistent flow rate through the system (Bolis *et al.*, 1992). Composted substrates should be at least 0.6 m in thickness but should not exceed 0.9 to 1.2 m; if not, the substrate tends to compact with depth and the permeability becomes too low for effective treatment (URS Report, 2003).

1.5.3 Effect of COD (Chemical Oxygen Demand)/Sulfate (SO_4^{2-}) ratio and nutrients

Several studies have been conducted to find the best chemical oxygen demand (COD)/SO_4^{2-} ratios for AMD treatment under sulfate-reducing conditions but the results are not consistent. With sludge as the organic carbon source (Al-Ani, 1994) the best performance was found for a COD/SO_4^{2-} ratio of 5.0, whereas other studies using natural or synthetic substrates found that SRB were predominant for a ratio below 1.7 (Choi and Rim, 1991; Prasad *et al.*, 1999). When complex organic carbon sources were used as substrate, higher optimal ratios were likely obtained because not all the carbon present was used by SRB (Prasad *et al.*, 1999). The assessment of the optimal

COD/SO_4^{2-} ratio for efficient operation of passive bioreactors under sulfate-reducing conditions deserves further investigation.

A C/N ratio around 10 is generally considered suitable for biological degradation of complex organic substrates (Reinertsen *et al.*, 1984; Béchard *et al.*, 1994). Higher ratios indicate an excessive carbon content or nitrogen deficiency, whereas lower ratios may suggest a lack of carbon (Prasad *et al.*, 1999). Nevertheless, Zagury *et al.* (2006) found that the C/N ratio of reactive mixtures was not a good indicator of the sulfate-reducing activity when they tested six natural organic materials. Moreover, when lactate is used as a substrate, the reported optimal values fluctuate from 15.7 to < 45 or between 45 and 120 (Gerhardt *et al.*, 1981; Okabe *et al.*, 1992).

1.5.4 Inhibitory/toxic effects of metals, sulfides (H_2S, HS^-, and S^{2-}), oxygen, and organic carbon

Several studies have been conducted to assess metal toxicity to SRB (Gray and O'Neill, 1997; Poulson *et al.*, 1997; Sani *et al.*, 2001a, 2001b, 2003; Utgikar *et al.*, 2001, 2002, 2003, 2004). Some studies were performed with artificially contaminated sulfate-rich waters similar to AMD (Elliott *et al.*, 1998; El Bayoumy *et al.*, 1999; Gibert *et al.*, 2004) or with mine waters pretreated with Na_2S in order to remove heavy metals (Glombitza, 2001). Results have clearly shown that the effect of heavy metals on SRB can be stimulatory at lower concentrations and inhibitory or even lethal at higher concentrations (Poulson *et al.*, 1997; Utgikar *et al.*, 2002; Sani *et al.*, 2003). Metals may inactivate the enzymes, denature the proteins and compete with essential cations (Utgikar *et al.*, 2002).

In the laboratory, the use of an artificial AMD without metals will simplify the biological system, by avoiding reactor clogging. However, an artificial AMD might affect the optimum operating conditions. Evaluation of potential toxic effects of dissolved metals is essential to successfully operate AMD biological treatment systems (Utgikar *et al.*, 2001). The following factors have been identified as important in

quantifying the toxicity of heavy metals to SRB - initial metal concentrations, metal sorption and precipitation, and metal complexation with organic ligands (Poulson *et al.*, 1997).

Toxic effects of metals have been reported for concentrations varying from a few mgL^{-1} to more than 100 mg L^{-1} (Utgikar *et al.*, 2002). Toxic levels are difficult to assess as several studies use "ill-defined" components such as strong chelators, buffers, or reductants in the media that can affect metal activity (Poulson *et al.*, 1997; Sani *et al.*, 2001a, 2001b, 2003). Without chelators in the bacterial growth media, a threshold Zn concentration of 13 mg L^{-1} was found toxic to *Desulfovibrio desulfuricans* (Poulson *et al.*, 1997). In the presence of these compounds, however, toxic concentrations of Zn ranging from 13 mg L^{-1} to 40 mg L^{-1} were reported (Hao *et al.*, 1994; Poulson *et al.*, 1997; Utgikar *et al.*, 2001). To more accurately assess the toxicity of metals to SRB by eliminating abiotic metal precipitation and minimizing formation of metal complexes in solution, a new metal toxicity medium (MTM) was developed and tested (Sani *et al.*, 2001a). Metals can also cause synergetic or cumulative toxic effects as in the case of Ni and Zn (Poulson *et al.*, 1997) or Cu and Zn (Utgikar *et al.*, 2004). During laboratory studies, toxic effects of binary mixtures of Cu and Zn were substantially higher than expected on the basis of additive individual metal toxicity (Utgikar *et al.*, 2004). Therefore, successful operation of a passive bioreactor for treatment of a heavily contaminated AMD might require metal concentrations below inhibitory/ toxic levels in order to maintain a maximum rate of sulphidogenesis.

Exposure to O_2 can inhibit SRB metabolism, although the inhibition is reversible (Nagpal *et al.*, 2000b). The presence of enzymes responsible for O_2 tolerance detected in some SRB can explain their tolerance to low levels of oxygen. In any case, pH was found more critical to bioreactor efficiency than dissolved oxygen (Willow and Cohen, 2003). Inhibition potential differs among sulfur compounds with toxicity increasing in the following order: sulfate < thiosulfate < sulfite < total sulfide < H_2S (Al-Ani, 1994). Hydrogen sulfide was reported to have a direct and reversible toxic effect on SRB (Reis *et al.*, 1992). Toxic effects were reported for H_2S concentrations varying from 477 to

617 mg L^{-1} (Okabe *et al.*, 1992; Reis *et al.*, 1992; Al-Ani, 1994; Kolmert *et al.*, 1997). These H_2S concentrations are, however, much higher than those observed in passive field bioreactors. The inhibition is caused by undissociated H_2S that easily permeates the cell membrane, and by the removal of nutrients as metal sulfides (Reis *et al.*, 1992; Nagpal *et al.*, 2000b; Hulshoff Pol *et al.*, 2001). Furthermore, sulfide toxicity is metal-concentration related because most metals react with sulfide to give insoluble metal sulfides (Hulshoff Pol *et al.*, 2001). According to Utgikar *et al.* (2002), metal sulfides can inhibit SRB activity by deposition on bacterial cells. Sulfide toxicity is also related to pH, temperature, and organic carbon source. Low pH and low temperature conditions entail higher toxicity because they favor non ionized hydrogen sulfide formation (Hulshoff Pol *et al.*, 2001). Undissociated acetic acid also entailed inhibitory effects to SRB, when hydrogen sulfide was continuously removed from the system. At lower pH (5.8), acetic acid exerted inhibitory effects, whereas at higher pH (6.6), hydrogen sulfide prevailed as the inhibitor of concern (Reis *et al.*, 1992).

Chemical characterization of the organic matter source can provide insights into potential inhibitory effects on SRB (Tassé and Germain, 2002). Phenolic compounds and plant-derived tannins can inhibit the activity of various enzymes, whereas compounds such as terpenoids, amino acids, or hydrophilic base extracted fractions may exert toxic effects (Chang *et al.*, 2000; Tassé and Germain, 2002; Marschner and Kalbitz, 2003). The exact mechanisms responsible for the observed effects have not been made clear yet.

1.5.5 Ecotoxicity assessment of treated effluent

To our knowledge, Song *et al.* (2001) is the only study that performed whole effluent toxicity assays on undiluted wetland effluent. In this study, a laboratory-scale wetland was used to treat slightly alkaline (8.0 to 8.5) synthetic lead mine drainage and synthetic lead smelter wastewater. A significant toxicity decrease in wetland effluent for all organisms studied and 100% survival of fathead minnows and *Daphnia magna* was observed. However, lethality of *Ceriodaphnia dubia* was 100% in an undiluted effluent.

Dilution of effluent to half strength increased survival to 75 to 100%. Wetlands thus offer encouraging promise for decreasing the toxicity of lead-contaminated wastewater.

1.5.6 Configuration of passive bioreactors

Vertical flow bioreactors have been used in numerous laboratory and field studies (Dvorak *et al.*, 1992; Cheong *et al.*, 1998; Elliott *et al.*, 1998; Drury, 1999; Tsukamoto and Miller, 1999; Chang *et al.*, 2000; Willow and Cohen, 2003; Tsukamoto *et al.*, 2004; Johnson and Hallberg, 2005b; Kuyucak *et al.*, 2006). In downward flow mode bioreactors, the influent is fed through the top, while in the upward flow mode it is fed through the reactor bottom (URS Report, 2003). Recently, flow in a horizontal plane was reported in a field study (Zaluski *et al.*, 2003). A three-step system separating SRB activity from metal precipitation units and from a pH control system was also proposed at the laboratory scale (Prasad *et al.*, 1999). The flow pattern can affect both the transport of metals and their interaction with the substrate (Song, 2003). Bioreactors with vertical flow may show preferential channels of influent AMD percolating through the reactive mixture. The upward flow bioreactors tend to last longer because upward flow limits compaction and preferential flow paths (URS Report, 2003). However, release of metals by treated effluent is a potential problem. A horizontally oriented bioreactor using a mixture of cow manure and cut straw did not show preferential flow patterns during a 32-mo field operation period (Zaluski *et al.*, 2003). This configuration seems more promising, whereas the three-step process requires higher maintenance costs.

1.6 Performance of passive bioreactors

1.6.1 Neutralization and alkalinity generation

Mine water acidity arises from low pH and from dissolved metals (Fe, Mn, and Al), which undergo hydrolysis reactions producing H^+ (Gazea *et al.*, 1996). AMD pH must generally be corrected before or during biological treatment. Limestone was

successfully used for increasing influent pH either in anoxic drains before alimentation into the bioreactor (Johnson and Hallberg, 2005b) or integrated into the composition of reactive mixture (Reisinger *et al.*, 2000; Cocos *et al.*, 2002; Reisman *et al.*, 2003; Zagury *et al.*, 2006; Kuyucak *et al.*, 2006). During biotreatment, acidity removal and effluent alkalinity are due to bicarbonate produced by bacterial sulfate reduction (Dvorak *et al.*, 1992). Furthermore, generation of ammonia can contribute to an increase in alkalinity (Béchard *et al.*, 1994).

1.6.2 Sulfate removal

Acid mine drainage frequently contains sulfate levels from 100 to 5 000 mg L^{-1} (Kolmert and Johnson, 2001). While several mechanisms are responsible for metal removal, sulfate reduction determines sulfate removal and is the best indicator of SRB activity (Johnson and Hallberg, 2005b). Until recently, sulfate reduction was considered as a single mechanism of sulfate removal (Lyew and Sheppard, 1997). However, at early times of treatment, loss of sulfate due to adsorption onto or coprecipitation with ferric(oxy)hydroxides may occur (Christensen *et al.*, 1996; Waybrant *et al.*, 1998; Zagury *et al.*, 2006). A distinction must be made between the factors affecting the amount of sulfate removed and the rates of its removal by sulfate-reduction. While the first is related to the available surface area and HRT, the second is dependent on the initial concentration of sulfate in AMD (Lyew and Sheppard, 1999; Chang *et al.*, 2000).

Under optimum field conditions, sulfate reduction occurs at rates of about 0.3 mol $^{-1}$ m^{-3} d^{-1} (URS Report, 2003). Results reported in the literature indicate higher sulfate removal in batch experiments compared to column and field bioreactors (Table 1.1). Comparison of laboratory and field passive bioreactors in terms of the sulphate removal rate is, however, not feasible because of several factors of influence specific to each study.

First, the HRTs in batch experiments are far longer than in columns and field-bioreactors. Second, there is the variability of the initial concentrations, ranging from

229 mg L^{-1} (Zaluski *et al.*, 2003) to 4800 mg L^{-1} (Waybrant *et al.*, 1998), which influences SRB growth and sulfate reduction kinetics (Moosa *et al.*, 2002). Third, some studies provide the calculated percentages of sulfate removal without presenting the initial and final concentrations (Cheong *et al.*, 1998; Elliott *et al.*, 1998; Johnson and Hallberg, 2005b). For example, a sulfate removal efficiency of 42% from 900 mg L^{-1} SO_4^{2-} translates to a residual sulfate concentration about 500 mg L^{-1} (Tsukamoto *et al.*, 2004), whereas an efficiency higher than 82% from 2 315 mg L^{-1} SO_4^{2-} yields about 400 mg L^{-1} of sulfate in the treated effluent (Jong and Parry, 2003).

Moreover, some researchers report sulfate removal taking into account initial sulfate concentration and equivalents of organic carbon consumed (Tsukamoto and Miller, 1999; Tsukamoto *et al.*, 2004). Therefore, great care should be taken when comparing the efficiency of passive systems in terms of sulfate removal.

1.6.3 Metal removal

As discussed earlier, heavy metals can be removed from AMD via various mechanisms. Increasing the pH of acidic water effectively removes some metals due to precipitation under the form of hydroxides. Bacterial H_2S produced during sulfate reduction results in precipitation of other metals such as Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ag^{2+}, and Fe^{2+} as sulfides.

The pH is important because it influences both the solubility of hydroxides and carbonates and the kinetics of hydrolysis and precipitation processes.

Very high initial concentrations of metals in AMD fed to bioreactors (Table 1.2) may lead to a higher metal load of treated effluent (Zaluski *et al.*, 2003) than in a less contaminated water (Johnson and Hallberg, 2005b). Generally, the proportion of metals removed via sorption has not been clearly quantified. In a monitoring study on the performance of a passive field bioreactor over 32 mo, only Zn, Cu, and Cd were removed as sulfides at thresholds independent of the initial concentrations in AMD. Iron, Mn, Al, and Zn seemed to be removed following precipitation or co-precipitation as hydroxides (Zaluski *et al.*, 2003).

Metals such as manganese and arsenic are more challenging. Their removal as sulfides is less effective in passive bioreactors (Dvorak *et al.*, 1992; Wildeman and Updergraff, 1997; Cheong *et al.*, 1998; Chang *et al.*, 2000; Jong and Parry, 2003; Zaluski *et al.*, 2003). In the case of manganese, this is related to the relatively high solubility of MnS, which forms only when the Mn concentrations are very high compared with others metals (Cheong *et al.*, 1998). Furthermore, Mn is generally weakly sorbed (Willow and Cohen, 2003). According to Hallberg and Johnson (2005b), a pH > 8 is required to abiotically oxidize Mn (II) to insoluble Mn (IV) and to form insoluble hydroxides and carbonates. Similarly, Zagury *et al* (2006) reported a rapid removal of Mn as $MnCO_3$ (initial concentration of 14 mg L^{-1}, pH around 8) during batch experiments with poultry manure. Johnson and Younger (2005) reported a novel enhanced bioremediation system that consists of a passively aerated subsurface gravel bed. The provision of air and the use of catalytic substrates helped overcome the slow kinetics of manganese oxidation.

The optimum condition for Mn removal using a SRB bioreactor was thoroughly investigated by Yoo *et al.* (2004a, 2004b). Results showed that an excess of H_2S was required to remove Mn^{2+}.

In the case of arsenic, the exact process responsible for its initial removal is not clear but adsorption or concomitant coprecipitation with other metal sulfides or with ferrihydrite has been suggested (Jong and Parry, 2003; Zaluski *et al.*, 2003). Formation of insoluble arsenic sulfide may occur later when reducing conditions are established. Bioreactors that efficiently removed arsenic along with other divalent metals (Fe, Ni) for a period of almost 2 yr were reported in a pilot-scale bioreactor (Tsukamoto and Miller, 1999). Proportions of As(III) and As(V) species in the AMD were suggested as a critical factor affecting the rate of arsenic reduction in different environments (Jong and Parry, 2003).

1.6.4 Long-term performance of pilot-scale and full-scale systems

Theoretically, AMD-contaminated water can be successfully treated using simple anaerobic passive bioreactors for many yr. However, between 3 and 4 yr is the maximum reported operating period (URS Report, 2003). In practice, several problems related to flow patterns, plugging, compacting, overloading and exhausting of carbon available to SRB occurred during the treatment. These problems affected both the longevity and the performance of bioreactors and ultimately resulted in their failure.

Treatment in a passive bioreactor filled with a mixture of rice stalk, cow manure and limestone succeeded for only 56 d in removing metals and reducing acidity (Cheong *et al.*, 1998). After 118 d of operation, the substrate thickness had compacted by 15 cm and the pipes for the conveyance of AMD were clogged by brownish iron hydroxide. Another pilot-scale anaerobic bioreactor utilizing a mixture of horse manure and sand as substrate removed < 10% of the influent sulfate and iron by the end of the second year. Its use was discontinued because of the exhaustion of available organic carbon (Tsukamoto and Miller, 1999).

In a full-scale study, two bioreactors using a mixture of sawdust and hay gave significantly greater iron concentrations in the effluent than in the source water, indicating net mobilization by reductive dissolution of colloidal and/or solid-phase ferric iron compounds (Johnson and Hallberg, 2005b). This phenomenon was attributed to the high proportion of recalcitrant organic materials used in the mixture. Clogging of bioreactors due to formation of sulfide precipitates and microbial biomass is an important operational problem, minimized by the use of a physical matrix with large pore spaces, a good hydraulic conductivity and the ability to flush out the precipitates (Lyew and Sheppard, 1997; Tsukamoto *et al.*, 2004). Overloading is another common reason for passive treatment failure (Reisman *et al.*, 2003).

Table 1.2 Metal removal in some passive bioreactors reported in the literature

Fe		Mn		Cu		Cd		Ni		Zn		As		References
I	E	I	E	I	E	I	E	I	E	I	E	I	E	
..... mg L^{-1}														
50-70	<25													Johnson and Hallberg (2005b)
0.008-8.7	</>†	0.7-3.8	≈‡	0.003-3.1	<2	0.003-0.042	<0.005			1-11	<1	0.001-0.011	>§	Zaluski *et al.* (2003)
				2-23	<2									Reisinger *et al.* (2000)
53	8	26	0.5			0.3	0.003	0.9	0.02	317	0.3			Dvorak *et al.* (1992)
92.5	0.22							0.3	0.05	0.98	0.07			Glombitza (2001)
126	65	70	53	1.51	0.08	0.15	0.02			11.4	1.88			Cheong *et al.* (1998)
10-20	<40	140-225	<200	3.5-7	<0.5	1.8-3.2	<0.2			500	<200	0.5-2.2	<1.2	Reisman *et al.* (2003)
10-50	<1	12-17	<5	20	<0.1	1	<0.01			90	<1	0.09-0.11	<0.03	Drury (1999)
		50	<33.1	8	0	0.5	<0.001			100	<0.9			Willow and Cohen (2003)
200	<200													Béchard *et al.* (1994)
5.1-50.8	0.9-9.1			5.1-50.3	<1.1			5.1-49.9	<1.2	5.1-50.4	<1.1	5.1-50.6	<11.3	Jong and Parry (2003)
300	<70			1.37	<1					15.3	<2			Beaulieu *et al.* (2000)
100	7													Tsukamoto *et al.* (2004)
500	<200			50	<5					100	<1			Chang *et al.* (2000)
26.3-29.0	<31.2	1.62-1.89	<21.6	4.52-9.88	<1.7					8.62-10.8	<3.54			Christensen *et al.* (1996)
1683	<125	14	<0.4			8.3	<0.1	15	<1.2	15	<1			Zagury *et al.* (2006)
400	<5													Tassé and Germain (2002)
1080	<10					135	0.01	480	0.03	0.81	0			Waybrant *et al.* (1998)
800	NR	47	NR	0.08	NR			0.8	<0.4	7.3	<2			Cocos *et al.* (2002)

† Fe concentrations in effluent were lower than those in influent during the initial 8 mo of operation and were higher for the remaining 28 mo.

‡ Mn concentrations did not change during the treatment in bioreactors.

§ As concentrations in effluent were higher than those in influent for most of the operating time.

I, influent; E, effluent; NR, not reported.

1.7 Conclusion and research needs

1.7.1 What we know

Passive on-site bioreactors offer promising perspectives for treatment of AMD-contaminated waters due to the relative simplicity of the system and low operating costs. These technologies are well suited for remediation of abandoned, remote mine sites or for sites located in cold areas. The efficiencies of passive bioreactors depend on the activity of SRB, which is mainly controlled by the composition of the reactive mixture.

The most important component is the organic carbon source. Many studies have attempted to predict the biodegradability of complex organic substrates by using chemical extractions; however, they have not been successful. Higher sulfate reduction rates have been reported with reactive mixtures containing more than one organic carbon source. Even though formation of metal sulphides is the preferred metal removal process, many metal removal mechanisms including adsorption and precipitation of metal carbonates and hydroxides occur in passive bioreactors. Furthermore, these mechanisms change during the life span of a passive bioreactor. High concentrations of dissolved metals and sulfides can be toxic to SRB. However, passive bioreactors can still operate at temperatures as low as 2 to 6^{o} C and pH 3.0. Furthermore, SRB can tolerate low levels of oxygen.

In pilot and field studies, several problems related to flow patterns, plugging, compacting, overloading, and exhausting of carbon available to SRB have been reported. In addition to reactive mixture composition and presence of SRB, reactor configuration, Eh, hydraulic retention time, and COD/sulfate ratios are critical factors for a long-term operation of passive sulfate-reducing bioreactors.

1.7.2 Research needs

Different aspects need to be further investigated for better design and operation of on-site passive treatment systems.

The depletion rate of organic matter is a key problem. An improved methodical analysis of natural organic substrates is warranted to assess their ability to promote sulfate reduction and metal removal. Anaerobic degradation of complex organic carbon compounds to simpler molecules by other microflora may limit the rate at which substrates become available to SRB. More work must be conducted to understand and differentiate the fundamental biochemical and microbiological reactions that occur in anaerobic bioreactors with complex natural organic substrates. Bioreactors are recommended to be allowed to "mature" before fed with AMD, especially when recalcitrant materials are included in the substrate to provide long-term provision of organic carbon. After maturation, however, the amount of colloids and DOM in pore water and within the effluent should be assessed. Metals bound to DOM and colloids are highly mobile and can flow out of the treatment system.

Additional work to accurately assess the various metal removal mechanisms occurring in passive bioreactors is strongly recommended. Geochemical modeling, solid phase speciation analysis, and mineralogical and microbial characterization should be performed to assess the various metal removal mechanisms. The inclusion of natural organic matter in geochemical equilibrium models and metal speciation analysis should be further studied. This knowledge should lead to a more efficient long-term operation of passive bioreactors.

Limited work has been done on the direct assessment of the ecotoxicological potential of biologically treated AMD waters. Characteristics of natural organic materials, high concentrations of dissolved organic carbon, low redox potential, high concentrations of dissolved sulfides, and enhanced metal availability are among the parameters that might influence effluent toxicity. Correlation of metal speciation in the treated effluent and in the reactive mixture with toxic effects of treated waters could help improve our understanding of passive bioreactor systems.

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CHAPITRE II

ARTICLE #2: BIOLOGICAL TREATMENT OF HIGHLY CONTAMINATED ACID MINE DRAINAGE IN BATCH REACTORS: LONG-TERM TREATMENT AND REACTIVE MIXTURE CHARACTERIZATION

2.1 Abstract

Passive bioreactors involving sulphate-reducing bacteria (SRB) are a practical alternative technology to treat acid mine drainage (AMD). Careful selection of the organic carbon source is important to ensure performance and long-term efficiency of the treatment. However, a rigorous and methodical characterization to predict the biodegradability of organic substrates by SRB still needs to be investigated. In the present study, four natural organic materials were thoroughly characterized to assess their ability to serve as substrates and to find a parameter that links organic carbon sources with their biodegradability. Three reactive mixtures were then comparatively evaluated for their performance to treat a highly contaminated AMD in long-term (152 days) batch experiments. All three mixtures were successful for sulphate reduction and metal (Fe, Ni, Cd, Zn, and Mn) removal (91.8-99.8%). Higher efficiencies were observed in the reactors with 30% (w/w) cellulosic wastes (maple wood chips and sawdust) which decreased sulphate concentrations from 5500 mg/L to < 1mg/L, than in reactors with 2-3% cellulosic wastes, where final sulphate concentrations were in the range 2000-2750 mg/L. Organic material characterization indicated that higher C/N ratios, COD (Chemical Oxygen Demand)/SO_4^{2-} ratios and DOC (Dissolved Organic Carbon)/SO_4^{2-} ratios were associated with better sulphate-reducing conditions and metal removal. This work suggests that C/N and DOC/SO_4^{2-} ratios considered together are key parameters to assess the biodegradability of natural organic wastes under sulphate-reducing conditions.

Keywords: Acid mine drainage; Sulphate-reducing bacteria; Batch reactors; Natural organic carbon source; DOC (Dissolved Organic Carbon)/SO_4^{2-} ratio.

2.2 Introduction

Prolonged exposure of reactive sulphide minerals (e.g. pyrite, pyrrhotite) to oxygen and water, in the absence of sufficient neutralizing minerals, generates acid mine drainage (AMD). AMD is characterized by low pH and high concentrations of sulphates and heavy metals that represent a potential hazard for the environment. Therefore, AMD contaminated waters must be collected and treated before being discharged into the environment (Neculita *et al.*, 2007).

Over the past 20 years, passive bioreactors were successfully used for the treatment of AMD in pilot and field-scale projects on remote sites (Dvorak *et al.*, 1992; Gusek *et al.*, 1999; Reisinger *et al.*, 2000; Reisman *et al.*, 2003; Kuyucak *et al.*, 2006). They rely on sulphate-reducing bacteria (SRB), which are anaerobic microorganisms capable of increasing the pH and alkalinity of water, and of immobilizing dissolved metals by precipitating them as metal sulphides, in the presence of a biodegradable organic carbon source. Under these conditions, organic carbon oxidation is coupled with sulphate reduction:

(1) $2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$

where CH_2O represents a short-chain organic carbon molecule available to SRB.

Soluble sulphides generated in reaction (1) react with metals (Me^{2+}) to form biogenic metal sulphides (MeS), reversing reactions that occurred to produce contaminated waters (Reisinger *et al.*, 2000):

(2) $H_2S + Me^{2+} \rightarrow MeS + 2H^+$

Passive bioreactors utilize a simple passive to semi-passive, flow-through design. AMD is fed horizontally or vertically over a solid reactive mixture into a pond or a tank and is

released treated, with higher alkalinity and pH and lower concentrations of heavy metals and sulphates (URS Report, 2003).

Reactive mixture composition is crucial for the efficiency of the treatment process (Cocos *et al.*, 2002). Efficient reactive mixtures generally contain an organic carbon source, a bacterial source or SRB inoculum, a solid porous medium, a nitrogen source and a neutralizing agent (e.g. limestone) (Cocos *et al.*, 2002; Waybrant *et al*, 1998, 2002; Zagury *et al.*, 2006).

Recently, consultants such as Golder Associates Ltd have installed efficient full-scale passive bioreactors containing mixtures of natural organic materials at several former mine sites (e.g. Ferris-Haggarty Mine, Wyoming, USA, and Cadillac Molybdenite, Northern Québec, Canada) (Kuyucak *et al.*, 2006).

However, efficiencies obtained in laboratory bioreactors are better than in pilot or full-scale bioreactors, none of which have remained operational without significant overhaul or modification for more than three to four years (URS Report, 2003). Assessment of organic material biodegradability in short-term batch experiments can therefore lead to overestimating their capacity to sustain SRB activity and remove metals during long-term operation.

Furthermore, for field implementation, selection of locally available organic carbon sources is preferred because they are less expensive than commercialized organic carbon sources such as hydrogen release compounds (Regenesis) or the use of molasses (ARCADIS). Additionally, it was proved that mixtures of several natural organic materials, both organic wastes (animal manure, compost) and cellulosic wastes (wood chips, sawdust) perform better than a single source (Cocos *et al.*, 2002; Waybrant *et al.*, 1998, 2002; Zagury *et al.*, 2006). Careful selection of a suitable carbon source is of great importance to ensure performance and longevity in biological AMD treatment (Zagury *et al.*, 2006). However, a rigorous and methodical test to predict the

biodegradability of organic substrates by SRB is still warranted and needs to be investigated (Gibert *et al.*, 2004; Zagury *et al.*, 2006).

Over the past decade, a few studies have attempted to link physicochemical composition of natural organic materials with their ability to promote sulphate reduction and metal removal (Prasad *et al.*, 1999; Chang *et al.*, 2000; Cocos *et al.*, 2002; Waybrant *et al.*, 1998, 2002; Gibert *et al.*, 2004; Zagury *et al.*, 2006). Results confirmed that the higher the content of lignin and cellulose in the organic substrate, the lower is its biodegradability and its capacity for developing and sustaining bacterial activity (Chang *et al.*, 2000; Waybrant *et al.*, 1998, 2002; Gibert *et al.*, 2004). Nevertheless, the recent study of Zagury *et al.* (2006), who assessed the biodegradability of a natural organic substrate mixture versus single substrates, reports a very low efficiency in a bioreactor containing poultry manure as a single organic carbon source, despite its highest DOC and easily available substances (EAS) content. On the other hand, poultry manure was very efficient for sulphate reduction and metal removal when used in a mixture with leaf compost and maple wood chips. Consequently, substrate characterization based solely on EAS and DOC, on an individual basis, does not give a clear indication of its ability to promote sulphate reduction and metal removal (Zagury *et al.*, 2006).

Further, few studies have attempted to link organic material C/N ratios with their efficiency for AMD biological treatment (Prasad *et al.*, 1999; Zagury *et al.*, 2006). A C/N ratio around 10 is generally considered suitable for biological degradation of complex organic substrates (Reinertsen *et al.*, 1984; Béchard *et al.*, 1994). Nevertheless, the C/N ratio taken alone was not a good indicator of the sulphate-reducing ability of a given mixture (Zagury *et al.*, 2006). Moreover, when lactate is used as substrate, the optimal reported C/N ratios are higher and vary greatly from 15.7 to < 45 or from 45 to 120 (Gerhardt *et al.*, 1981; Okabe *et al.*, 1992).

Finally, other studies have attempted to link COD/SO_4^{2-} ratios with the suitability of a natural organic material to act as a substrate during biological treatment of AMD contaminated waters (Al-Ani, 1994; Greben *et al.*, 2000; Henry and Prasad, 2000;

Greben and Maree, 2005). As in the case of C/N ratios, if the COD/SO_4^{2-} ratio is taken alone, data interpretation must consider the complexity of the organic carbon in the substrate used. The theoretical COD/SO_4^{2-} ratio for SRB is 0.67, while typical observed values range between 0.7 and 1.5, depending on the type of carbon source (Hao *et al.*, 1996). Thus, when ethanol is used as the substrate, the optimal COD/SO_4^{2-} ratio is 0.55-0.84 (Greben *et al.*, 2000; Greben and Maree, 2005), whereas when organic wastes (e.g. municipal compost, activated sludge) are used as substrate, not all of the carbon present is used by the SRB and the optimal COD/SO_4^{2-} ratio has been reported to be 1.6 (Henry and Prasad, 2000) or 5 (Al-Ani, 1994).

In fact, COD does not directly reflect organic carbon availability to anaerobic bacteria because it is determined in aerobic conditions. Additionally, it does not quantify complex dissolved organic carbon because the standard analysis is performed at 150°C. Nevertheless, C/N ratios and COD/SO_4^{2-} ratios, eventually coupled with other parameters, are still promising indicators of an organic material's capacity to perform as a substrate in biofilters for AMD passive treatment.

Consequently, the present study is divided in two parts. In the first part, four organic waste materials (maple wood chips, maple sawdust, composted poultry manure, and leaf compost) were thoroughly characterized in terms of biodegradability and ability to serve as organic carbon sources for SRB during AMD treatment in passive bioreactors. The objective was to find the key parameter that links natural organic material composition with its biodegradability. In the second part, two reactive mixtures previously reported as efficient in short-term batch bioreactors (41-71days), and a third mixture developed for the purpose of this study, were comparatively assessed for their performance to treat a highly contaminated AMD in longer term batch bioreactors (120-152 days). The objective was to select the most efficient reactive mixture in order to test it further in column bioreactors.

2.3 Materials and methods

2.3.1 Physicochemical and microbiological characterisation of solid organic materials

In the first part of the study, maple wood chips and sawdust (P.W.I. Industries, Canada), composted poultry manure (Fertilo de Fafard, Canada), and leaf compost (city of Montreal) were thoroughly characterized. These four organic materials as well as creek sediment (Cupra mine site, QC), which was used as a source of acclimated SRB, were refrigerated at 4°C prior to their analysis. All analyses were performed in triplicate, with wet samples. The reported results were corrected for moisture content.

Solid organic materials were characterized for *physical parameters* (pH and water content), *elemental analysis* (total C, N as TKN (total Kjeldahl nitrogen), and total P), and *biodegradation parameters* (total volatile solids (TVS), total organic carbon (TOC), waxes and resins, easily available substances (EAS), hot water soluble substances, hemicellulose, cellulose, and lignin content). Water extracts (1:10 solid: liquid ratio) were analyzed for total organic carbon (TOC), dissolved organic carbon (DOC), and chemical oxygen demand (COD).

Enumeration of heterotrophic anaerobic fermentative bacteria was performed in the solid organic materials, while sulphate reducing bacteria (SRB) counts were carried out in both the organic materials and the creek sediment.

The characterisation is presented in Table 2.1. The pH was determined in deionized water using a solid to liquid ratio of 1:10 according to Method D 4972-95a (American Society for Testing and Materials [ASTM], 1995) using a portable pH/mV/temperature meter (HACH, model sensION1) with a gel-filled pH electrode and a combination Ag/AgCl redox potential electrode (HACH, Hampton, NH). Water content was determined at 105°C according to Method D 2216-92 (ASTM, 1995). Volatile solids were determined at 550°C according to Karam (1993).

Total carbon was measured by combustion with an induction furnace (LECO Corporation, 1975). Total kjeldahl nitrogen (TKN) and total P were determined by Standard Methods 4500-N_{org} and 4500-P, respectively (American Public Health Association [APHA], 1998).

A phosphoric acid treatment followed by an infrared determination of CO_2 evolved was performed to determine total inorganic carbon (Ministère de l'Environnement et de la Faune du Québec, 1996). Organic carbon was calculated by the difference between total carbon and total inorganic carbon. Total organic carbon (TOC) and dissolved organic carbon (DOC) in leachate were analyzed after mixing (shaking for 2 h at room temperature with a customized rotary agitator) 20 g (wet weight) of each organic substrate with 200 ml of deionised water (18.2 Mohms). The extracts were then centrifuged (13800 x *g*) for 10 min and analyzed for TOC or filtered (0.45 µm) and then analyzed for DOC. TOC of non-filtered/filtered extract was determined at 680°C, after acidification of samples with H_3PO_4, according to Standard Method 5310 B (APHA, 1998) using a TOC analyzer (DOHRMAN, model DC-190). COD analysis was performed using the dichromate reflux method with a COD reactor (HACH Procedure Manual, 1998) and a spectrophotometer (HACH model DR/2010).

Easily available substances (EAS) and waxes and resins were analyzed by a modified forage fibre analysis (FFA), as per Zagury *et al.* (2006). Hot water soluble substances, hemicellulose, cellulose, and lignin content were determined according to Harper and Lynch (1981).

Enumeration of heterotrophic anaerobic fermentative bacteria and of SRB in organic materials and the creek sediment was performed using the Most Probable Number technique as per Standard Methods (1998) and ASTM (1990), respectively.

Table 2.1 Physicochemical and microbiological characteristics of natural organic materials used in batch reactors

	Cellulosic wastes		Organic wastes	
	Maple wood chips	Maple sawdust	Composted poultry manure	Leaf compost
Physical parameters				
pH	5.75±0.10	5.32±0.01	7.91±0.03	9.32±0.42
Water content (% w/w)	6.5±0.1	5.2±0.0	67.2±1.6	37.4±1.3
Elemental analysis (% w/w dry weight)				
C	47.7	47.9	28.2	15.6
N (TKN)	5.1×10^{-3}	ND	1.3	0.7
P	6.3×10^{-4}	4.3×10^{-4}	1.2	0.1
Biodegradation parameters of solid materials (% w/w dry weight)				
Total volatile solids	99.7±0.4	100.0±0.0	71.4±1.3	25.8±0.9
Total organic carbon (TOC)	44.0	45.3	25.7	1.3
Waxes, resins	1.1±0.5	5.0±0.3	3.5±0.8	7.1±2.4
Easily available substances (EAS)	31.3±0.5	31.9±0.6	25.6±1.2	40.7±15.7
C/N ratio	8627	-	20	2
Hot water soluble substances	4.8±0.8	7.4±0.3	11.9±1.0	7.0±0.7
Hemicellulose	24.8±1.6	37.2±1.5	21.5±2.7	10.3±3.5
Cellulose	64.9±2.3	48.4±0.2	25.3±0.8	4.8±0.7
Lignin	2.7±0.0	6.0±1.0	19.5±2.6	41.4±2.3
Ash	2.8±0.1	1.0±0.0	21.8±1.9	36.4±4.4
Water extracts (1:10 solid: liquid ratio) analysis (mg/L)				
Total organic carbon (TOC)	367	892	198	66
Dissolved organic carbon (DOC)	305	692	139	53
Chemical oxygen demand (COD)	842±53	1798±22	182±25	83±5
Microbial counts in solid materials (cells/100mL)				
Heterotrophic anaerobic fermentative bacteria	5.0×10^{5}	3.3×10^{4}	$> 1.6 \times 10^{7}$	$> 1.6 \times 10^{7}$
Sulphate reducing bacteria (SRB)	< 2	< 2	5.0×10^{4}	5.0×10^{4}

2.3.2 Batch experiment description

The capacity of maple wood chips, maple sawdust, composted poultry manure, and leaf compost to promote sulphate reduction and metal removal was assessed during a 150-day batch experiment. The study was performed with three reactive mixtures, in duplicate, in 2L glass reaction flasks, at room temperature (22±1°C). The mixture proportions (%w/w, dry weight) are given in Table 2.2.

Mixture #1 and Mixture #2 contain three organic carbon sources (maple wood chips, composted poultry manure, and leaf compost) and have been previously tested in short-term batch experiments (Cocos *et al.*, 2002; Zagury *et al.*, 2006). Mixture #3 contains maple sawdust as a fourth organic carbon source. Mixture #1 was added in reactor R1, mixture #2 in reactor R2, and mixture #3 in reactor R3. Synthetic AMD was then added to the six reactors (in duplicates for each reactive mixture). The reactors contained 250g or 300g (dry weight) of reactive mixture, for a final solid: liquid ratio of 1:3 in R1 and of 1:4 in R2 and R3. Reactors were then sealed and thoroughly shaken. Synthetic AMD preparation has been described in detail in the study of Zagury *et al.* (2006).

AMD characterization is presented in Table 2.3. Sampling was performed every 8 to 16 days for a total period of 120 days (R3) or 152 days (R1 and R2). Batch reactors contained sampling ports fitted with Teflon-lined septa. All sampling was performed under anaerobic conditions ($N_{2(g)}$ high purity atmosphere) in a glove bag. SRB counts and analysis of pH, oxidation reduction potential (ORP), alkalinity, TOC, DOC, COD, sulphate, Fe^{2+}, total sulphides, and metals (Fe, Mn, Cd, Ni, Zn) were also carried out.

Measurements of pH and the redox potential (HACH electrode, Ag/AgCl) were determined directly in the sampling solution (within the glove box) immediately after collection. Except for TOC and SRB counts, all other parameters were determined on separate 0.45 µm filtered samples. Sulphides, sulphate, and ferrous iron were determined during the first 1-2 hours after collection, using a spectrophotometer (HACH, model DR/2010) and Standard Methods (APHA, 1998).

Table 2.2 Composition of three reactive mixtures assessed in batch reactors

Component	Mixture #1 (Cocos *et al.*, 2002)	Mixture #2 (Zagury *et al.*, 2006)	Mixture #3 (this study)
	.....% w/w dry weight		
Organic carbon sources			
Maple wood chips	3	2	10
Maple sawdust	0	0	20
Composted poultry manure	20	18	10
Leaf compost	30	30	20
Nitrogen source			
Urea	3	3	3
Bacterial source			
Creek sediment	37	15	15
Porous medium			
Sand	5	30	20
pH neutralizer			
Calcium carbonate	2	2	2

Alkalinity was analyzed using Standard Method 2320-B (APHA, 1998). TOC of non-filtered extract and of filtered extract (DOC) was determined after acidification of samples with H_3PO_4, according to Standard Method 5310 B (APHA, 1998) using a TOC analyzer (DOHRMAN, model DC-190). COD analysis was performed by the dichromate reflux method using a COD reactor (HACH Procedure Manual, 1998) and a spectrophotometer (HACH, model DR/2010). Metal concentrations were determined using an atomic absorption spectrometer (Perkin Elmer, model AAnalyst 200) (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) after sample acidification at pH 2 with concentrated HCl. Samples for SRB enumeration were taken every 16 to 32 days.

To avoid H_2S accumulation due to dropping of metal concentrations, a 10 mL spike of metals (24 g/L Fe, 0.6 g/L Cd, 0.7 g/L Mn, 0.9 g/L Ni, and 0.9 g/L Zn), was added at day 74. Metal concentrations in the spike were calculated in order to reach the initial concentrations in the reactors (Table 2.3).

Table 2.3 Composition of synthetic AMD added in batch reactors

Component	Concentration (mg/L)	Source
Ca^{2+}	487.8±10.5	$CaSO_4 \cdot 2H_2O$
Cd^{2+}	12.6±0.9	$CdSO_4 \cdot 8/3H_2O$
Fe^{2+}	1670±66	$FeSO_4 \cdot 7H_2O$
K^+	67.1±1.4	K_2SO_4
Mg^{2+}	98.9±5.0	$MgSO_4$
Mn^{2+}	13.5±1.2	$MnSO_4 \cdot H_2O$
Na^+	87.1±1.7	Na_2SO_4
Ni^{2+}	16.8±1.8	$NiSO_4 \cdot 6H_2O$
Zn^{2+}	18.9±1.1	$ZnSO_4 \cdot 7H_2O$
SO_4^{2-}	5500±250	-
pH	5.45-5.51	-

After the spike, the first sampling was performed on day 75 (the day after the spike) and then sampling was performed every 8-16 days until the end of the experiments.

All laboratory ware used during the analytical procedures was cleaned sequentially with a phosphate-free detergent, soaked in 10% (v/v) nitric acid for 24 h, then in distilled water, and finally rinsed three times with deionized water (18.2 Mohms). Unless otherwise stated, all reagents were of analytical grade (ACS) or better.

2.3.3 Geochemical modeling

Geochemical modeling using the thermodynamic chemical equilibrium model VMINTEQ version 2.51 was performed on supernatant samples collected on days 0, 8, 16, 24, 32, and 40 to help in the assessment of the metal removal mechanisms observed during the early phase of the batch experiment. For the mixtures where sulphate reduction was not evident, additional samples were collected later in the experiment. VMINTEQ calculates saturation indices of various mineral phases taking into account geochemical processes such as dissolution/precipitation, complexation, oxidation/reduction, ion exchange and gas equilibrium. However, this model does not take into account SRB activity and subsequent precipitation of metal sulphides.

2.4 Results and discussion

2.4.1 Physicochemical characterisation of natural organic materials

Table 2.1 presents the characterisation of the natural organic materials used in the batch experiment. For the purpose of discussion, natural organic materials are divided in two groups: celullosic wastes (maple wood chips and maple sawdust) and organic wastes (composted poultry manure and leaf compost). As indicated in Table 2.1, the pH of the cellulosic wastes was slightly acidic (5.32 - 5.75), whereas the organic wastes had an alkaline pH ranging from 7.91 to 9.32. Elemental analysis indicated that total carbon (TC) was lower in leaf compost (15.6%) and composted poultry manure (28.2%) than in cellulosic wastes (up to 47.9%). Similarly, TOC was lower in leaf compost (1.3%) and in composted poultry manure (25.7%), while higher values were measured in cellulosic wastes (up to 45.3%). However, organic wastes were characterized by a higher P content (0.1-1.2%) and N content (0.7-1.3%) than cellulosic wastes (10^{-3}-10^{-4}%). One may calculate a C/N ratio (expressed as TOC/TKN), which gives values of 2 in leaf compost, 20 in composted poultry manure, and > 9 x 10^3 in cellulosic wastes. A C/N ratio less than 10, together with the highest EAS content of leaf compost (40.7%) might indicate this organic substrate as the most available for SRB (Prasad *et al.*, 1999). Nevertheless, leaf compost alone was not successful when used as single substrate for AMD treatment in batch and column bioreactors (Gibert *et al.*, 2003; 2004; Zagury *et al.*, 2006). One explanation could be related to a lower TC of leaf compost compared to other organic wastes (e.g. animal manure, municipal compost). Additionally, in leaf compost, high percentages of TC can be in the form of carbonates and bicarbonates (TIC). Leaf compost used in the present study contained 15.6% TC, from which 1.3% was TOC and 14.3% was TIC. Such a high percentage of TIC can interfere during an EAS analysis and give misleading results about the capacity of leaf compost to act as a good substrate for bacterial activity.

Hot water soluble substances showed little variation from 4.8% in maple wood chips to 11.9% in composted poultry manure. As expected, higher contents of hemicellulose (up to 37.2%) and cellulose (up to 64.9%) were found in cellulosic wastes.

Water extract analysis indicated that a higher content of TOC and of DOC characterized cellulosic wastes compared to organic wastes. The lowest (53 mg/L) and the highest (692 mg/L) DOC contents were found in leaf compost and maple sawdust, respectively.

Organic carbon from sawdust is not easily available for SRB and long acclimatization periods can be required for passive bioreactors filled with the sawdust as the sole organic carbon source before becoming efficient for AMD treatment (Johnson and Hallberg, 2005). However, after an acclimatization period, better results in terms of metal and sulphate removal were reported with sawdust alone (Johnson and Hallberg, 2005; Tuttle *et al.*, 1969) than with compost alone (Gibert *et al.*, 2003, 2004; Zagury *et al.*, 2006). Therefore, sawdust can be a good source of organic carbon for SRB during long-term operation of passive bioreactors.

2.4.2 Microbial enumeration

Bacterial counts (Table 2.1) showed a high density of heterotrophic anaerobic fermentative bacteria in all solid organic materials: 10^4-10^5 cells/100mL in celullosic wastes to 10^7 cells/100mL in organic wastes. Counts also showed the presence of SRB in organic wastes (10^4 cells/100mL), which confirmed previous assumptions about their presence in natural organic wastes (Zagury *et al.*, 2006). However, SRB were not detected in maple wastes (< 2 cells/100mL). Creek sediment, which is generally used as a potential bacterial inoculum, showed a fair count of SRB (10^2 cells/100mL), which is lower compared with the sediment used by Cocos *et al.* (2002) (3.0×10^4 cells/100mL) but higher compared to the sediment used by Zagury *et al.* (2006) (25 cells/100mL).

2.4.3 Batch experiments

2.4.3.1 Long-term efficacy

There was little difference between the duplicate reactors throughout the experiment (Figures 2.1 to 2.3): results are therefore presented as the average between bioreactors that contained the same reactive mixture. Generally, the graphs indicate two patterns in physicochemical quality of treated water. First, a lag period of about 80 days was observed before the occurrence of sulphate reduction and the increase in SRB counts for all mixtures. This could be related to higher initial ORP values compared to previously published studies (Waybrant *et al.*, 1998; Cocos *et al.*, 2002; Zagury *et al.*, 2006) that retarded the start of sulphate-reduction. Second, the evolution of principal water quality parameters indicated three phases of AMD treatment: one between day 0 and day 32, a second phase between day 32 and day 75, and the last phase between day 75 and the end of the experiments (day 120-152).

First phase (0-32 days)

Important changes in water quality were recorded in this phase. The pH increased sharply from 5.5 to between 8 and 9, as well as alkalinity (results not shown), which rose from 6-40 mg/L $CaCO_3$ to around 2500 mg/L $CaCO_3$. ORP decreased from 33-50 mV to values as low as -177 mV. TOC and DOC decreased from around 2000 mg/L to about 500 mg/L in R1 and R2, whereas values around 1000 mg/L were recorded until day 56 in R3. Sulphate concentrations varied around 5000 mg/L, whereas metal concentrations dropped in all bioreactors. On day 32, metal removal varied from 98.5% to 99.9% (Cd, Fe, Mn, and Zn) and from 94.7% to 98.4% (Ni). As modeling results showed, early metal removal might be explained by (oxy)hydroxide and carbonate mineral precipitation. Saturation indices calculated with VMINTEQ using water chemistry from day 0, day 8, and day 16 indicated that metal removal could be attributed to the precipitation of (oxy)hydroxide minerals such as ferryhydrite, goethite, K-jarosite, Na-jarosite, lepidocrocite, maghemite, magnetite, as well as siderite

($FeCO_3$). Between day 16 and day 40, precipitation of carbonate minerals such as calcite ($CaCO_3$), magnesite ($MgCO_3$), rhodocrosite ($MnCO_3$), otavite ($CdCO_3$), smithsonite ($ZnCO_3$), and $NiCO_3$ was also indicated. These results are in agreement with other batch experiment studies (Waybrant *et al.*, 1998; Zagury *et al.*, 2006).

Second phase (32-75 days)

Water quality was less variable during this phase. Alkalinity increased steadily and reached the highest values on day 75 (12-13 g/L $CaCO_3$). The ORP dropped to values around -300 mV on day 75, while sulphate concentrations increased to initial concentrations (5500 mg/L) or more. Before the spike on day 74, metal concentrations remained stable. However, after the spike, metal concentrations decreased rapidly and remained low until the end.

Third phase (75-152 days)

From day 75 until the end of the experiment, all parameters showed very little variation, except for sulphate, which drastically decreased from about 5500 mg/L to < 1mg/L in R3 (day 120), and to values in the range 2250-2750 mg/L in R1 and 2000-2375 mg/L in R2 (day 152). A sharp decrease in sulphate concentrations such as observed in R3 have already been reported in other batch experiment studies, where concentrations up to 5000 mg/L SO_4^{2-} decreased to < 163mg/L in only 35 days or less (Waybrant *et al.*, 1998; Zagury *et al.*, 2006). In addition, sulphide concentrations up to 2.7 mg/L were measured.

The results of sulphate reduction were well correlated with SRB counts, which grew progressively during the experiment. Initial (day 0) SRB counts in the reactors were lower than 2 cells/100 mL in R1, 80 cells/100mL in R2, and 230 cells/100 mL in R3.

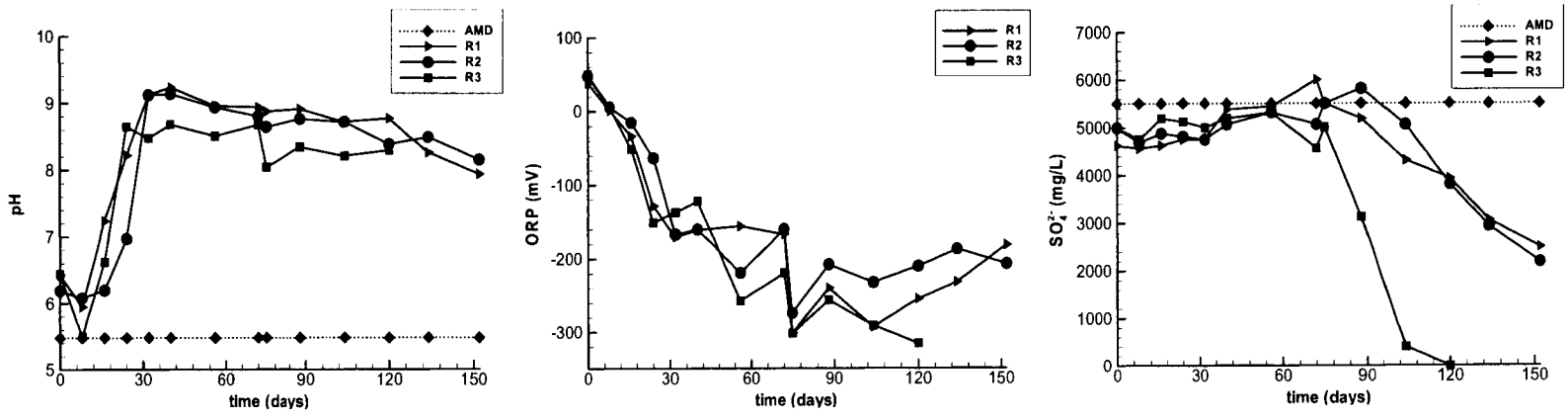

Figure 2.1 Variation of pH, ORP and sulphate in batch reactors containing three different reactive mixtures (R1, R2, and R3)

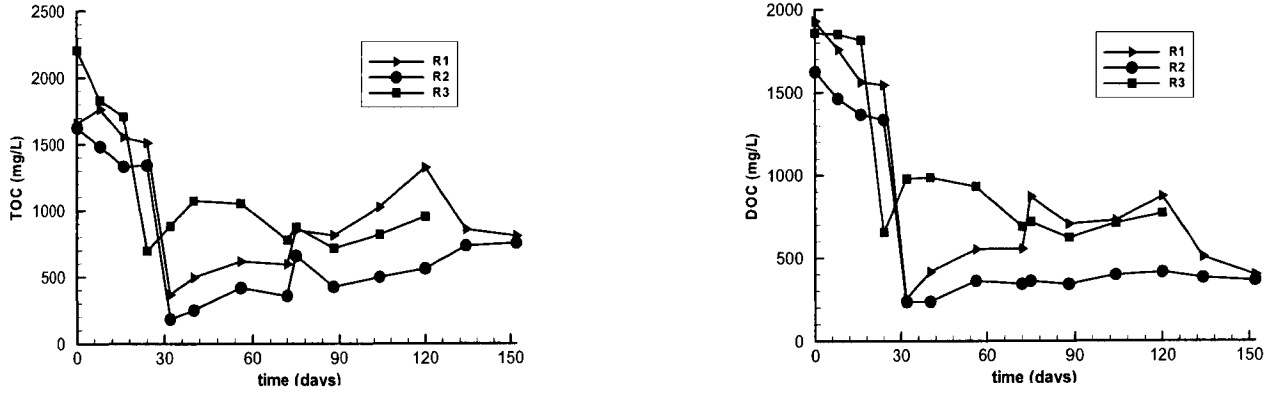

Figure 2.2 Variation of total organic carbon (TOC) and dissolved organic carbon (DOC) in batch reactors containing three different reactive mixtures (R1, R2, and R3)

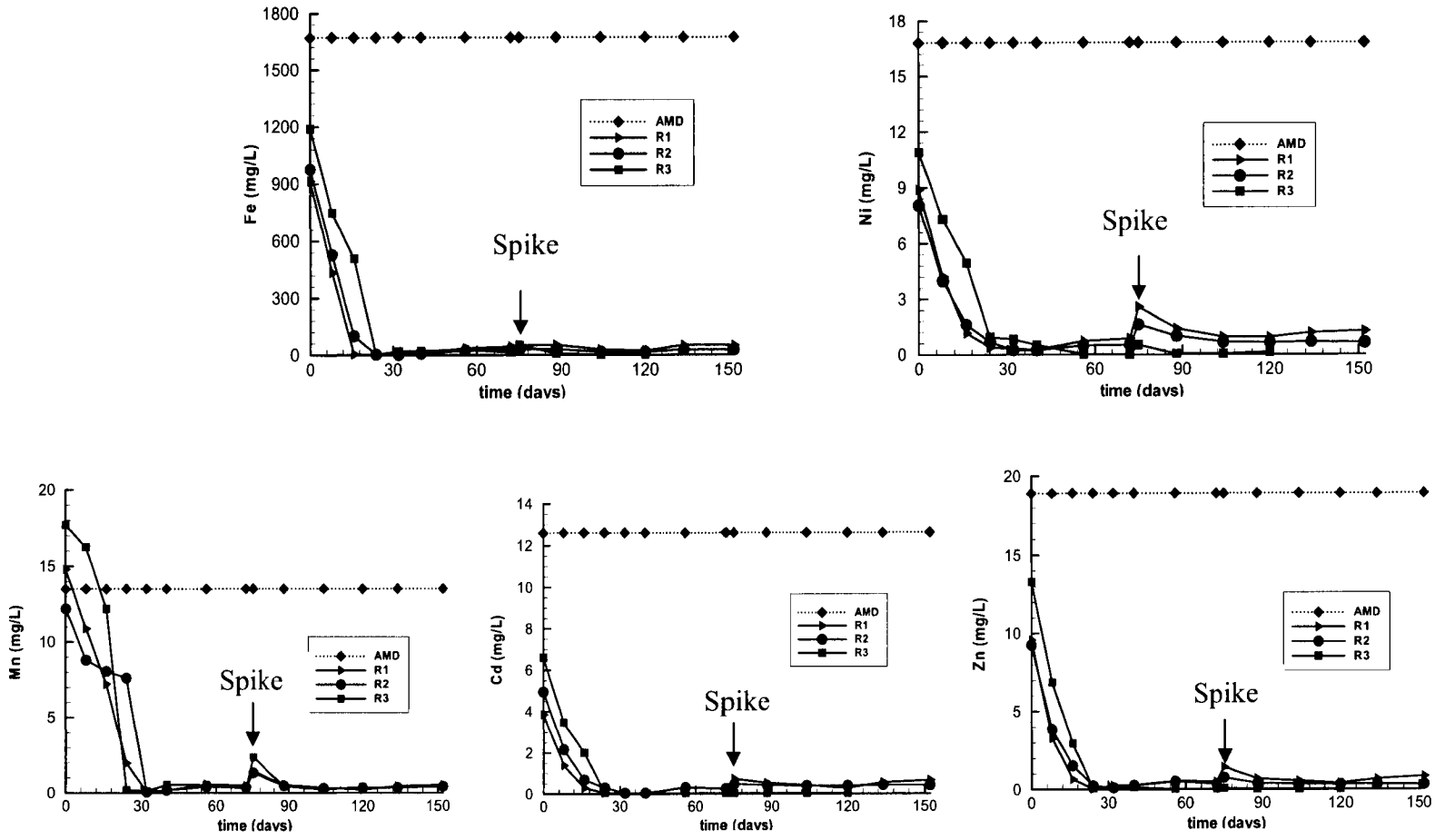

Figure 2.3 Metal concentrations in batch reactors containing three different reactive mixtures (R1, R2, and R3) as a function of time

However, after the lag period, SRB counts increased in all reactors, despite there being no evidence of sulphate removal in reactors R1 and R2.

On day 88, SRB counts were 8.0 x 10^4 cells/100mL (R1), 2.3 x 10^4 cells/100mL (R2), and 2.3 x 10^6 cells/100mL (R3). Final SRB counts (day 120) were 1.3 x 10^5 cells/100mL (R1), 2.3 x10^4 cells/100mL (R2), and 5.0 x10^4 cells/100mL (R3). As results indicated, SRB counts yielded the highest values in R3, which contained the most reactive mixture in terms of sulphate-reduction and metal removal.

In all reactors, metal removal efficiencies were generally high (Figure 2.3). Metal concentrations were lowest in reactor R3, with total Fe final values around 3 mg /L. Metal removal was up to 99.8% for all metals, except for Mn that reached 96.9%. In reactors R1 and R2, metal concentrations were higher, especially Fe, with final values around 27 mg/L in R2, and around 51 mg/L in R1. In these reactors, metal removal yielded values ranging from 91.8% (Ni) to 98.1% (Zn). In a related study performed with a very similar reactive mixture, a mineralogical analysis using scanning electron microscopy and X-ray elemental mapping on a spent solid mixture after 350 days of batch AMD treatment clearly indicated the presence of iron sulphides (Neculita *et al.*, 2006). This finding supports the removal of metals through formation of metal sulphides once the sulphate-reducing conditions are fully established.

Sulphate-reduction rates

Sulphate reduction rates were calculated using the least squares regression method [as per Cocos *et al.* (2002) and Zagury *et al.* (2006)] on data from the period when bioreactors demonstrated sulphate-reduction. The data between day 88 and day 152 (R1 and R2), and between day 56 to day 120 (R3) were used. Sulphate reduction rates were 39-43 mg/L per day in R1, 55-59 mg/L per day in R2, and 80-86 mg/L per day in R3. The sulphate reduction rates measured in R3 are comparable to the rates previously reported (Cocos *et al.*, 2002; Waybrant *et al.*, 1998, 2002; Zagury *et al.*, 2006).

Comparative efficiencies of the three reactive mixtures

In terms of sulphate and metal removal, mixture #3 was the most efficient and mixture #1 was the least efficient. The differences between reactors were in the solid: liquid ratio used, which was 1:3 (R1) and 1:4 (R2 and in R3). More importantly, mixture #3 contained 30% of each group of organic materials (organic and cellulosic), whereas mixtures #1 and #2 contained 48-50% organic wastes and only 2-3% cellulosic wastes (Table 2.2). It is worth noting that organic wastes had a higher EAS content than cellulosic wastes, which had the highest content of recalcitrant organic carbon (e.g. hemicellulose, cellulose) (Table 2.1). As a result, mixtures #1 and #2 had a higher content of easily available organic carbon than mixture #3 and could be expected to perform better. Moreover, TOC and DOC contents calculated from characterization data (Table 2.1) showed values of 20.6 g and 52.9 mg (R1), of 13.4 g and 47.0 mg (R2), and of 40.7 g and 483.5 mg (R3), respectively. Batch mixture #3 had much higher contents of TOC and of DOC than mixtures # 1 and #2. Additionally, more than half of the organic carbon in reactor R3 was released from cellulosic wastes, and this type of organic carbon is reportedly not easily available to SRB (Cocos *et al.*, 2002; Waybrant *et al.*, 1998, 2002). However, in long term batch tests, mixture #3 with 30% cellulosic wastes yielded higher efficiencies. Therefore, the usual characterization parameters could not predict the most efficient reactive mixture. These findings are different from Cocos *et al.* (2002) who reported that a higher percentage of poultry manure entails a better reactivity. It must be noted that the experiment of Cocos *et al.* (2002) only lasted 40 days.

Furthermore, in all bioreactors, AMD treatment started in the first 80 days, when pH increased to 8-9, alkalinity increased to 12-13 g/L, and heavy metal removal reached values as high as 99.9%. Modeling results indicated that up to day 40, metal removal mechanisms can be attributed to precipitation of (oxy)hydroxide and carbonate minerals. Sulphate reduction occurred after this lag period of about 80 days, as indicated by increased SRB counts and by decreasing sulphate concentrations.

Long-term batch experiments can thus lead to a more realistic evaluation of a reactive mixture's potential efficiency in long-term AMD treatment. However, batch results must be confirmed by continuous flow column experiments.

2.4.3.2 Role of C/N, COD/SO_4^{2-}, and DOC/SO_4^{2-} ratios

C/N and COD/SO_4^{2-} ratios were calculated using data available from natural organic material characterization and from water quality during batch experiments.

Initial C/N ratios (expressed as TOC/TKN) were 4.0, 3.5, and 10.1 for mixture#1, mixture #2, and mixture#3, respectively. Therefore, mixture #3, which performed the best, had the highest C/N ratio. These results are in agreement with other studies, which indicated that a C/N ratio around 10 is suitable for biological degradation of complex organic substrates (Reinertsen *et al.*, 1984; Béchard *et al.*, 1994) but not with the findings of Zagury *et al.* (2006), who reported the best efficiencies for a C/N ratio around 3. However, mixture #3 contained a proportion of cellulosic wastes (30%) 10 fold higher compared to the 3% used in the study of Zagury *et al.* (2006). As already mentioned, cellulosic wastes release a higher content of DOC in water (Table 2.1 and Figure 2.2). As a result, the choice of the organic carbon source for an efficient biological AMD treatment can not be made based solely on the C/N ratio.

Initial COD/SO_4^{2-} ratios were then calculated, giving relatively similar values of 0.07, 0.06, and 0.15, for mixture#1, mixture #2, and mixture#3, respectively. The COD/SO_4^{2-} ratios were also calculated over the duration of the batch tests (120-152 days; Figure 2.4). The results showed similar trends for all three mixtures with COD/SO_4^{2-} ratios that decreased slightly until day 32 and then increased to values up to 0.65 (R1 and R2) or to 3.88 (R3) on day 104, due to the decrease in sulphate concentrations to 400 mg/L. It is worth noting that the initial COD/SO_4^{2-} ratios were less than the theoretical value (0.67) (Hao *et al.*, 1996) in all reactors. However, sulphate reduction was observed even at COD/SO_4^{2-} ratios less than 0.67. This can be explained by the presence of cellulosic

wastes in mixture compositions that released complex dissolved organic carbon, which was not quantified during the COD determination.

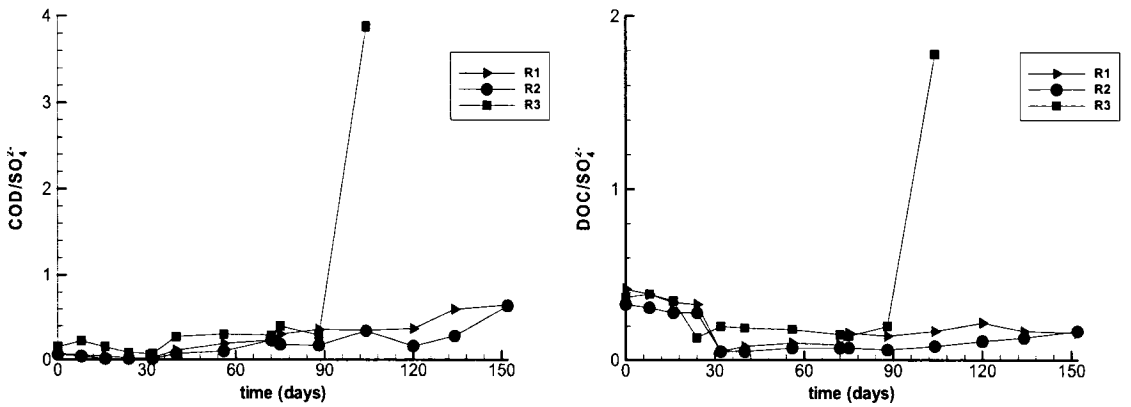

Figure 2.4 Chemical oxygen demand (COD)/SO_4^{2-} ratio and dissolved organic carbon (DOC)/SO_4^{2-} ratio in batch reactors containing three different reactive mixtures (R1, R2, and R3) as a function of time

Additionally, the high alkalinity generated (up to 13 g/L) could interfere during the test analysis. Furthermore, COD is not an accurate measure of organic carbon availability to anaerobic bacteria because it is determined under aerobic conditions.

From these findings, we suggest that DOC could be a more appropriate indicator for organic carbon availability to SRB than COD. The DOC/SO_4^{2-} ratio was therefore calculated to confirm this hypothesis. Initial DOC/SO_4^{2-} ratios were similar in all bioreactors and varied from 0.32 to 0.38. DOC/SO_4^{2-} ratios calculated over the duration of the batch tests showed similar trends as in the COD/SO_4^{2-} ratio (Figure 2.4), with DOC/SO_4^{2-} ratios that decreased until day 32 and then increased slowly to values up to 0.17 (R1 and R2) or more sharply to 1.78 (R3) on day 104. Therefore, higher ratios of both COD/SO_4^{2-} and DOC/SO_4^{2-} seemed to be better correlated with sulphate-reducing

conditions. However, DOC is more easily and accurately quantified in the complex system of a passive bioreactor than the chemical oxygen demand (COD). For that reason, C/N and DOC/SO_4^{2-} ratios taken together are suggested as key parameters that link an organic mixture composition with its biodegradability under anaerobic conditions.

2.5 Conclusions

The three reactive mixtures tested were successful in promoting sulphate reduction and metal removal (91.8-99.8%) but higher efficiencies were observed in the reactor (R3) that contained a mixture of equal proportions (30%, w/w) of organic and cellulosic wastes. A lag period of about 80 days was observed before the occurrence of sulphate reduction stressing the drawbacks of short-term (less than three months) batch experiments for mixture selection. Organic material characterization indicated that for C/N, COD/SO_4^{2-} and DOC/SO_4^{2-} ratios, higher values were associated with better sulphate-reducing conditions. In addition, C/N and DOC/SO_4^{2-} ratios taken together are easily measurable parameters that link natural organic material composition with its biodegradability under anaerobic conditions.

Because hydraulic retention time is an essential parameter for an efficient design of a full-scale passive bioreactor, the most efficient reactive mixture (#3) is currently being tested in column bioreactors that will be operated at different hydraulic retention times for more than a year.

2.6 Acknowledgements

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CHAPITRE III

ARTICLE #3: EFFECTIVENESS OF SULPHATE-REDUCING PASSIVE BIOREACTORS FOR TREATING HIGHLY CONTAMINATED ACID MINE DRAINAGE: I. EFFECT OF HYDRAULIC RETENTION TIME

3.1 Abstract

Sulphate-reducing passive bioreactors have proved to be an effective technology for the treatment of acid mine drainage (AMD) contaminated waters over relatively short periods of time (1-5 years). However, long-term efficiency can be limited by organic carbon availability to the anaerobic microflora and by problems related to the hydraulic properties of the reactive mixture. In the present study, the effect of two hydraulic retention times (HRTs) of 7.3d and 10d on the performance of passive bioreactors was evaluated over an 11-month period for the treatment of a highly contaminated AMD. Evolution of the porosity and hydraulic conductivity of the reactive mixture was also evaluated during the 15-month operation of two bioreactors. Results indicated that bioreactors were effective at both HRTs for increasing the pH and alkalinity of contaminated water and for sulphate and metal removal (60-82% for Fe and up to 99.9% for Cd, Ni, and Zn). Although the quality of treated effluent was significantly improved with the 10d HRT compared to the 7.3d HRT, results showed that the higher HRT reduced the porosity and the permeability of the reactive mixture which might lead to hydraulic related problems and, eventually, to limited efficiency in long-term operation compared to a shorter HRT. A compromise must therefore be found for the design of a long-term effective passive bioreactor in order to respect the discharge limits in treated effluent and to limit the problems related to the hydraulic properties of the reactive mixture.

Keywords: Acid mine drainage (AMD), Sulphate-reducing passive bioreactor; Hydraulic retention time (HRT), Porosity, Hydraulic conductivity

3.2 Introduction

Acid mine drainage (AMD) contaminated waters have low pH and high sulphate and metal concentrations which need to be treated before being discharged into the environment (Blowes *et al.*, 2003). Sulphate-reducing passive bioreactors are preferred to traditional water treatment plant technologies because they allow higher metal removal at low pH and generate more stable sludge with lower operation costs and minimal energy consumption (Zaluski *et al.*, 2003). Generally, passive bioreactors operated over relatively short periods of time (up to 5 years) meet their treatment objectives in terms of increasing the pH and alkalinity, and for sulphate and metal removal (Dvorak *et al.*, 1992; Reisinger *et al.*, 2000; Zaluski *et al.*, 2003; Kuyucak *et al.*, 2006; Figueroa *et al.*, 2007). However, in long-term operations, their efficiency is sometimes limited by organic carbon availability to anaerobic microflora, including the sulphate-reducing bacteria (SRB). While it is known that organic carbon availability is mainly controlled by the reactive mixture composition (Cocos *et al.*, 2002; Neculita *et al.*, 2007), the lack of consensus on design criteria and of specifications for organic substrate and inorganic material fractions represents an important issue for passive bioreactors that needs more research (Figueroa *et al.*, 2007).

The effectiveness of passive bioreactors has been proven to depend on substrate composition (Beaulieu *et al.*, 2000; Chang et al., 2000; Waybrant *et al.*, 2002; Zagury *et al.*, 2006; Neculita *et al.*, 2008), hydraulic retention time (HRT) (Younger *et al.*, 2002), as well as AMD toxicity and variations in flow rate (Reisman *et al.*, 2003; Kuyucak *et al.*, 2006). In a field-bioreactor, optimization of HRT is the most important design objective, and yet the most difficult to achieve (Younger *et al.*, 2002). Recent research suggests that the nominal HRT of field-bioreactors should be at least 40 hours, while a HRT of four or more days is required for efficient treatment of highly contaminated

AMD (Younger *et al.*, 2002). Finally, the long-term efficiency of passive bioreactors is also limited by problems related to the hydraulic properties of the reactive mixture such as clogging, compaction, segregation, and development of preferential flow paths (Younger *et al.*, 2002). Suspended solids from AMD, (oxy)hydroxide, carbonate and sulphide minerals formed by metal precipitation, and biomass and metabolic products generated by bacterial activity can induce changes in substrate properties (Rockhold *et al.*, 2002). These solids potentially decrease the porosity and permeability, affect the longevity and the performance of bioreactors and, ultimately, can result in their failure (Neculita *et al.*, 2007). Saturated hydraulic conductivity (k_{sat}) of the substrate materials is therefore an important variable to be determined (Bolis *et al.*, 1992). Moreover, k_{sat} needs to be evaluated before installing the bioreactor because laboratory tests can more easily be carried out. For field-bioreactors, however, hydraulic parameters are very difficult to evaluate because the installation of the large number of piezometers to monitor the system may change the hydraulic behaviour of the system itself (Younger *et al.*, 2002). Usually, values of k_{sat} are substrate-related which typically vary from 10^{-4} cm/s (compost) to between 10^{-2} and 10^{-3} cm/s (sawdust) (URS, 2003). The volume of substrate needed to achieve a given nominal HRT is related to its hydraulic conductivity and porosity by Darcy law (assuming a saturated medium), which can be expressed as follows:

(1) $v = k_{sat} \times i$, with $k_{sat} = f(n)$

where v = seepage velocity (cm/s), k_{sat} = saturated hydraulic conductivity (cm/s), i = hydraulic gradient, and n = porosity of the porous medium.

The porosity of the substrates must be estimated prior to final selection of mixtures and to design appropriate dimensions for the treatment system (Younger *et al.*, 2002). Usually, field-tested porosities are in the range 0.15 to 0.35, while laboratory-based values are in the range 0.35 to 0.63 (Younger *et al.*, 2002; Amos *et al.*, 2003; Tsukamoto *et al.*, 2004).

Several studies have evaluated the influence of biofilm growth and related changes in the physical properties of porous media (Taylor and Jaffé, 1990; Taylor *et al.*, 1990; Rockhold *et al.*, 2002; Anello *et al.*, 2005; Polo *et al.*, 2006). However, they used very simple matrices (e.g. sand), liquid organic carbon sources (e.g. lactate, methanol) and sometimes only aerobic media. Moreover, some studies found that there appears to be a limit beyond which no further permeability reductions can occur (Taylor and Jaffé, 1990). Expressed as the ratio of the hydraulic conductivity k_{sat} to the initial hydraulic conductivity ($k_{sat\ 0}$), this limit has been reported to be on the order of 10^{-4} (Taylor and Jaffé, 1990). Limited results are, however, available with respect to the evolution of the hydraulic properties during the long-term operation of laboratory and field-scale passive bioreactors filled with mixtures constituted from complex organic carbon sources and mineral waste materials (Bolis *et al.*, 1992; Reisinger *et al.*, 2000).

The main objective of the present work was to study the evolution of effluent quality, as well as the evolution of hydraulic parameters during the operation of column bioreactors treating a highly contaminated AMD, at two different HRTs. To help the interpretation, porosity and hydraulic conductivity were evaluated before, during, and after the testing period.

3.3 Materials and methods

3.3.1 Column bioreactor design, set-up and operation

Six Plexiglas columns (length 45 cm, with inner diameter 10 cm, volume 3.5L) were used for the sulphate-reducing bioreactors (Figure 3.1), with downward flow. Initially (for the first 12 weeks), two nominal HRTs of 2.5d and 5d were tested in triplicate. Each column was packed with the same reactive mixture consisting of 60% (w/w, dry weight) organic materials and 40% (w/w) inorganic materials (sand, creek sediment, urea, and calcium carbonate).

Natural organic materials were constituted from equal proportions (30%, w/w) of cellulosic (maple wood chips and sawdust) and organic (leaf compost and poultry manure) wastes. This reactive mixture was successfully tested in long-term (120d) batch bioreactors. More details on the reactive mixture composition and its selection are given in Neculita (2008) and Neculita and Zagury (2008).

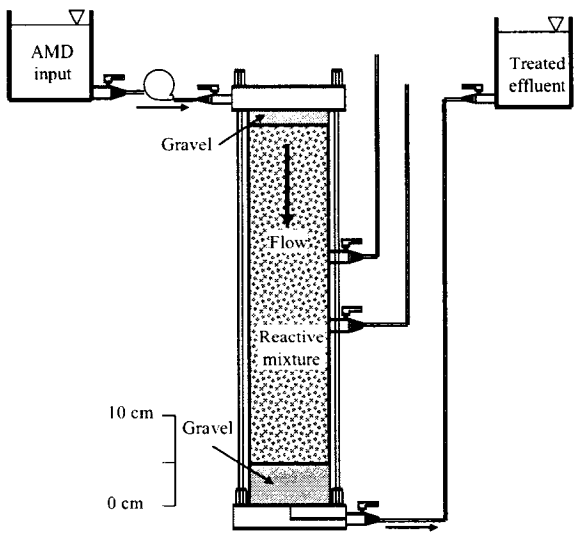

Figure 3.1 Design of down-flow sulphate-reducing column bioreactors equipped with piezometers, as used in the present study

The reactive mixture was slightly compacted and sandwiched between two layers of gravel 2.5 cm and 5 cm thick, at the top and bottom of the reactors, respectively. The packed material was enclosed at the top and bottom with a fine-mesh geotextile and with porous-plates and covers. The top layer of gravel allowed uniform dispersion of influent AMD across the reactor, while the fine-mesh geotextile prevented materials from being washed out of the columns. After set-up, the bioreactors were saturated with Postgate B medium (Postgate, 1984), which was previously sterilized, and the columns were enveloped in aluminum foil to exclude light and to simulate conditions in field

bioreactors, as well as to prevent growth of phototrophic bacteria (Waybrant *et al.*, 2002). The Postgate B medium was prepared in distilled water and had the following composition: 3.5 g/L sodium lactate; 2.0 g/L $MgSO_4 \cdot 7H_2O$; 1.0 g/L NH_4Cl; 1.27 g/L $CaSO_4 \cdot 2H_2O$; 1.0 g/L yeast extract; 0.5 g/L KH_2PO_4; 0.5 g/L $FeSO_4 \cdot 7H_2O$; 0.1 g/L thioglycolic acid, and 0.1 g/L ascorbic acid. After addition of the Postgate B medium, the columns were incubated for five weeks before starting their operation (acclimation period). This acclimation period is necessary to grow a large population of SRB that would produce enough sulphide and alkalinity in order to withstand the shock of the AMD feed (Waybrant *et al.*, 2002). Continuous flow was then started and flow through the columns was maintained using Masterflex® L/S® precision peristaltic pumps, which were calibrated prior to starting the tests. A double-switch valve allowed collection of the treated effluent either in 5L container, which were emptied every week, after sampling, or in 50mL vials for instantaneous analysis. After the first 12 weeks, the treated effluent quality deteriorated (e.g. pH, alkalinity, Fe^{2+}, SO_4^{2-}) and the HRTs were increased from 2.5d and 5d to 7.3d and 10d, respectively. Four columns (in duplicate for each of the two HRTs) were decommissioned after 11 months of operation and a thorough evaluation of the metal precipitate mineralogy and potential for metal remobilization was carried out (see Part II of the present study, Neculita *et al.*, 2008; this journal). The last two columns were dismantled after 15 months of operation. All experiments were carried out at laboratory temperature, which was not controlled to reflect field conditions and ranged from 18 to 27°C.

3.3.2 Acid mine drainage quality

Artificial AMD with high sulphate and metal concentrations was used as influent for the six column bioreactors (Table 3.1). This AMD, with a pH of 5.5-5.6 was prepared every week and stored in 5L open containers. The AMD was not bubbled with nitrogen (as in many other column-bioreactor experiments) in order to more realistically reproduce aerobic conditions in a field-settling pond. As a result, measurements indicated that the

pH of the AMD feed decreased from its initial value to as low as 2.9 between day 0 and day 7, when a new solution of AMD was prepared. The AMD had a yellow-reddish color, which indicated the presence of soluble ferric minerals. Therefore, ferrous to ferric iron oxidation and its subsequent precipitation could explain the pH decrease.

It is worth noting that AMD quality was also changed after 12 weeks of operation, when iron concentrations were reduced from 1066±78 mg/L to 504±83 mg/L, while sulphate concentrations were maintained at their initial levels using Na_2SO_4 (Table 3.1).

Table 3.1 Composition of synthetic AMD feed in column bioreactors over a 44-week period

Component	Concentration (mg/L)	Source
Ca^{2+}	372±75	$CaSO_4 \cdot 2H_2O$
Cd^{2+}	9.8±1.8	$CdSO_4 \cdot 8/3H_2O$
Fe^{2+} (weeks: 0-12)	*1066±78*	$FeSO_4 \cdot 7H_2O$
Fe^{2+} (weeks: 13-44)	*504±83*	
K^+	66.3±40.0	K_2SO_4
Mg^{2+}	85.8±10.5	$MgSO_4$
Mn^{2+}	10.1±2.6	$MnSO_4 \cdot H_2O$
Na^+ (weeks: 0-12)	*135±46*	Na_2SO_4
Na^+ (weeks: 13-44)	*625±231*	
Ni^{2+}	13.7±1.0	$NiSO_4 \cdot 6H_2O$
Zn^{2+}	14.5±2.1	$ZnSO_4 \cdot 7H_2O$
SO_4^{2-}	4022±583	Na
pH	2.89-5.69	Na

[a] Na – Not applicable

3.3.3 Physicochemical and microbiological analyses

Samples of AMD and treated effluent were collected from the 5L containers and analyzed on a weekly basis over an 11-month period. Analyses included pH, ORP (oxydoreduction potential), ferrous iron (Fe^{2+}), sulphate, and metals (Ca, Mg, Na, K, Fe, Mn, Cd, Ni, and Zn). Physicochemical analysis of the treated effluent also included total organic carbon (TOC), dissolved organic carbon (DOC), alkalinity, dissolved sulphides,

and ammonia. Moreover, SRB counts were carried out every month. All parameters were analyzed according to standard methods (APHA, 1998). pH and ORP were determined in unfiltered samples using a portable pH/mV/temperature meter (HACH, model sensION1) with a gel-filled pH electrode and a combination Ag/AgCl redox potential electrode (HACH, Hampton, NH). Alkalinity was determined by sulphuric acid titration on non-filtered samples. Ferrous iron, sulphate, sulphides, and ammonia analyses were performed on 0.45µm filtered samples using a HACH spectrophotometer (HACH, model DR/2010). TOC and DOC of the effluent were determined at 680°C using a TOC analyzer (DOHRMAN, model DC-190). Metal concentrations were determined using an atomic absorption spectrometer (Perkin Elmer, model AAnalyst 200) after sample acidification at pH 2 with concentrated HCl. Enumeration of SRB in treated effluent was carried out by using the Most Probable Number technique and the norm D 4412 – 84 (ASTM, 1990), and Standard Method 9240 (APHA, 1998).

3.3.4 Hydraulic parameter evolution

Porosity and saturated hydraulic conductivity were evaluated before the columns' set-up, on the freshly constituted reactive mixture and finally, on the substrate recovered at the end of the tests (week 60). Saturated hydraulic conductivity evolution was also determined during the operation of the columns at weeks 26 and 38 (10d HRT) and at weeks 33 and 39 (7.3d HRT) on two columns, one for each HRT used. The designated columns were equipped mid-way down with open standpipe-piezometers (Figure 3.1) placed 10 cm apart for measuring the pore-water gradients of the saturated reactive mixture during operation. The initial saturated hydraulic conductivity was evaluated in rigid wall permeameters using the falling-head and constant-head methods (sections D 4511-92, D 2434-68, and D 5856-95 from ASTM, 1994, 1995, and 1996, respectively), while the k_{sat} determined during operation and the final k_{sat} were evaluated directly in the columns using the constant-head method (e.g. McCarthy, 1998). Porosity was calculated as the ratio between void volume and total volume of the reactive mixture

samples used in the permeability tests. The void volume of the reactive mixture was calculated considering a specific gravity (G_s) of 0.7, similar to peat.

At the end of the test (after 60 weeks of operation), a low saturated hydraulic conductivity severely limited the gravitational flow through the reactive mixture and, as a result, no further measurements could be taken from the 10d HRT column. In order to evaluate if the k_{sat} decrease was uniform throughout the column, a middle layer of reactive mixture (about 16 cm) was carefully transferred to a classic 1.5 rigid-wall permeameter and k_{sat} was measured. After 13 months of operation, other hydraulic tests (e.g. tracer tests) were also unsuccessfully attempted. Also, the open standpipe piezometers (Figure 3.1) intended for measuring of the water gradients in pores of the saturated reactive mixture did not allow the recording of valid measures (hydraulic head differences between the ports were very small) for hydraulic behavior evolution during operation of the bioreactors.

3.4 Results and interpretation

3.4.1 Bioreactor performance during the first 12 weeks of operation

Within one day of starting the AMD feed, a black precipitate developed at the top of each bioreactor which then spread throughout the solid phase. Over time, the top layer of reactive mixture (5-10 cm) changed from black to yellow-brown, a specific color for iron (oxy)hydroxide minerals, and maintained this color until the end of the tests (week 44). The formation of black precipitate is similar to observations reported in other studies (Chang *et al.*, 2000; Christensen *et al.*, 1996). This is an indication that, once the AMD feed started, the hydrogen sulphide which was generated during the acclimation period by SRB reacted with the metals. Indeed, the initial sulphide concentrations in the treated effluent were up to 2 mg/L, despite the high load of metals in the AMD feed. However, low counts of SRB (10^3 cell/100 mL) were found in samples collected from treated effluent immediately after starting column operation (Figure 3.2). One explanation could be related to competition between SRB and other anaerobic microbial

populations (e.g. methane producing bacteria), which are very active in highly reducing environments (ORP<-300mV). Such environments are also characterized by high concentrations of easily available organic carbon and limited sulphate concentrations, such as conditions found in the present study at the end of the acclimation period. Moreover, once the AMD feed began, the effluent quality steadily and continuously deteriorated during the first 12 weeks of column operation, regardless of the HRT used (Figure 3.2). The decrease in pH (from 9.0 to 5.5) and alkalinity (from 3.5 g/L $CaCO_3$ to as low as 23 mg/L $CaCO_3$) were noted, while the ORP kept increasing from values as low as -342 mV to around 50 mV. Important variations in the ferrous iron (from 40 mg/L to between 1200 mg/L to 1400 mg/L) and sulphate concentrations (from between 1000 mg/L to 1700 mg/L to around 4000 mg/L) were also recorded. At the same time, TOC and DOC decreased from around 7 g/L to between 6 to 22 mg/L on week 12. Moreover, total Fe concentrations increased to the value of Fe in the AMD feed, while Mn concentrations increased to values 3 times higher than the influent concentrations. Only concentrations of Ni and Zn remained very low, with removal rates in the range 96.5-99.9% (Figure 3.3), while Cd was removed to below detection limits (<0.03 mg/L). By week 12, SRB counts reached their lowest levels (<2 cell/100 mL) in all reactors which indicated that bacterial activity was also severely affected (Figure 3.2).

A potential cause of the declining bioreactor effectiveness was overloading. Either excessive Fe concentrations in the AMD feed and/or too short HRTs, which gave insufficient contact time between the reactive mixture and AMD, could explain these results. Therefore, Fe concentrations were decreased from around 1000 mg/L to 500 mg/L and the HRTs were increased from 2.5d and 5.0d to 7.3d and 10d, respectively, starting in week 13 (Table 3.1). The HRT increase was based on the fact that a longer HRT of 20d was previously reported to be effective for the treatment of AMD containing 500 mg/L of iron (Chang *et al.*, 2000).

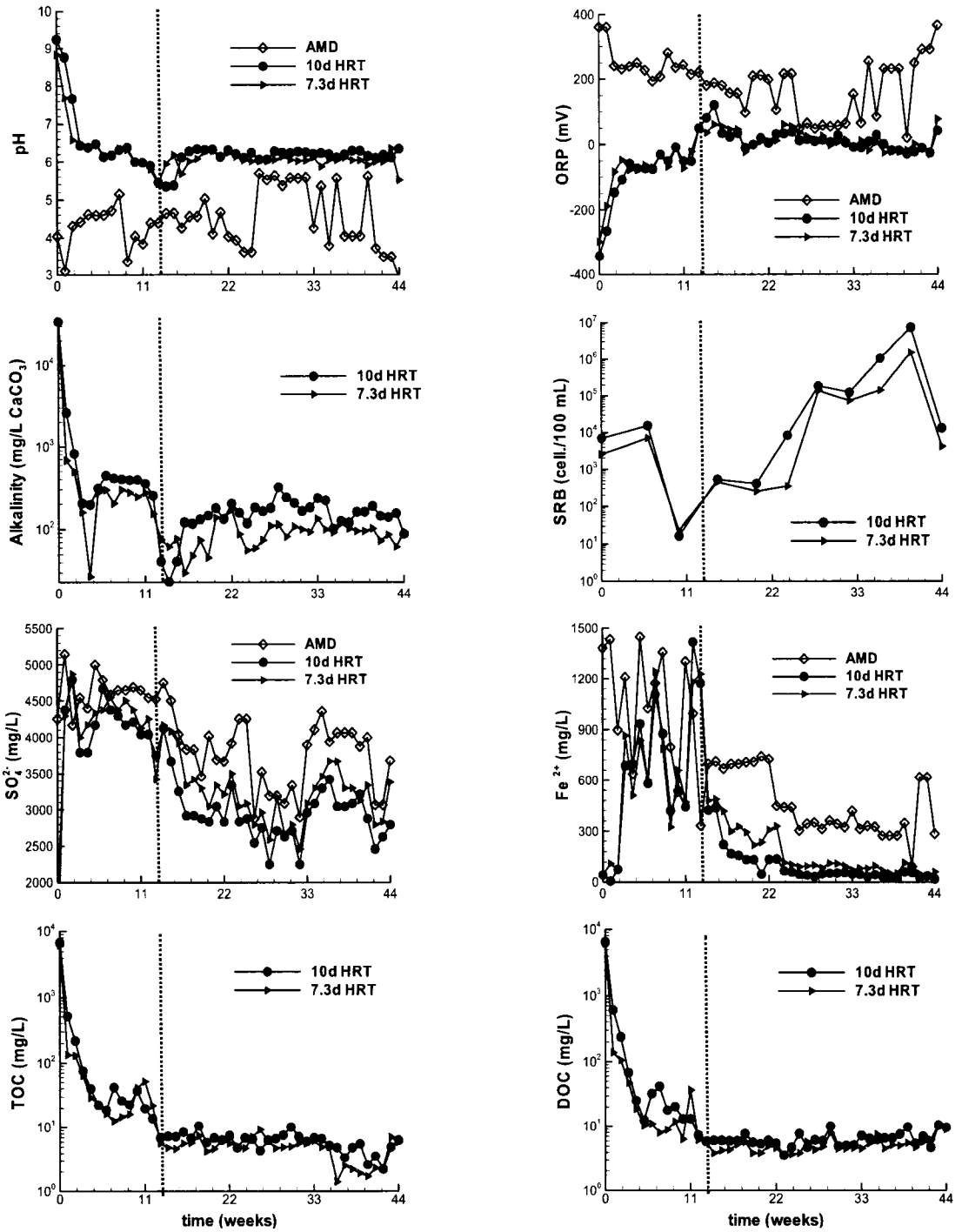

Figure 3.2 Evolution of physicochemical parameters and SRB in sulphate-reducing column bioreactors (up to week 13, the bioreactors were operated at HRTs of 2.5d and 5.0d, which were then increased to 7.3d and 10d HRTs, respectively)

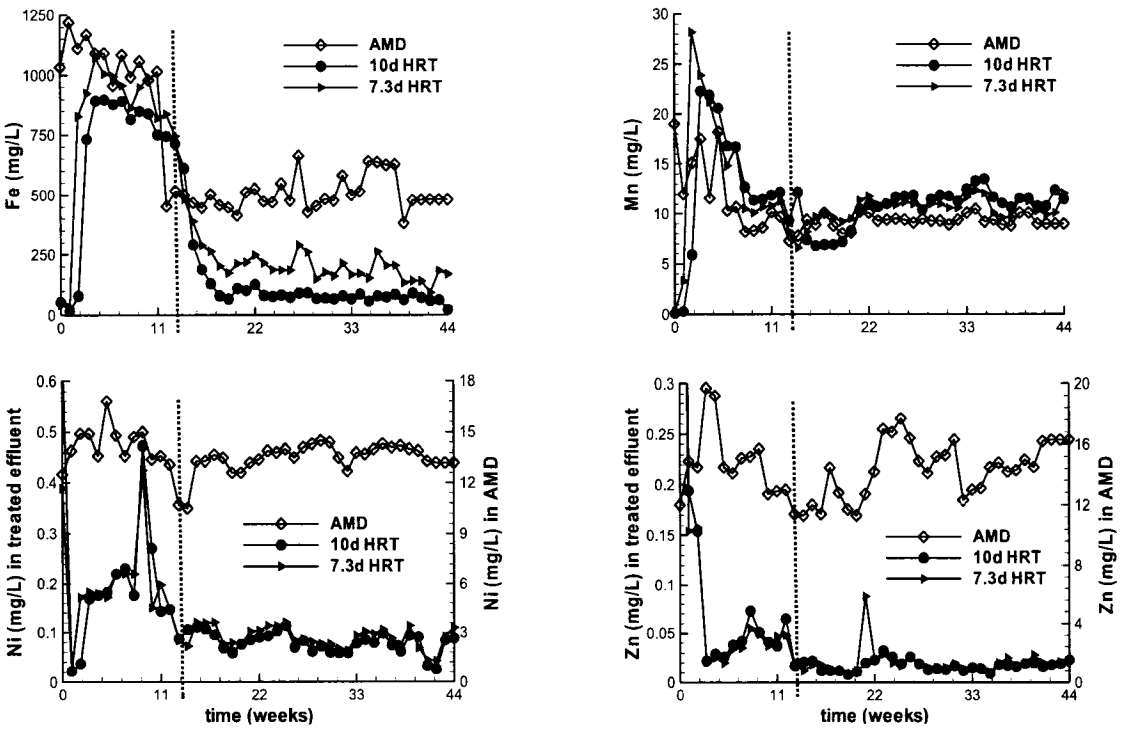

Figure 3.3 Evolution of metal concentrations in sulphate-reducing column bioreactors (up to week 13, the bioreactors were operated at HRTs of 2.5d and 5.0d, which were then increased to 7.3d and 10d HRTs, respectively)

3.4.2 Bioreactor performance from week 13 to the end of the tests

After the changes were completed, treated effluent quality began to improve by week 14. A steady state condition was obtained for all bioreactors, which was maintained until the end of the tests, regardless of the HRT used (Figures 3.2 and 3.3). This translated into low coefficients of variation (standard deviation to mean ratio) for pH (3-4%), sulphate (13%), alkalinity (33-37%), TOC (29-37%), DOC (25-29%), Mn (9-18%), Ni (24-26%), Fe (29-52%), and Zn (31-67%). Other parameters, directly related to transformations occurring during effluent exposure to air, showed higher variation, such as Fe^{2+} (80-110%) and ORP (210-290%).

Also, starting in week 14, high percentages of metal removal were recorded in all reactors with average values of 60.0%, 99.3%, and 99.9% (7.3d HRT) and of 81.9%, 99.4%, and 99.9% (10d HRT) for Fe, Ni, and Zn, respectively. Cadmium concentrations in the effluent was always below detection limit (<0.03 mg/L).

Treated effluent from bioreactors operated at both HRTs contained high Mn concentrations, which is a very challenging metal to remove in sulphate-reducing passive bioreactors (Neculita *et al.*, 2007). In the early phase of bioreactor operation, Mn can be removed as carbonate minerals, while MnS (solubility product, $[K_{sp}] \sim 10^{-16}$) is eventually formed later during bioreactor operation, if high concentrations of sulphides are generated in the treatment system. Indeed, the solubility of rhodochrosite (~ 65 mg/L) is higher than the concentration of Mn in the AMD used in the present study. Poor removal of Mn is often reported in pilot and field-scale bioreactors (Zaluski *et al.*, 2003; Hallberg and Johnson, 2005; Kuyucak *et al.*, 2006). Therefore, Mn removal in passive remediation systems is often less effective than removal of iron due to a higher solubility of MnS compared to other metal sulphides and, therefore, it is the last metal to be removed through formation of MnS (Waybrant *et al.*, 2002).

The SRB counts progressively increased after week 14, to yield their highest levels by week 40 (10^7 cell/100 mL). This is not well correlated with sulphate concentrations, whose lowest concentrations were found between week 25 and week 33 (Figure 3.2). However, an alternative sink for sulphate removal is the formation of gypsum ($CaSO_4 \cdot 2H_2O$) (Willow and Cohen, 2003). In the present study, Ca was added as a major cation during AMD preparation (Table 3.1). Also, Ca could have been released from the poultry manure and the leaf compost that were used in the reactive mixture and which contained 23 g/kg and 15g/kg Ca, respectively. Generally, starting in week 14, more sulphate (21±6%) and ferrous iron (81±15%) were removed at higher HRT (10d) than at 7.3d HRT (14±7% and 65±21%, respectively), which is in agreement with previous studies (Chang *et al.*, 2000). Moreover, significantly better quality (t-student, p <0.05) of 10d HRT effluent compared to 7.3d HRT effluent was found in terms of pH,

alkalinity, total and ferrous iron, sulphate, and Ni concentrations between week 14 and the week 44. Therefore, higher HRTs yielded better effectiveness in terms of increasing pH and alkalinity, and sulphate and metal removal, and which therefore produced a better physicochemical quality of treated effluent. Moreover, based on metal removal efficiencies, the columns did not lose significant treatment capacity after 15 months of continuous operation. A final analysis of treated effluent (results not shown), carried out in week 60, indicated that the columns were still effective for increasing pH and alkalinity, and for sulphate and metal removal.

3.4.3 Iron related problems

Over the duration of the study, a common observation was that once the treated effluent was exposed to air, ferrous iron oxidized to ferric iron, precipitated as (oxy)hydroxide minerals and decreased the pH. This process was directly related to effluent alkalinity, where higher alkalinities increased the buffer capacity and yielded less significant decreases in pH in 10d HRT effluent. Evidence of this process was found on samples collected from 50 mL vials used for instantaneous analysis. In these vials, the effluent pH was 0.9 units higher and alkalinity increased 10-fold compared to the quality in the 5 L containers designated for effluent collection.

This behavior of iron has not been previously reported. Nevertheless, in the study of Chang *et al.* (2000), treated effluent had a pH >7 and contained concentrations of total Fe up to 400 mg/L over a 35-week period of column bioreactor operation. In the present study, the pH was lower (6.0-6.3) and the maximum concentrations of total Fe were 131 mg/L (10d HRT) and 234 mg/L (7.3d HRT), between week 17 and the end of the tests.

3.4.4 Hydraulic parameter evolution

The initial saturated hydraulic conductivity of the freshly constituted, slightly compacted reactive mixture was 2.3×10^{-2} cm/s (using the constant-head permeameter). The value is consistent with reported values for similar substrates (Bolis *et al.*, 1992;

URS, 2003). Over time, the saturated hydraulic conductivity decreased more significantly in the 10d HRT columns than in the 7.3d HRT columns. In fact, by week 38, in the 10d HRT columns, the k_{sat} dropped from its initial value (2.3 x 10^{-2} cm/s) to values as low as 7.3 x 10^{-9} cm/s (determined using rigid wall, falling head permeameters) (Figure 3.4). The measurements of k_{sat} during week 38 must be interpreted with caution because they were not obtained using the most appropriate technique (triaxial cells) for such low values. However, the low saturated hydraulic conductivity and/or the presence of a gas layer at the top of the 10d HRT column severely limited the gravitational flow through the reactive mixture and, as a result, no further permeability tests were performed afterward (k_{sat} was too low to be measured). Moreover, a permeability test performed on a 16 cm portion of the middle layer of the column gave a k_{sat} of 2.0 x 10^{-5} cm/s (Figure 3.4) confirming that these top and/or bottom portions controlled water flow in the column after a few weeks.

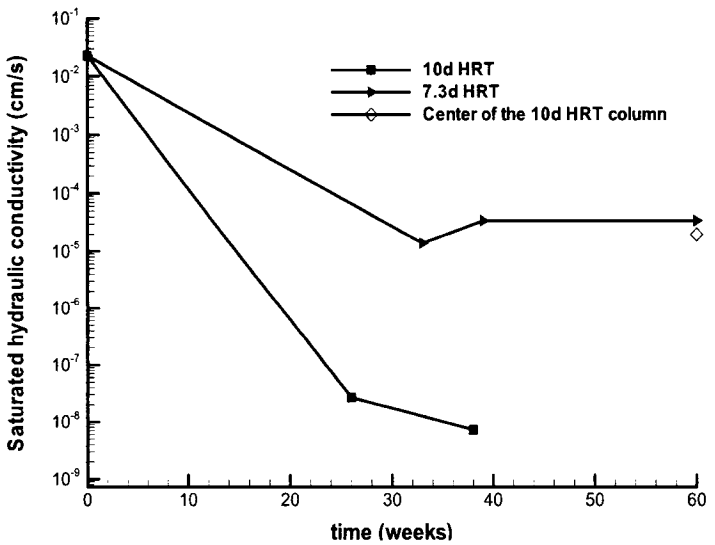

Figure 3.4 Saturated hydraulic conductivity evolution over a 60 week period in two sulphate-reducing column bioreactors operated at 7.3d and 10d HRTs (up to week 13, the bioreactors were operated at HRTs of 2.5d and 5.0d, which were then increased to 7.3d and 10d HRTs, respectively)

After dismantlement, it was observed that the geotextiles used at both the top and bottom of the 10d HRT column were not plugged by solids (mineral precipitates, biofilms etc.).

At the same time, a less significant decrease of substrate saturated hydraulic conductivity was recorded in the column operated at a 7.3d HRT. Values of k_{sat} were typically in the order of 10^{-5} cm/s by week 33 and were relatively stable until the end of the column tests (week 60). The observed decrease of k_{sat} was consistent with that reported by Taylor and Jaffé (1990), in which reductions in saturated hydraulic conductivity by factors of 10^3 were assumed to be due to enhanced biological growth in the porous medium. However, the column reactors used in the Taylor and Jaffé (1990) study were filled with sand and used methanol as substrate during a 356-day operation, with upward flow, at a 1.1-3.4d HRT. On the other hand, insignificant decreases of k_{sat} (from 7.0×10^{-3} cm/s to 1.5×10^{-3} cm/s) were reported by Reisinger *et al.* (2000) in the first eight months of operation of their field-scale bioreactor which was filled with a substrate consisting of cattle manure, sawdust, limestone, hay, and alfalfa, and which was operated entirely by gravity. After the initial decrease, however, the k_{sat} remained relatively constant over 2 years of efficient treatment of a weakly contaminated AMD (pH 3.5-7, 20-60 mg/L SO_4^{2-}, and 2.5-22.5 mg/L Cu).

Initial porosities of freshly constituted, slightly compacted reactive mixtures were evaluated to be in the range 0.338-0.427. The porosity decreased less in the 7.3d HRT columns, where a value of 0.365 was determined in week 60 compared to the 10d HRT columns, in which the porosity eventuality dropped to very low values.

Hydraulic parameter evolution in bioreactors operated over a 15-month period for AMD treatment thus suggests that a higher HRT could more easily lead to problems related to the hydraulic properties of the reactive mixture and could limit long term bioreactor effectiveness. As a result, an appropriate HRT should be determined to treat a given quality of AMD.

However, based on the results obtained in the present study, a compromise must be found for the design of a long-term effective passive bioreactor in order to respect the discharge limits in treated effluent and to limit the problems related to the reduction of k_{sat} in reactive mixtures.

3.5 Conclusion

Steady and continuous deterioration of effluent quality was recorded during the first 12 weeks of column bioreactors treating a highly contaminated AMD, regardless of the hydraulic retention time (HRT). Either insufficient HRTs (2.5d and 5d) or excessive iron concentrations (around 1000 mg/L) may explain the decrease in effluent quality. Higher HRTs (7.3d and 10d, respectively) and lower iron concentrations (around 500 mg/L) significantly improved the bioreactor effectiveness and helped achieve a steady-state quality of treated effluent from all bioreactors between week 14 and the end of the tests (week 44). Mean percent of metal removal for Fe, Ni, and Zn at a 7.3d HRT were 60.0%, 99.3%, and 99.9%, respectively, and were 81.9%, 99.4%, and 99.9%, respectively at a 10d HRT. Cadmium concentrations in the effluent were always below detection (<0.03 mg/L). Overall, significantly better quality in terms of pH, alkalinity, total and ferrous iron, sulphate, and Ni concentrations were found for 10d HRT effluent compared to 7.3d HRT effluent. However, the bioreactors were not effective for Mn removal either of the tested HRTs. Over the duration of the study, SRB counts increased progressively and reached the highest levels (10^7 cell/100 mL) by week 40.

Hydraulic parameter evolution over a 15-month period indicated that higher HRTs could more easily lead to problems related to the hydraulic properties of the reactive mixture and, therefore, could limit bioreactor effectiveness. In columns operated at a 10d HRT, the hydraulic conductivity dropped from initial values of about 10^{-2} cm/s to values as low as 10^{-9} cm/s (by week 38), which severely limited gravitational flow through the reactive mixture. Less significant decreases of k_{sat} were measured in

columns operated at a 7.3d HRT, for which values on the order of 10^{-5} cm/s were maintained from week 33 until week 60.

Results obtained in the present study indicate that to optimize the design of a passive bioreactor, a compromise must be made which combines the best conditions for respecting discharge limits in treated effluent with the most appropriate hydraulic parameters.

3.6 Acknowledgements

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CHAPITRE IV

ARTICLE #4: TOXICITY AND METAL SPECIATION IN ACID MINE DRAINAGE TREATED BY PASSIVE BIOREACTORS

4.1 Abstract

Sulfate-reducing passive bioreactors treat acid mine drainage (AMD) by increasing its pH and alkalinity and by removing metals as metal sulfide precipitates. In addition to discharge limits based on physicochemical parameters, however, treated effluent is required to be nontoxic. Acute and sublethal toxicity was assessed for effluent from 3.5 L column bioreactors filled with mixtures of natural organic carbon sources and operated at different hydraulic retention times (HRTs) for the treatment of a highly contaminated AMD. Effluent was first tested for acute (*Daphnia magna* and *Oncorhynchus mykiss*) and sublethal (*Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia*, and *Lemna minor*) toxicity. Acute toxicity was observed for *D. magna*, and a toxicity identification evaluation (TIE) procedure was then performed to identify potential toxicants. Finally, metal speciation in the effluent was determined using ultrafiltration and geochemical modeling for the interpretation of the toxicity results. The 10 d HRT effluent was nonacutely lethal for rainbow trout but was acutely lethal for *D. magna*. The toxicity to *D. magna*, however, was removed by 2 h of aeration, and the TIE procedure suggested iron as a cause of toxicity. Sublethal toxicity of the 10-d HRT effluent was observed for all test species, but it was reduced compared to the raw AMD and to a 7.3-d HRT effluent. Data regarding metal speciation indicated instability of both effluents during aeration and were consistent with the toxicity being caused by iron. Column bioreactors in operation for more than nine months efficiently improved the physicochemical quality of highly contaminated AMD at different HRTs. The present study, however, indicated that design of passive treatment should include sufficient HRT and posttreatment aeration to meet acute toxicity requirements.

Keywords-Acid mine drainage, Sulphate-reducing passive bioreactors, Acute/sublethal toxicity, Toxicity Identification Evaluation, Metal speciation

4.2 Introduction

Acid mine drainage (AMD), characterized by low pH and high concentrations of sulfate and metals, represents a very serious environmental issue for the mining and metallurgical industry around the world (Blowes *et al.*, 2003). To avoid significant environmental impacts, AMD must be collected and treated before being discharged into the environment.

Over the past 15 years, passive bioreactors have been used successfully for AMD treatment (Dvorak *et al.*, 1992; Kuyucak *et al.*, 2006). They rely on sulfate-reducing bacteria, capable of oxidizing short-chain organic carbon to bicarbonate and of reducing sulfate to hydrogen sulfide. In bioreactors, bicarbonate increases the pH and alkalinity of AMD, and hydrogen sulfide removes metals through formation of metal sulfide precipitates (Neculita *et al.*, 2007).

Field passive bioreactors are effective for increasing pH and alkalinity and for removing metals and sulfate for periods up to four years (United Registrar of Systems, 2003) (http://www.epa.gov/ne/superfund/sites/elizmine/43547.pdf). Besides discharge limits based on physicochemical analysis, however, nontoxicity of treated mine effluent is very important, because a worldwide trend exists to implement toxicity-based discharge limits (Power and Boumphrey, 2004). Toxicological parameters need to be integrated in effluent management to assess recovery and effectiveness of remedial activities and to plan future remediation strategies (Deanovic *et al.*, 1999).

In Canada, the Metal Mining Effluent Regulations (MMER) (Environment Canada, 2002) (http://canadagazette.gc.ca/partII/2002/20020619/html/index-e.html) require that all Canadian mines produce effluent that is acutely nonlethal to rainbow trout (*Oncorhynchus mykiss*). In addition, mine operations also are required in some

Canadian provinces to discharge effluent that is acutely nonlethal to cladoceran *Daphnia magna*. Since 2003, most metal mines are required to conduct, from two to four times per year, a series of four freshwater sublethal toxicity tests, as follows: growth inhibition of green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), growth inhibition of macrophyte *Lemna minor*, reproduction inhibition of water flea *Ceriodaphnia dubia*, and finally, a larval growth test with fathead minnow (*Pimephales promelas*). If the nondiluted undiluted effluent fails the trout lethality test, the MMER requires its physicochemical characterization and increased frequency of toxicity testing.

To our knowledge, only two studies have been published regarding the toxicity of biologically treated mining effluents. In the study of Song *et al.* (2001), which was performed on laboratory-scale constructed wetlands for treating low-contaminated neutral mine and smelter waters, no acute toxicity on fathead minnows and *D. magna* was found. Although 100% mortality for *C. dubia* was observed in undiluted effluent, survival increased to between 75 to 100% after its dilution to half-strength. A second study, which compared toxicity of treated AMD, indicated that biologically treated water from a sulfate-reducing bioreactor was as effective as chemically treated water using lime and sulfide precipitation to remove toxicity to *P. subcapitata* (Riesen *et al.*, 2005)(http://biomine.brgm.fr/Documents/4BioMinEProducts/Publications/30_Riesen_et_al_IBS2005Proceedings.pdf).

Furthermore, for effluent that failed toxicity requirements, investigation of the cause of acute lethality may be warranted. To help with such investigations, the U.S. Environmental Protection Agency developed a toxicity-based identification approach using the response of organisms to isolate and identify toxicity-causing agents (Norberg-King *et al.*, 1991; Durham *et al.*, 1993; Mount, 1989).

Toxicity identification evaluation (TIE) identifies substances responsible for acute lethality through specific treatments, and it often is initiated to correct a noncompliance issue. For example, the ethylenediaminetetra-acetic acid (EDTA) chelation test is used

to complex metals and to discriminate between toxicity from metals and that from other sources (Hockett and Mount, 1996; Van Sprang and Janssen, 2001). Based on the U.S. Environmental Protection Agency approach, a guidance document regarding TIE methods also was developed for Canadian mines (Environmental Services Group, 2002) (http://www.nrcan.gc.ca/ms/canmet-mtb/mmsl-lmsm/enviro/time/docs/GuidanceDocumentforConductingTRE.PDF).

Toxicity identification evaluation procedures already have been used to identify metal toxicity in natural/artificial effluents and freshwater samples (Deanovic *et al.*, 1999; Hockett and Mount, 1996; Van Sprang and Janssen, 2001; Tietge *et al.*, 1997).

In addition to TIE procedures, metal speciation can help to address toxicity of biologically treated mining effluent. Total metal concentration, however, is a poor predictor of metal uptake and toxicity, because it does not account for metal bioavailability (Ure and Davidson, 2002). Moreover, assessment of metal speciation is important in treated effluent from passive bioreactors because of transformations that can occur during posttreatment aeration.

The present study evaluates acute and sublethal toxicity of AMD treated using column bioreactors filled with mixtures of natural organic carbon sources. The bioreactors were operated at two hydraulic retention times (HRT; 7.3 and 10 d) for the treatment of an artificial AMD. The first objective was to assess the bioreactors effectiveness for removal of acute and sublethal toxicity from AMD. Because acute toxicity was observed, the present study also aimed to identify potential causes of toxicity using a TIE. Finally, metal speciation in the AMD and treated effluent was evaluated using ultrafiltration and geochemical modeling in order to support the interpretation of the toxicity and TIE results.

4.3 Materials and methods

4.3.1 Sampling

Samples used in the present study were the influent (AMD) and two effluents collected from six column bioreactors (3.5 L each), which continuously treated a highly contaminated artificial AMD for seven months before the toxicity tests began. The synthetic AMD used in the present study is similar to a real AMD from a former mine site in Northern Quebec (Canada), with some modifications in order to maintain metal concentrations below their toxic values to sulfate-reducing bacteria (Neculita *et al.*, 2007). Two HRTs of 7.3 and 10 d were tested in triplicate in the six bioreactors; for the purpose of discussion, the two effluents will be referred to as 7.3- and 10-d effluent.

Bioreactors were filled with a reactive mixture consisting of 60% (w/w, dry wt) natural organic materials (10% maple wood chips, 20% sawdust, 10% poultry manure, and 20% leaf compost) and 40% of inorganic materials (20% sand, 15% creek sediment, 3% urea, and 2% calcium carbonate). Further details about AMD preparation and the tested reactive mixtures are given in Neculita and Zagury (2008), and details about the column operation have been given Neculita (2008).

Effluent sampling was performed on a weekly basis during seven weeks. For *D. magna*, two tests were performed on effluent sampled from two consecutive weeks to assess reproducibility of results. Fresh, artificial AMD was prepared every week (using metal sulfates and distilled water) for feeding column bioreactors and for toxicity testing. Samples were stored less than 24 h in glass vials at 4°C before their testing.

4.3.2 Sample physicochemical characterisation

Acid mine drainage and effluent analysis included pH, oxydoreduction potential (ORP), ferrous iron (Fe^{2+}), sulfate, major cations (calcium, magnesium, sodium, and potasium), and metals (iron, manganese, cadmium, nickel, and zinc). Treated effluent analysis also included total organic carbon, dissolved organic carbon (DOC), alkalinity, dissolved

sulfides, and ammonia. All parameters were analyzed according to standard methods (APHA, 1998). The pH and ORP were determined in unfiltered samples using a portable pH/mV/temperature meter (model sensION1; Hach, Hampton, NH, USA) with a gel-filled pH electrode and a combination Ag/AgCl redox-potential electrode (Hach). Alkalinity was determined by sulfuric acid titration on nonfiltered samples. Ferrous iron, sulfate, sulfides, and ammonia analyses were performed on filtered samples (pore size 0.45μm) using a Hach spectrophotometer (model DR/2010). Total organic carbon and DOC of effluent were determined at 680°C using a total organic carbon analyzer (model DC-190; Dohrman, Santa Clara, CA, USA). Metal concentrations were determined using a flame atomic absorption spectrometer (model AAnalyst 200; PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) after sample acidification at pH 2 with concentrated HCl.

Effluent quality from three columns (for each HRT) was monitored weekly during the seven-week duration of the toxicity study. Values are presented as mean ± standard deviation (n = 21). For AMD, the monitoring also was done weekly, with n = 7.

4.3.3 Toxicity tests

Toxicity tests were conducted with three samples - AMD, 10-d HRT effluent, and 7.3-d HRT effluent - except for the rainbow trout test that was only performed on 10-d HRT effluent.

Acute toxicity

Test species and methods allowed by MMER protocol (Environment Canada, 2002) were used to determine the acute toxicity of AMD and treated effluent. Acute toxicity to rainbow trout and to the *D. magna* was measured based on Environment Canada test protocols (Environment Canada, 2000a, 2000b).

Rainbow trout were acclimated to dechlorinated tap water more than two weeks before starting the test. Continuous aeration at a flow rate of 6.5 ml/min was maintained during 96 h of exposure to treated effluent. The same dechlorinated tap water was used as control and for preparing serial dilutions. Exposure occurred in a static test system at 15±2°C. Insufficient test water did not allow the use of the standard method. Therefore, a modified protocol (ESG, 2002) was used in which the sample volume during exposure and the minimal height were less than in the standard method (6 vs 10 L and 12 vs 15 cm, respectively).

Daphnia magna were obtained from Aquatic Research Organisms (Hampton, NH, USA) and then cultured and tested in our laboratory using reconstituted hard water (U.S. EPA, 1993). Ten neonates (age, < 24 h) were exposed during 48 h in a 150-ml sample either in a serial dilution including at least five dilutions (100, 50, 25, 12.5, and 6.25%, v/v) or in an undiluted triplicate toxicity test. As prescribed in the test method, pre-aeration was performed if dissolved oxygen in the sample was less than 40% for up to 30 min before starting each toxicity test. Exposure occurred in a static test system at 20±2°C. Controls consisting of reconstituted hard water were run in parallel with samples in every assay to ensure the validity of the tests. For the acute toxicity tests, the median lethal concentrations (LC50s) were calculated either by log-normal regression or by the untrimmed or trimmed Spearman-Karber method depending on the data set (Environment Canada, 2005) using the specialized computer code Comprehensive Environmental Toxicology Information System, Ver 1.025b (Tidepool Scientific Software, McKinleyville, CA, USA).

Sublethal toxicity

Sublethal toxicity of AMD and treated effluents to the green algae, macrophyte (plant) and cladoceran (water flea) was determined as prescribed by the mandatory environmental effects monitoring of the MMER test protocol. The growth inhibition test with the green algae *P. subcapitata* was conducted according to the Environment

Canada test method (Environment Canada, 1992a), including the use of double-deionized water, spiked with nutrients as the exposure medium. *Pseudokirchneriella subcapitata* (UTCC 37) was obtained from the University of Toronto Culture Collection (Toronto, ON, Canada). An electronic particle counter was used to measure cell concentrations in the inoculum and in the control and test solutions at the end of the tests (Z2 Coulter® particle counter and size analyzer, Beckman Coulter Canada, Mississauga, ON, Canada).

Growth of the macrophyte *L. minor* in the AMD and treated effluent was also measured in a modified American Public Health Association medium according to the MMER protocol (Environment Canada, 1999). Axenic plants (UTCC 490) were obtained from the University of Toronto Culture Collection. Sublethal toxicity tests on plant *L. minor* were conducted after the seven-week study period. At that time, AMD had similar quality and the two effluents had relatively similar iron concentrations (7.3-d HRT, 203.5 mg/L; 10-d HRT, 68.5 mg/L) but lower alkalinity (10-d HRT, 134 mg/L as $CaCO_3$; 7.3-d HRT, 28 mg/L as $CaCO_3$). The tests were conducted with two three-frond plants (for a total of six fronds per test vessel), which were exposed to 150 ml of sample in a serial dilution including seven dilutions (100, 50, 25, 12.5, 6.25, 3.13, and 1.56%, v/v) with three to four replicate samples. Control growth exceeded the minimum requirement for test validity. The 25 % inhibition concentration (IC25) is the reported endpoint as part of the MMER test protocol. The IC25 values for algal and macrophyte growth were calculated by linear interpolation using the Comprehensive Environmental Toxicology Information System based on measured concentrations.

Finally, inhibition of reproduction in *C. dubia* was tested (Environment Canada, 1992b). As for *D. magna*, organisms were obtained from Aquatic Research Organisms. For *C. dubia*, however, the M4 medium (Elendt, 1990) was used for cultures and for the control. The C. *dubia* test was conducted as a comparison between control and undiluted effluent, with $n = 10$. For both tests, no mortalities were observed in the

control, and the total number of neonates in the control exceeded the test validity requirements.

4.3.4 Toxicity Identification Evaluation (TIE)

Iron and ammonia were the substances suspected of causing toxicity on *D. magna*, based on their high concentrations (total ammonia, > 4.2 mg/L; total iron, 79-198 mg/L) in the treated effluents (Table 4.1) and on reference toxicity values for *D. magna* for the metals and ammonia (U.S. EPA, 2000). Treatments of samples included metal chelation with EDTA, aeration, pH modification, and spikes of ammonia or iron to discriminate between the two toxicants. The first treatment consisted of an EDTA spike (final concentration, 0.238 mM or 0.08 g/L) and the same spike followed by a pH adjustment to 9.3 and aeration for 1 h (ESG, 2002). After each treatment, the sample pH was readjusted to its initial pH just before toxicity testing. In the second treatment, only aeration for 2 h was used. An ammonia spike test was then carried out on 10-d HRT effluent using NH_4Cl (final concentration, 10 mg/L NH_4^+). Finally, an iron-only effluent, which was prepared to match the highest iron concentration (100 mg/L Fe) of 10-d HRT effluent using $FeSO_4 \cdot 7H_2O$ and the same water used as control in the serial dilution preparation, and the iron-only effluent spiked with EDTA (final concentration, 0.238 mM or 0.08 g/L) were tested for toxicity. The iron-only effluent did not contain ammonia. Control tests using unmodified samples were performed with each series of modified samples to confirm the absence of toxicity changes caused by TIE manipulations alone.

4.3.5 Metal speciation

Metal partitioning in AMD and treated effluent from passive bioreactors was assessed by measuring metal concentrations in nonfiltered samples (total recoverable fraction), in samples filtrated at 0.45μm (colloidal and soluble fraction), and in samples ultrafiltrated at 1kDa (soluble fraction). Filtrations/ultrafiltrations were performed under a nitrogen

atmosphere using a stirred ultrafiltration cell (Amicon model 8200; Millipore, Bedford, MA, USA) and regenerated cellulose membranes (YM1, 63.5 mm diameter, Millipore). The objective was to evaluate changes in the concentrations of dissolved (< 0.45µm) and ultra-dissolved (< 1 kDa) metals for conditions representative of the toxicity tests.

Table 4.1 Physicochemical characterization of columns' feed (acid mine drainage [AMD]) and treated effluent from bioreactors [a]

Parameter (mg/L, if not otherwise specified)	AMD	7.3 d HRT effluent	10 d HRT effluent
pH	5.4±0.4	6.0±0.1	6.2±0.2
Oxydoreduction potential (mV)	70±40	23±21	17±19
Alkalinity (mg/L $CaCO_3$)	NA	92±32	212±106
SO_4^{2-}	3,316±210	2,863±234	2,619±287
S^{2-}	NA	< DL	< DL
Fe^{2+}	349±51	99±12	48±17
NH_3-NH_4^+	NA	32±7	6±5
Na^+	844	881±7	874±27
K^+	72	79±1	77±3
Ca^{2+}	409	547±13	536±79
Mg^{2+}	105	110±1	105±12
Fe	555±86	198±51	79±36
Mn^{2+}	10.2±0.2	10.9±0.3	11.6±1.0
Cd^{2+}	12±4	< 0.03	< 0.03
Ni^{2+}	15.4±0.4	0.09±0.02	0.07±0.02
Zn^{2+}	17±1	0.018±0.004	0.017±0.006
Total organic carbon	-	6±2	7±3
Dissolved organic carbon	-	5±2	6±2

[a] Values are presented as the mean ± standard deviation of physicochemical parameters determined weekly during a seven-week period.

< DL = less than the detection limit (0.01 mg/L of S^{2-})

HRT = Hydraulic Retention Time

NA = Not applicable; - = not analyzed.

Metals were analyzed in samples collected just before starting the toxicity tests (0 h), after 48 h of static exposure (as in the *D. magna* toxicity test), and after 96 h of aeration (as in the rainbow trout toxicity test). The retentate was not analyzed. The filtrate was acidified at 2% (v/v) with concentrated HCl, and nine metals (sodium, potassium, calcium, magnesium, iron, manganese, cadmium, nickel, and zinc) were analyzed.

Metal speciation of nonfiltered samples was also estimated using the thermodynamic chemical equilibrium model VMINTEQ (Ver 2.52) (http://www.lwr.kth.se/English/OurSoftware/vminteq/), which allows direct identification of potential precipitates. The model calculates saturation indices of various mineral phases based on geochemical processes, such as complexation, dissolution/precipitation, oxidation/reduction, ion exchange, and gas equilibrium.

For the discussion and interpretation of the measured metal speciation, equilibrium calculations were also conducted using the WHAM VI geochemical model (Tipping, 1998). Two particulate fractions were considered (humic and fulvic acids) as well as three colloidal fractions (humic and fulvic acid as well as iron oxides). Default hypotheses were used regarding the nature of the organic carbon: 50% of the organic carbon being humic substances, a 9:1 fulvic to humic acid ratio, and a fulvic acid and humic acid carbon content of 50%. Dissolved inorganic carbon concentrations were estimated based on the measured pH and alkalinity for the two effluents or calculated based on a pCO_2 of 3.2×10^{-4} atm for the AMD.

The particulate organic carbon was estimated by the difference between the total organic carbon and DOC. The entire DOC was considered to be colloidal. The calculated Fe^{3+} concentration (difference between total iron and Fe^{2+}) was used as an estimate for the colloidal iron oxide concentration. All other parameters were taken directly from Table 4.1.

4.4 Results

4.4.1 Sample physicochemical characteristics

The highly contaminated AMD treated in bioreactors (Table 4.1) had a pH appropriate for sulfate-reducing bacteria growth (pH>5), high sulfate concentrations (3,316±210mg/L) and metal concentrations of 10.2±0.2mg/L for manganese, approximately 15 mg/L for cadmium, nickel and zinc, and 555±86 mg/L for iron. The quality of 10-d HRT effluent was better than that of 7.3-d HRT effluent (Table 4.1). The 10-d HRT effluent had significantly higher pH and alkalinity as well as lower sulfate, ammonia, total iron, and ferrous iron concentrations compared to those of 7.3-d HRT effluent; however, ORP, metals (nickel and zinc) and major cations (sodium, potassium, calcium, and magnesium) had comparable concentrations in both effluents.

Moreover, nickel, cadmium, and zinc concentrations were very low ($\leq$ 0.085 mg/L) compared to the AMD concentrations, whereas manganese concentrations were high (10.9-11.6 mg/L), which was an indication that this metal was not successfully removed from AMD (10.2 ± 0.2 mg/L). During the seven-week study period, the coefficient of variation of quality parameters for the two effluents was the highest for ORP (91-114%) and the lowest for pH (1-2%). Other parameters recorded relatively small variation, such as Fe^{2+} (12-35%), SO_4^{2-} (8-11%), alkalinity (35-50%), and metals (manganese, 3-9%; nickel, 22-28%; zinc, 23-33%; and iron, 26-45%). These values confirm the relative treatment system stability during the seven weeks of sampling and toxicity testing.

4.4.2 Toxicity tests

Acute toxicity

The 10 d HRT effluent manifested no mortality for rainbow trout even in the undiluted effluent (Table 4.2). For the cladoceran *D. magna*, AMD was the most toxic, with an LC50 of 9%, whereas 10-d HRT effluent was less toxic than 7.3-d HRT effluent, with

average LC50s of 53 and 25%, respectively (n = 2). For each effluent, toxicity results from the two tests were similar, with overlapping 95% confidence intervals for their respective LC50 estimates.

Table 4.2 Acute toxicity on rainbow trout (*Oncorhynchus mykiss*) and water flea (*Daphnia magna*) with unmodified samples from passive bioreactors [a]

Organism	Test No.	Sample	LC50 (%, v/v)
O. mykiss	1	10d HRT effluent	>100
D. magna	1	AMD	9 (7–11)
		7.3d HRT effluent	27 (22-33)
		10d HRT effluent	44 (33–58)
	2	7.3d HRT effluent	23 (19-29)
		10d HRT effluent	62 (52-73)

[a] Toxicity is expressed as the 48-h median lethal concentration (LC50) with 95% confidence intervals given in parentheses

AMD = Acid Mine Drainage; HRT = Hydraulic Retention Time.

Sublethal toxicity

As for *D. magna*, toxicity data regarding the algae *P. subcapitata* also indicated that the 10-d HRT effluent was less toxic than AMD and the 7.3-d HRT effluent, with an IC25 of 17% for the 10-d HRT effluent compared with 1.2% for the AMD (Table 4.3). It was noteworthy that the IC25 of 0.8 % for 7.3-d HRT effluent was not statistically different compared to AMD, which indicates that this treatment did not significantly reduce the toxicity to the algae.

The 10-d HRT effluent was also tested for growth inhibition to *L. minor* using frond count and dry weights. Based on both endpoints, *L. minor* was less sensitive than *P. subcapitata* to the effluent. For the water flea *C. dubia* (results not shown), all samples were lethal after less than 24 h of exposure. This could be considered as acute lethality.

Table 4.3 Sublethal toxicity on *Pseudokirchneriella subcapitata* and *Lemna minor* with unmodified samples from passive bioreactors [a]

Organism	Test No.	Sample	IC25 (%, v/v)	IC50 (%, v/v)
P. subcapitata	1	AMD	1.2 (0.8–2.2)	5.3 (1.5–20.9)
		10d HRT effluent	17 (12–20)	24.2 (22.6–N/A)
		7.3d HRT effluent	0.8 (0.7-1.0)	7.3 (N/A-10.0)
L. minor	2	10d HRT effluent	Frond count: 66 (52-74)	Frond count: 90.5 (82.2-99.5)
			Total dry weight: 82 (70-104)	Total dry weight: >100 (N/A-N/A)

[a] Toxicity is expressed as the 25 and 50% inhibition concentration (IC25 and IC50), with 95% confidence interval given in parenthesis.

AMD = Acid Mine Drainage; HRT = Hydraulic Retention Time; NA = Not Available.

Because undiluted AMD and both effluents failed the acute nonlethality requirement on *D. magna* (Table 4.2), the present study was continued with modified effluent during a TIE procedure.

4.4.3 Toxicity Identification Evaluation (TIE)

When EDTA addition was performed alone to modify the samples, it did not reduce toxicity on *D. magna* for both treated effluents, suggesting that metal complexation had little or no effect on the toxicity of these effluents (Table 4.4).

Moreover, toxicity of iron-only effluent increased after EDTA addition. When the EDTA addition was coupled with 1h aeration at pH 9.3 (ESG, 2002), however, the toxicity was eliminated from the 10-d and 7.3-d HRT effluents. Toxicity removal was also observed after 2h of aeration alone for the 10-d HRT effluent.

Table 4.4 Survival of cladoceran *Daphnia magna* when exposed to modified samples during toxicity identification evaluation [a]

Test No.	Sample	Treatment	Survival [b] (%)
1	10 d HRT effluent	Control	40 ± 26
		+0.238mM EDTA	43 ± 31
		+0.238mM EDTA+1 h aeration at pH 9.3	100 ± 0
	7.3 d HRT effluent	Control	0 ± 0
		+0.238mM EDTA	0 ± 0
		+0.238mM EDTA+1 h aeration at pH 9.3	100 ± 0
2	10 d HRT effluent	Control	23 ± 15
		+2 h of aeration	100 ± 0
	7.3 d HRT effluent	Control	0 ± 0
		+2 h aeration	0 ± 0
3	10 d HRT effluent	Control	7 ± 12
		+10 mg/L NH_4^+	7 ± 12
		10 mg/L NH_4^+ +1 h aeration	33 ± 42
4	Iron-only effluent [c]	Control	92 ± 1
		+0.238mM EDTA	35 ± 18

[a] EDTA = ethylenediaminetetra-acetic acid; HRT – Hydraulic Retention Time.

[b] Survival refers to the proportion (%) surviving at 48h and it is presented as the mean value ± standard deviation (n = 3).

[c] Reconstituted hard water plus 100 mg/L of iron.

In contrast, toxicity was unaffected by the 2h of aeration alone for the for 7.3-d HRT effluent, in which no survival was observed. The addition of ammonia (10 mg/L NH_4^+) either alone or with aeration did not significantly affect the toxicity of the 10-d HRT effluent.

A common observation made during toxicity tests was that iron oxidation (from Fe^{2+} to Fe^{3+}) and its subsequent precipitation as (oxy)hydroxide minerals increased turbidity of all samples.

4.4.4 Metal speciation

Major cations (sodium, potassium, calcium, and magnesium) and metal concentrations (iron, manganese, cadmium, nickel, and zinc) in the AMD and both effluents, expressed as the total recoverable fraction, as well as in the less-than-0.45-μm and in the less-than-1-kDa fractions are presented in Figures 4.1 to 4.3. Generally, in all samples, data indicate that colloidal (< 0.45 μm and > 1 kDa) and truly soluble (< 1 kDa) metal concentrations decreased slightly after 48 h of static exposure compared to their initial concentrations (0 h) but increased after 96 h of aeration. In both effluents (Figs. 4.2 and 4.3), however, the total recoverable fraction was close to the 0.45-μm filtrate fraction and the 1-kDa ultrafiltrate fraction for cadmium, manganese, nickel, and zinc. In the case of AMD, total recoverable concentrations of cadmium, manganese, nickel, and zinc after 96 h of aeration were lower than the 0.45-μm filtrate fraction. A nonhomogenous sample or an incomplete dissolution of all the precipitate in the sample analyzed for total recoverable metal concentrations could explain this observation.

Results on metal speciation obtained by geochemical equilibrium modeling (VMINTEQ) on all unfiltered samples (0 h) indicated iron (oxy)hydroxide minerals could precipitate as ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), goethite (FeO(OH)), K-jarosite ($KFe_3(SO_4)_2(OH)_6$), lepidocrocite (FeO(OH)), maghemite (Fe_2O_3), magnesioferrite ($MgFe_2O_4$), and magnetite (Fe_3O_4).

Moreover, the model predicted precipitation of carbonate minerals, such as siderite ($FeCO_3$) in both effluents, whereas in 10 d HRT effluent, nickel carbonate ($NiCO_3$) and rhodochrosite ($MnCO_3$) were also possible precipitates. Results obtained with the VMINTEQ model also indicated that for cadmium, nickel, and zinc, the dissolved fraction in unmodified samples varied from 80 to 98.5% in 10-d HRT and 7.3-d HRT effluents, respectively.

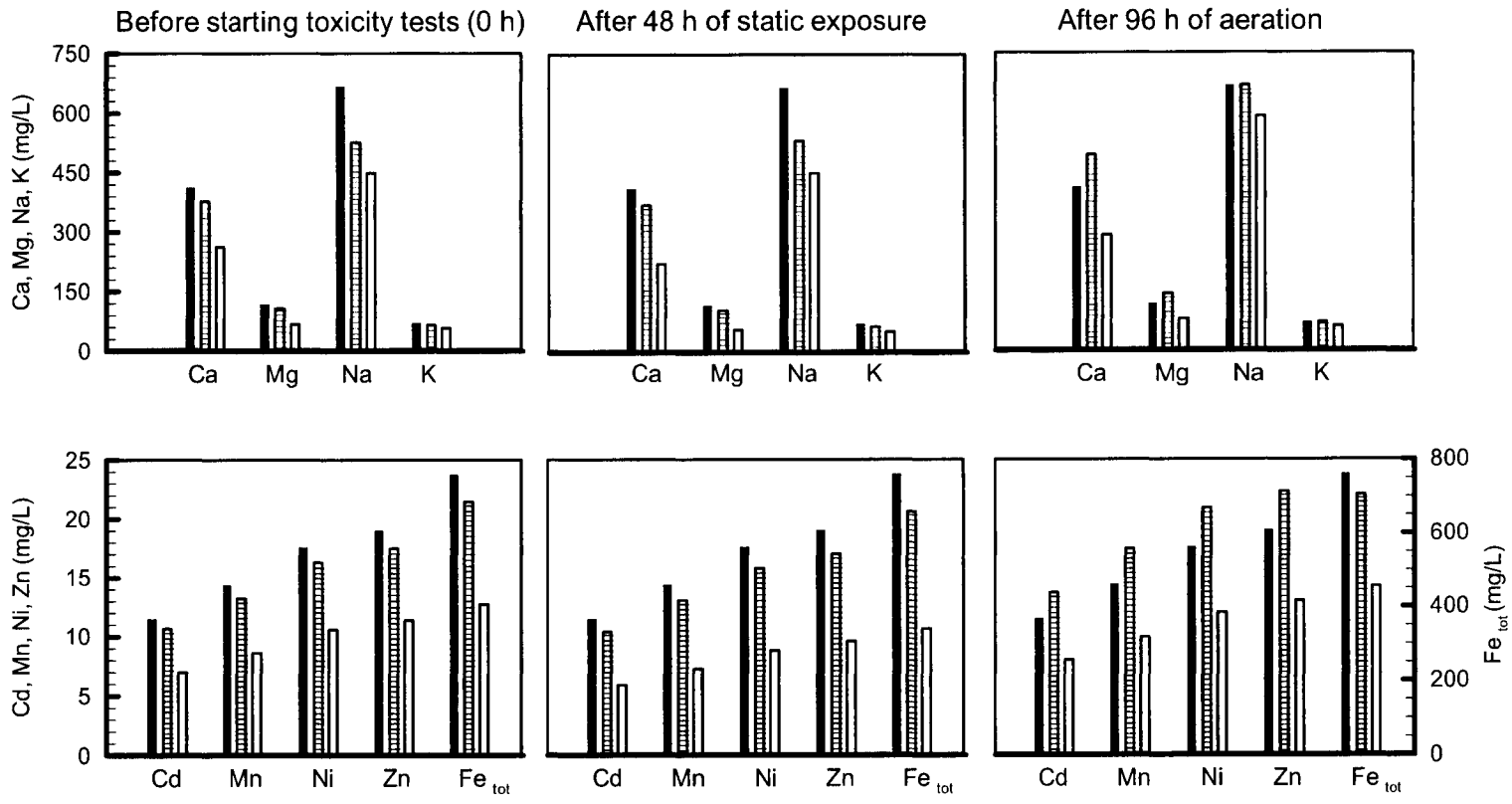

Figure 4.1 Metal partitioning in highly contaminated acid mine drainage.(■ = Total recoverable (unfiltered); ▤ = less-than-0.45-μm fraction; □ = less-than-1-kDa fraction)

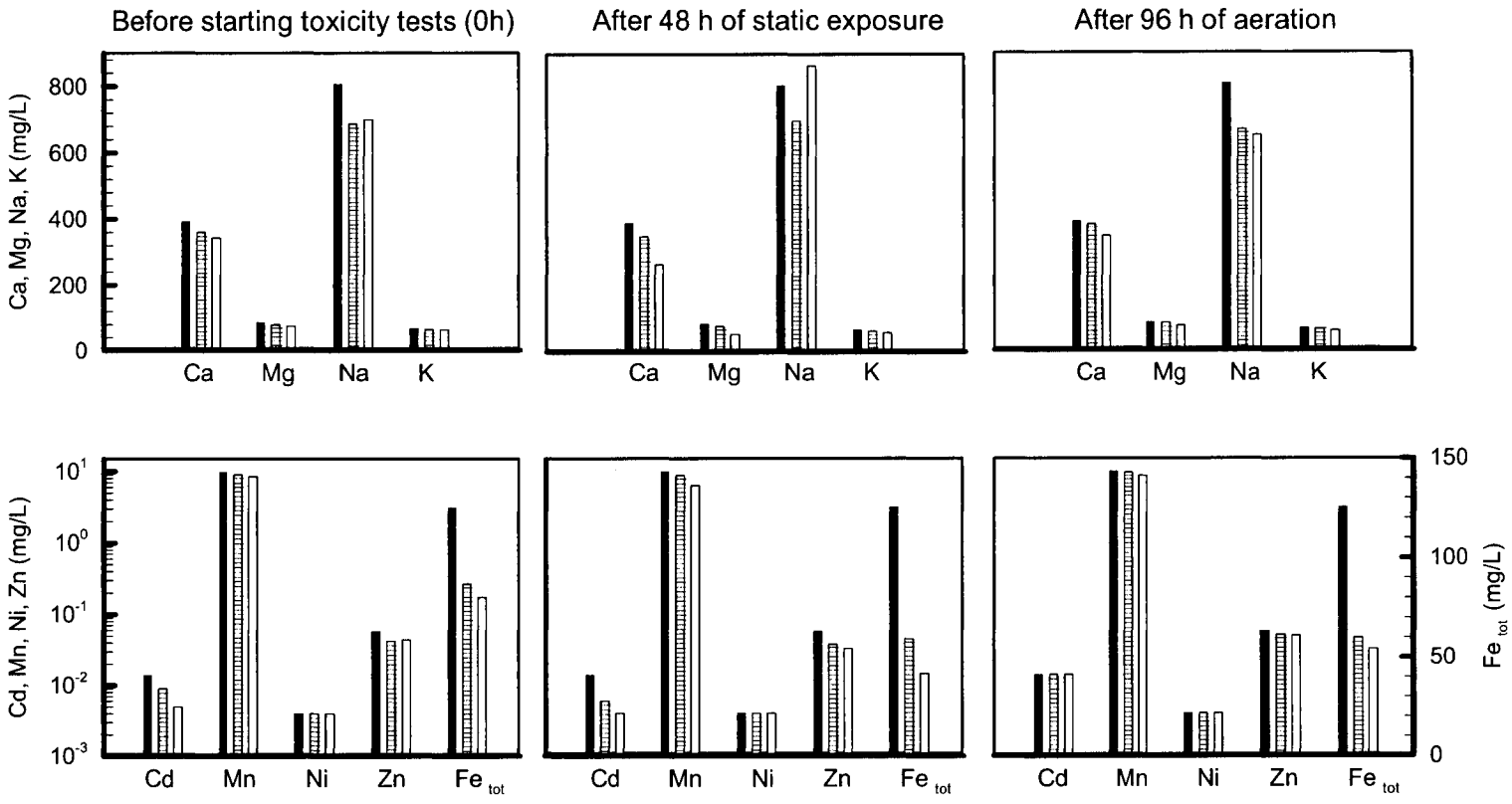

Figure 4.2 Metal partitioning in treated effluent from bioreactors operated at 10 d HRT (hydraulic retention time). (■ = Total recoverable (unfiltered); ▤ = less-than-0.45-μm fraction; □ = less-than-1-kDa fraction)

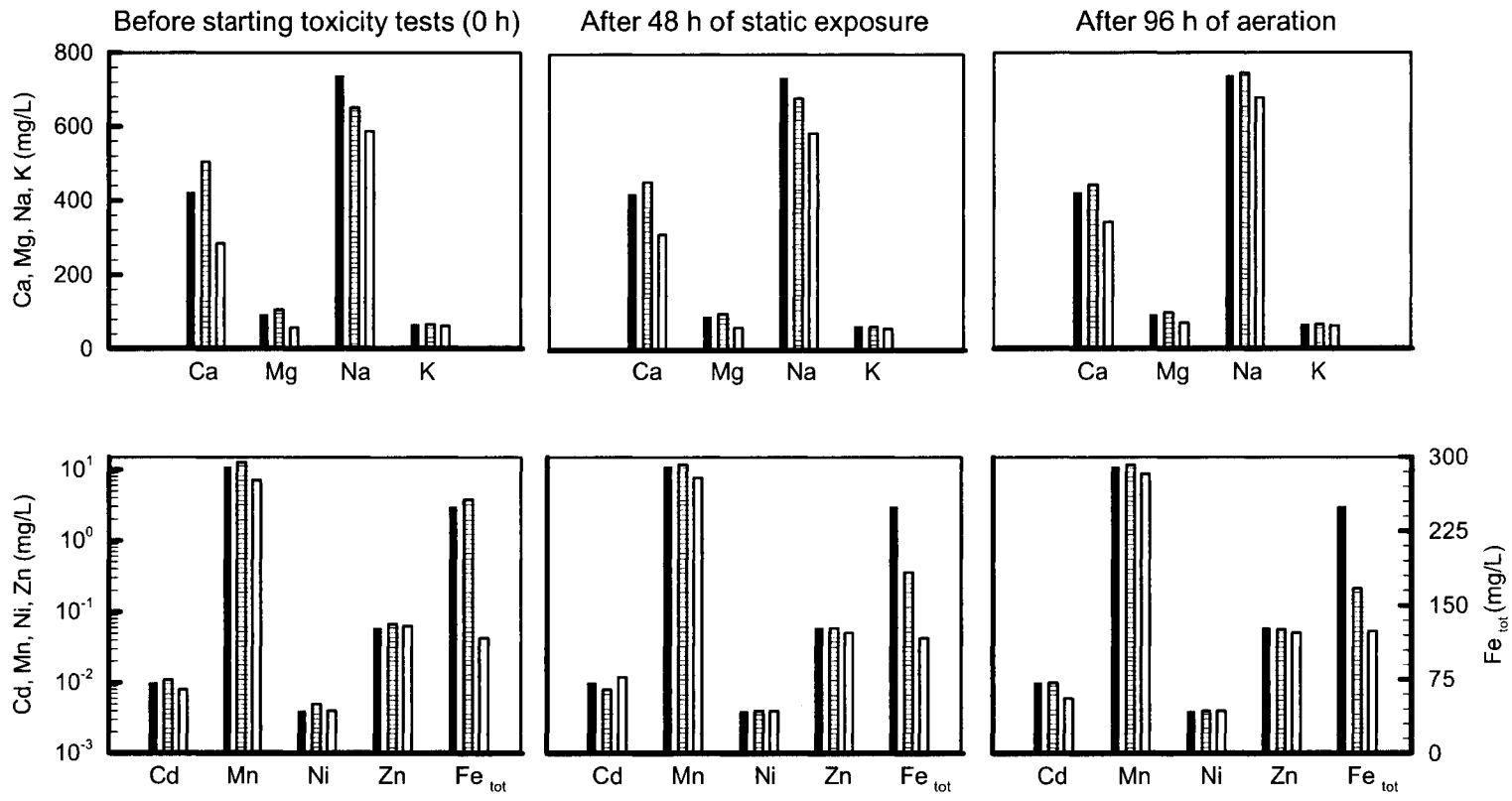

Figure 4.3 Metal partitioning in treated effluent from bioreactors operated at 7.3 d HRT (hydraulic retention time).
(■ = Total recoverable (unfiltered); ▤ = less-than-0.45-μm fraction; □ = less-than-1-kDa fraction)

Complexation of cadmium, nickel, and zinc (19-20% of dissolved fraction) by DOC found in effluents was also indicated by estimation using VMINTEQ. Further estimations with WHAM VI confirmed that nickel, zinc, and cadmium are expected to be predominately in the ultradissolved fraction of both treated effluents.

For the 10 d HRT effluent, 98, 96, and 97% of the total recoverable fraction was estimated to be in the ultradissolved fraction, either as free ions or complexed by inorganic ligands. In terms of potential colloidal and solid phases for these metals, colloidal iron oxides would be 3- to 40-fold more important than colloidal humic substances and 20- to 110-fold more important than particulate humic substances. Only a small fraction of the metals, however, would be bound to iron oxides ranging from 0.019% for cadmium in the 10-d HRT effluent to 0.075% for zinc in the 7.3-d HRT.

4.5 Discussion

Physicochemical characterization of the treated AMD effluent indicates that both HRTs successfully treated the highly contaminated AMD by raising pH and alkalinity and by removing sulfate and metals, but the 10-d HRT was more effective. In both effluents tested, very high removal of cadmium, nickel, and zinc was observed (99.8, 99.5, and 99.9%, respectively). Treated effluent from the bioreactors operated at HRT of 10 d, however, had a significantly higher pH and alkalinity compared with treated effluent from bioreactors operated at a HRT of 7.3 d. Moreover, iron and sulfate removal was better in 10-d HRT effluent (85.7 and 21.0%, respectively) than in 7.3-d HRT effluent (64.4 and 13.7%, respectively). Therefore, a higher HRT (10 d) was more effective than a lower HRT (7.3 d). It should be noted that manganese was not removed from AMD regardless of the treatment retention time; in fact, manganese concentrations slightly increased. This is not unusual, because the column bioreactors were filled with a mixture of four natural organic materials that contained up to 380 mg/kg of manganese. Moreover, low manganese removal during biological treatment of AMD has previously been reported (Neculita *et al.*, 2007), being attributed to a higher solubility of

manganese sulfide (solubility product, [K_{sp}] ~ 10^{-16}) compared to other metal sulfides (K_{sp} = 10^{-19} to 10^{-38}). In the early phase of bioreactor operation, manganese can be removed as carbonate minerals (K_{sp} ~ 10^{-11} for rhodochrosite, [$MnCO_3$]), while MnS is formed later, during bioreactor operation, if high concentrations of sulfides are generated in the treatment system.

The treated effluent with a 10-d HRT would meet the Canadian discharge limit on acute lethality for rainbow trout but not the provincial requirements for *D. magna* without adding post-passive treatment aeration. The 10-d HRT effluent exhibited no mortality to rainbow trout; however, the effluent was acutely lethal to *D. magna* without a substantial aeration (2 h) (Table 4.4). Canadian regulations contain no requirements in terms of sublethal toxicity; nevertheless, they are used to monitor effluent quality. For the algae *P. subcapitata*, both effluents were more toxic (IC50, 7 and 24%, for 7.3-d HRT and 10-d HRT, respectively) than in the study of Riesen *et al.* (2005), who reported an IC50 greater than100% for a biologically treated AMD from a sulfate-reducing bioreactor. A highly contaminated AMD (the worst-case scenario), however, was treated in the present study, and the effluent samples were tested without adjustments of pH and conductivity, as prescribed in the Canadian regulations. The macrophyte *L. minor* was approximately four-fold less sensitive than *P. subcapitata* (Table 4.3), and toxicity would be mitigated more rapidly by dilution. As for *D. magna*, *C. dubia* was very sensitive to the treated effluent, which in fact was acutely lethal for this species, with 100% mortality rates observed within 24 h. Song *et al.* (2001) also have reported complete mortalities for *C. dubia* for undiluted passive treatment effluents.

In accordance with data on physicochemical characterization, the toxicity data also indicated that the 10-d HRT was more effective. Ecotoxicological endpoints for the cladoceran *D. magna* and the algae *P. subcapitata* showed similar profiles, with the highest toxicity manifested by AMD (and, in the case of *P. subcapitata*, the 7.3-d HRT effluent) and the lowest by 10-d HRT effluent. Iron oxidation and precipitation was

obvious during the course of the toxicity tests, with increased turbidity of the samples. Pre-aeration, which was performed in *D. magna* toxicity tests, or aeration as required in the rainbow trout toxicity testing, accelerated the iron oxidation and precipitation kinetics. Iron oxidation during pre-aeration was accompanied by a decrease of up to one pH unit for AMD and 7.3-d HRT effluent, from around pH 6.0 to pH 5.0, likely because the alkalinity was insufficient to buffer the acidity generated by ferric (oxy)hydroxide precipitation. In contrast, the pH of the 10-d HRT effluent increased up to 1.3 units, from pH 6.2 to pH 7.5, which likely is related to the lower iron concentrations and higher alkalinity that characterized the 10-d HRT effluent (Table 4.1).

Based on the comparison of concentrations in the treated effluents with toxicity values from the literature, it was concluded that only NH_4^+ and Fe were potential toxicants for the observed acute toxicity to *D. magna*. Iron was identified as a potential toxicant for *D. magna*, because iron concentrations in all tested samples were three- to six-fold higher than the reported EC50s (15-17 mg/L) for this organism (Hockett and Mount, 1996; Sorvari and Sillanpää, 1996). For ammonia, the concentrations in the 7.3-d HRT also exceeded the reported toxicity endpoints (LC50, 25.4 mg/L) (Parkhurst *et al.*, 1979). This was not the case, however, for the other metals and sulfate. Acute toxic effects were not assessed for cadmium because its concentrations were lower than the reported EC50s (0.980 mg/L; Sorvari and Sillanpää, 1996). Concentrations of zinc and nickel were also much lower than the expected LC50 for *D. magna* (4.0 mg/L for zinc (Guilhermino *et al.*, 2000) and 2.8 mg/L for nickel (Belabed *et al.*, 1994)). Finally, sulfate was also ruled out as a potential toxicant based on the available toxicity data (LC50 of 1.82 to 6.29 g/L (Mount *et al.*, 1997) when tested as Na_2SO_4, K_2SO_4, $CaSO_4$, or $MgSO_4$).

The treatments performed during TIE allowed the elimination of toxicity on *D. magna* and suggested Fe as the toxicant. Depending on the metal load, EDTA concentrations between 0.1g/L and 0.8g/L are suggested for toxicity removal when metals are suspected as a source of toxicity (ESG, 2002). In the present study, the EDTA spike

(final concentration, 0.08g/L) did not eliminate toxicity (Table 4.4). This does not rule out iron as a potential toxicant, because we later estimated, using WHAM VI with a modified database, that only approximately 5% of the dissolved Fe^{2+} would be complexed by EDTA in the treated effluents at the EDTA concentration used. Furthermore, other studies have reported little or no effect of EDTA addition on Fe toxicity (Hockett and Mount, 1996; Sorvari and Sillanpää, 1996). When the EDTA spike was performed at pH 9.3 and followed by 1 h of aeration before testing (ESG, 2002), the toxicity to *D. magna* was eliminated from both effluents (Table 4.4). Increased toxicity of iron-only effluent after EDTA addition was also observed (Table 4.4). The lower hardness in the reconstituted hard water used for the iron-only effluent, compared to the treated effluent could be associated with an increase in sensitivity of *D. magna* to EDTA (Sorvari and Sillanpää, 1996). Based on the TIE testing conducted for *D. magna*, we presume that the observed acute toxicity to *C. dubia* is also related to Fe^{2+} oxidation.

Following this observation, aeration alone was tested, because it is relatively easy to perform in situ on treated effluent from passive bioreactors. Results showed that 2 h aeration removed toxicity from 10-d HRT effluent, whereas 7.3-d HRT effluent became nontoxic if aeration for 2 h was performed after increasing the pH to 9.3.

Metal speciation analysis supports the hypothesis of iron toxicity. Results indicated that after 48 h of static exposure without aeration (Fig. 4.2) - that is, in conditions similar to the *D. magna* test - the ultradissolved fraction (<1kDa) of iron, cadmium, manganese, and zinc decreased in 10-d HRT effluent and the effluent showed no toxicity to *D. magna*. As mentioned previously, it is worth noting that cadmium, manganese, and zinc concentrations in the total recoverable fraction already were lower than toxicity thresholds. Iron concentrations in the ultradissolved fraction after 48 h of static exposure of 10d HRT effluent, however, were higher (41.8 mg/L) than the iron LC50 (15-17 mg/L). Moreover, after 96 h of aeration, in conditions similar to the rainbow trout test, all other metals except iron (i.e., cadmium, manganese, nickel, and zinc) had a

total recoverable fraction that was equal to the ultradissolved fraction (<1kDa) and lower than their expected toxicity thresholds. As for the 48 h treatment without aeration, the 96 h aeration, residual iron concentrations of 41.8 mg/L (10-d HRT effluent) and 117.3 mg/L (7.3-d HRT effluent) in the ultradissolved fraction were higher than the LC50 of 20.8 mg/L (U.S. EPA, 2000). We actually have measured no rainbow trout mortality in the undiluted 10-d HRT effluent, which could be related, in part, to complexation. Estimations with WHAM VI suggest that approximately 50% of the Fe^{2+} would be complexed by dissolved ligands in this effluent.

In the 7.3-d HRT effluent (Fig. 4.3), all metals were already in the ultradissolved fraction after 48 h of static exposure except for iron, the concentrations of which increased, and for cadmium, the concentrations of which decreased in the less-than-1-kDa fraction. In the case of iron, its oxidation and precipitation affected AMD and the two effluents in different ways. The buffering capacity of all samples influenced the dissolved iron concentrations and toxicity. As a consequence, iron concentrations in the ultradissolved fraction were lower after 48 h of static exposure or after 96 h of aeration (AMD, 341 mg/L vs an initial 456 mg/L; 10-d HRT effluent, 42 mg/L vs an initial 54 mg/L; 7.3-d HRT effluent, 117 mg/L vs an initial 124 mg/L). As expected, in AMD, the absence of alkalinity resulted in a higher ultradissolved fraction of iron after 96 h of aeration. In 7.3-d HRT effluent, the fraction less-than-1-kDa was the same at the beginning (i.e., 0 h) as it was after 48 h of static exposure, whereas in 10-d HRT effluent the same fraction initially was higher (iron, 80 mg/L), and decreased steadily during the aeration process. Results of theoretical metal speciation as modeled on unmodified samples with VMINTEQ were in agreement with results of metal fractionation analysis. Initially (i.e., at 0 h), between 0.3 and 23.2% of the dissolved fraction of all metals was complexed with DOC, whereas after 48 h of static exposure (7.3-d HRT effluent) or after 96 h of aeration (10-d HRT effluent), the ultra-dissolved fraction was equal to the total recoverable fraction.

4.6 Conclusion

The six 3.5-L column bioreactors filled with mixtures constituted from several natural organic carbon sources (maple wood chips and sawdust, leaf compost, and poultry manure) and continuously operated (in triplicate) at two different HRTs of 10 d and 7.3 d efficiently treated highly contaminated AMD (the worst-case scenario) by increasing its pH and alkalinity and by removing sulfate and metals. Physicochemical and toxicological parameters measured on treated effluent from columns operated at both HRTs indicated good reproducibility and proved the stability of the treatment systems under continuous operation. The treated effluent collected from columns operated at 10-d HRT, however, had better physicochemical quality in terms of pH, alkalinity, and iron and sulfate concentrations compared to the effluent collected from columns operated at 7.3-d HRT.

The 10-d HRT effluent passed the Canadian mine effluent regulations requirement regarding the absence of acute lethality on rainbow trout. Additional toxicity testing indicated that AMD toxicity to *D. magna*, however, was not eliminated during treatment in bioreactors at both tested HRTs (7.3 and 10d). Acute toxicity also was observed for *C. dubia.* In terms of sublethal toxicity, the growth of the algae *P. subcapitata* was inhibited by the 10-d HRT treated effluent as was the growth of *L. minor* to a smaller extent. The toxicity to *D. magna* was eliminated during the TIE procedure, and iron was suggested as a source of toxicity. Based on the present results, AMD with high concentrations of iron (>500 mg/L) could be very challenging for treatment in passive bioreactors. Iron oxidation (from Fe^{2+} to Fe^{3+}) can decrease the pH and buffer capacity of treated water and lead to increased toxicity. Metal speciation analysis proved helpful to link iron with toxicity and in highlighting transformations that occur over the course of the toxicity tests. Finally, the present study indicated that the addition of a short aeration step to the effluent treatment would eliminate lethality to *D. magna* by increasing the dissolved oxygen concentrations and precipitating iron as (oxy)hydroxide minerals.

4.7 Acknowledgement

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CHAPITRE V

ARTICLE #5: EFFECTIVENESS OF SULPHATE-REDUCING PASSIVE BIOREACTORS FOR TREATING HIGHLY CONTAMINATED ACID MINE DRAINAGE: II. METAL REMOVAL MECHANISMS AND POTENTIAL MOBILITY

5.1 Abstract

Column bioreactors were used for studying mechanisms of metal removal, assessment of long-term stability of spent reactive mixtures, as well as potential metal mobility after treating highly contaminated acid mine drainage (AMD; pH 2.9-5.7). Several physicochemical, microbiological, and mineralogical analyses were performed on spent reactive mixtures collected from four bioreactors, which were tested in duplicate for two hydraulic retention times (7.3d and 10d), with downward flow over a 11-month period. Consistent with the high metal concentrations in the AMD feed, and with low metal concentrations measured in the treated effluent, the physicochemical analyses indicated very high concentrations of metals (Fe, Mn, Cd, Ni, and Zn) in the top and bottom layers of the reactive mixtures from all columns. Moreover, the concentrations of Fe (50.8-57.8 g/kg) and Mn (0.53-0.70 g/kg) were up to twice as high in the bottom layers, whereas the concentrations of Cd (6.77-13.3 g/kg), Ni (1.80-5.19 g/kg), and Zn (2.53-13.2 g/kg) were up to 50-times higher in the top layers. Chemical extractions and elemental analysis gave consistent results, which indicated a low fraction of metals removed as sulphides (up to 15% of total metals recovered in spent reactive mixtures). Moreover, Fe and Mn were found in a more stable chemical form (residual fraction was 42-74% for Mn and 30-77% for Fe) relative to Cd, Ni or Zn, which seemed more weakly bound (oxidisable/reducible fractions) and showed higher potential mobility. Besides identifying (oxy)hydroxide and carbonate minerals, the mineralogical analyses identified metal sulphides containing Fe, Cd, Ni, and Zn. Metal removal mechanisms

were, therefore, mainly adsorption and other binding mechanisms with organic matter (for Cd, Ni, and Zn), and the precipitation as (oxy)hydroxide minerals (for Fe and Mn). After 15 months, however, the column bioreactors did not lose their capacity for removing metals from the AMD. Although the metals were immobile during the bioreactor treatment, their mobility could increase from spent reactive mixtures, if stored inappropriately. Metal recovery by acidic leaching of spent substrates at the end of bioreactor operation could be an alternative.

Keywords: Acid mine drainage (AMD), Sulphate-reducing passive bioreactor; Metal removal mechanisms, Remobilization potential, Mineralogical analysis

5.2 Introduction

Mining and metallurgical industries deal with contaminated acidic waters (often called acid mine drainage AMD or acid rock drainage ARD), which are generated by sulphide oxidation during exposure of tailings to oxygen and water, in the absence of sufficient neutralizing minerals. Acid mine drainage is characterized by low pH and high sulphate and metal concentrations (e.g. Ritcey, 1989; Blowes, 2003). To avoid significant environmental impacts, AMD contaminated waters require effective technologies for their long-term treatment (Neculita *et al.*, 2007).

Lately, there has been an increasing interest in sulphate-reducing passive bioreactors as an alternative technology to traditional water treatment plants for AMD treatment at closed sites, without electricity, and under extreme winter conditions (Kuyucak *et al.*, 2006). Passive bioreactors use sulphate-reducing bacteria (SRB) which are capable through their metabolism, of increasing the pH and alkalinity of contaminated water and of immobilizing dissolved metals by precipitating them as metal sulphides. The main mechanism of metal removal in bioreactors is precipitation in the form of (oxy)hydroxide, carbonate, and sulphide minerals (Neculita *et al.*, 2007). Sorption mechanisms (e.g. adsorption, surface precipitation) and co-precipitation with (or adsorption onto) Fe and Mn oxides can also occur.

Metal removal mechanisms change during the life of a passive bioreactor (Neculita *et al.*, 2007). Upon start-up, the adsorption of dissolved metals onto organic sites in the substrate material, as well as (oxy)hydroxide and carbonate mineral precipitation are important processes of metal removal (Machemer and Wildeman, 1992; Gibert *et al.*, 2005; Zagury *et al.*, 2006). Over time, the adsorption sites become saturated and, once sulfate-reducing conditions are established, sulphide precipitation becomes the predominant mechanism of metal removal (Machemer and Wildeman, 1992). In the case of iron, the sulphides (H_2S and HS^-) generated through SRB metabolism react with dissolved Fe and precipitate amorphous sulphides (Pósfai *et al.*, 2001). The more reduced the environment, the more reduced are the forms of sulphides (Herbert *et al.*, 1998). Amorphous Fe sulphides such as greigite, and mackinawite are very common metastable iron sulphides generated by biologically induced mineralization. They act as precursors in the formation of pyrite (FeS_2) in highly reducing environments through a series of solid-state transformations (Machemer *et al.*, 1993; Pósfai *et al.*, 2001).

The SRB favour the creation of an optimal chemical environment for sulphide precipitation; however, SRB do not control the growth of sulphide particles which can have a broad size and irregular spatial distribution (Pósfai *et al.*, 2001). Pyrite formation can, however, be limited by the rate of SO_4^{2-} reduction, and by Fe availability (Machemer *et al.*, 1993).

Solid phase analysis is therefore an important step for elucidating metal removal mechanisms. Moreover, an important objective of passive bioreactors is to ensure the stability of the spent reactive mixtures which contain metal precipitates. The stability of spent reactive mixtures depends on the metals' potential mobility, which is related to the quality of AMD treated, as well as to metal removal mechanisms. Results from geochemical modeling confirm that adsorption and precipitation of metal carbonates and (oxy)hydroxides can occur in batch organic-based bioreactors (Waybrant *et al.*, 1998; Zagury *et al.*, 2006; Neculita and Zagury, 2008).

Post-treatment analysis of reactive mixtures can provide information on how efficient the system is in removing metals, how long it will last, how available the metals are to remobilization, and on stability of the environment over time (Machemer *et al.*, 1993).

Mineralogical analyses may help to identify the chemical form of metals retained in the solid phase. Few techniques are appropriate for the mineralogical analysis of spent reactive mixtures due to the poor crystallinity of the precipitates and/or the relatively low concentrations of metal sulphides (Song, 2003; Gibert *et al.*, 2005). Among these methods, scanning electron microscopy equipped for backscattered electron imaging (SEM-BSE) has been the most successful technique, whereas X-ray diffraction or iron Mossbauer analyses have been less effective in detecting amorphous metal sulphides (Machemer *et al.*, 1993). The SEM approach coupled with X-ray microanalysis has been proven successful for identifying sulphides in reactive mixtures from constructed wetlands (Machemer *et al.*, 1993; Song, 2003), in reactive permeable walls (Herbert *et al.*, 1998) and in passive bioreactors (Gibert *et al.*, 2005; Neculita *et al.*, 2006). Also, amorphous iron and lead sulphides were reported by Song (2003) using SEM, while makinawite and greigite were found in the study of Herbert *et al.* (1998). However, sulphides were not detected in spent reactive mixtures after 158 days of bioreactor operation (Gibert *et al.*, 2005) whereas in a more recent study, SEM analysis indicated the presence of amorphous pyrite in the solid phase from a 350-day batch bioreactor (Neculita *et al.*, 2006).

Chemical analyses such as sequential extraction procedures (SEPs) combined with simultaneous determination of acid volatile sulphides and extracted metals (AVS-EM) are also efficient tools for assessing metal fractionation and potential mobility (Song, 2003; Jong and Parry, 2004). The SEP represents an important, widely used, and useful tool for gaining information on the potential mobility, potential bioavailability and toxicity of metals in the environment (Bacon and Davidson, 2008). The ranking of metal mobility in SEPs is based on metal concentrations in the water-soluble and exchangeable fraction, as well as fractions that are reducible, bound to carbonate, and

bound to organic matter or sulphides. The results from SEPs therefore provide additional information on metal removal mechanisms. However, due to their operational nature and the difficulty of data interpretation, sequential extractions are not particularly suited for absolute studies, without reference to other analyses (Bacon and Davidson, 2008). The interpretation of results from AVS-EM is based on the theory that metals are stable or potentially mobile if the EM/AVS molar ratio is less than one or greater than one, respectively (Yu *et al.*, 2001). Metal stability or potential mobility is related to the chemical form; at an EM/AVS molar ratio less than or equal to one, metals are mainly in the form of sulphide minerals, whereas at an EM/AVS molar ratio greater than one the (oxy)hydroxide and carbonate minerals are predominant. In addition, some authors have reported that the AVS-ES theory allows predicting the acute toxicity of Cd and Ni in sediments (Di Toro *et al.*, 1992).

There are limited data on metal removal mechanisms and potential mobility in complex reactive mixtures from bioreactors which treat highly contaminated AMD, with metal concentrations (e.g. Mn, Cd, Ni, Zn) of 10-15 mg/L and up to 500 mg/L Fe. Analysis of reactive mixtures collected from a bioreactor filled with acid-washed sand (very simple matrix) and fed with lactate (the best organic carbon source for SRB) for the treatment of mildly contaminated AMD for 14 days, indicated that the organic matter/sulphides bound fraction was the most important scavenging phase of all metals (Jong and Parry, 2004). On the contrary, sulphides were not detected in a spent reactive mixture from a bioreactor filled with a mixture of municipal compost, calcite, and river sediment, and which treated a comparable AMD over 158 days (Gibert *et al.*, 2005). Additional work is therefore required to properly evaluate metal removal mechanisms and potential mobility in bioreactors filled with complex reactive mixtures and that are used for highly contaminated AMD treatment.

The first part of the present study (Neculita *et al.*, 2008, this journal) provides details on the set-up and operation of six sulphate-reducing column bioreactors over an 11-15 month period for the treatment of a highly contaminated AMD at two different

hydraulic retention times (HRTs). In this second part of the study, four of the bioreactors (in duplicate for each HRT) were then dismantled, and several physicochemical, microbiological, and mineralogical analyses were performed on reactive mixtures collected from the first 10 cm in the top and bottom layers. The main objective of this part of the study was to gain insight into the mechanisms of metal removal, and to assess long-term stability of reactive mixtures and the potential mobility of metals after the treatment of a highly contaminated AMD.

5.3 Materials and methods

5.3.1 Sampling

Samples of reactive mixtures were collected from four 3.5 L sulphate-reducing column bioreactors after 44 weeks of treating highly contaminated AMD. The AMD was prepared on a weekly basis using distilled water and metal sulphates and had the following composition: 372 mg/L Ca, 9.8 mg/L Cd, 504 mg/L Fe, 66.3 mg/L K, 85.8 mg/L Mg, 10.1 mg/L Mn, 625 mg/L Na, 13.7 mg/L Ni, 14.5 mg/L Zn, and 4022 mg/L SO_4^{2-} at pH 2.9-5.7. The sampling of solids was carried out from the first 10 cm layer of the top and bottom of four reactors, which tested (in duplicate) two hydraulic retention times (HRTs) of 7.3d and 10d, in a downward flow configuration. The reactors were filled with a reactive mixture consisting of 60% (w/w, dry) organic materials and 40% inorganic materials (sand, creek sediment, urea, and calcium carbonate). Organic materials were constituted from equal proportions (30%, w/w) of cellulosic wastes (maple wood chips and sawdust) and organic wastes (leaf compost and poultry manure). The reactive mixture was selected after being tested in long-term (120 day) batch bioreactors. Further details on the AMD preparation and reactive mixture selection are given in Neculita and Zagury (2008), while the bioreactor set-up and operation is presented in a companion paper (Neculita *et al.*, 2008, this journal).

5.3.2 Physicochemical and microbiological analyses

Physicochemical analyses of the spent reactive mixtures included pH, moisture content, total volatile solids, total carbon (TC), total organic carbon (TOC), total sulphur (S), soluble sulphate, and metals. Microbiological analyses included counts of total anaerobic heterotrophic bacteria (TAHB) and sulphate-reducing bacteria (SRB). The pH was determined in deionized water using a solid to liquid ratio of 1:10 according to the standard D4972-95 (ASTM, 1995) using a portable pH/mV/temperature meter (HACH, model sensION1) with a gel-filled pH electrode and a combination Ag/AgCl redox potential electrode (HACH, Hampton, NH). The water content was determined at 105°C according to the standard D2216-92 (ASTM, 1995). Volatile solids were determined at 550°C according to Karam (1993). Total carbon and total sulphur were measured by combustion with an induction furnace (LECO Corporation, 1975). A hydrochloric acid treatment followed by the same combustion as in the case of the total carbon analysis was performed to determine total organic carbon (Tiessen and Moir, 1993). Inorganic carbon was calculated by the difference between total carbon and total organic carbon. Soluble sulphate was analyzed as sulphur extracted in hydrochloric acid (40%, v/v) and using ICP-AES, as per Sobek *et al.* (1978). Based on the fact that the total metal concentrations are related to the extraction conditions, the metals in the spent reactive mixtures (Fe, Mn, Cd, Ni, and Zn) were determined using four digestion methods (A, B, C, and D). These metal analyses were performed either by atomic absorption spectrometry (AAS, Perkin Elmer, model AAnalyst 200), where method detection limits for Fe, Mn, Cd, Ni, and Zn were, respectively, 0.05, 0.02, 0.03, 0.02, and 0.02 mg/L or by atomic emission spectroscopy (ICP-AES, Perkin Elmer Optima 3100), where the method detection limit was <0.01% (w/w). The analyses were carried out on wet samples, which were accurately weighed, and used the following reagents:

- A – 1 g of solid and 51 mL of 30 HNO_3 to 20 $HClO_4$ to 1 HF (Zagury *et al.*, 1997);
- B – 1 g of solid and 71 mL of 30 HNO_3 to 20 $HClO_4$ to 20 HCl to 1 HF.

- C – 0.5 g of solid and 33-36 mL of 10 HNO_3 to 1 Br_2 to 20 HCl to 2-5 HF (Potts, 1987).
- D – 1.25 g of solid and 25 mL of 6M HCl (Brouwer and Murphy, 1994).

In digestion method B, samples of spent reactive mixtures were weighed in Teflon vessels with 20 mL concentrated HCl. After 24h of reaction at room temperature and a under fume hood, other acids were added and the mixture was then treated as in digestion method A (Zagury *et al.*, 1997).

The counts of heterotrophic anaerobic fermentative bacteria and of SRB used the Most Probable Number technique as per Standard Methods (APHA, 1998) or/and ASTM (1990), respectively. All laboratory glassware used during the analytical procedures was sequentially cleaned with a phosphate-free detergent, soaked in 10% (v/v) nitric acid for 24 h, then in distilled water, and finally rinsed three times with deionized water (18.2 Mohms). Unless otherwise stated, all reagents were of analytical grade (ACS) or better. All analyses were carried out in duplicate, for both top and bottom layers of each reactor. The reported concentrations were corrected for moisture content.

5.3.3 Stability of metal precipitates and potential mobility

Stability of metal precipitates and potential mobility in the reactive mixtures were assessed with a sequential extraction procedure (SEP) and a simultaneous determination of acid volatile sulphides and extracted metals (AVS-EM).

The SEP (Zagury *et al.*, 1997) used in the present study is based on the classical Tessier *et al.* (1979) scheme, with some modifications, especially on the extraction of the residual fraction. The five operationally defined fractions and the reagents employed were as follows: soluble and exchangeable metals (extracted with 0.5M $MgCl_2$, pH 7), carbonate bound (leached by 1M NaOAc buffered with HOAc, pH 5), reducible or bound to Fe-Mn oxides (extracted with 0.04M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc), oxidisable or bound to organic matter (released by HNO_3, H_2O_2, and NH_4OAc), and

residual metal fraction (dissolved by acid attack with HNO_3, HF, and $HClO_4$). The procedure was conducted with 1 g of solid accurately weighed in 50mL polypropylene centrifuge tubes, to which 8mL of extracting solution was added. Between each of the successive extractions, separation was carried out by centrifuging (Beckman J2-21) at 12,000 x *g* for 30 min. The supernatant extract was removed and collected in 50mL vials. The residue was rinsed twice with 8 mL deionized water, centrifuged for 30 min, and the supernatant mixed with the initial extract and analyzed for metal concentrations by AAS.

The AVS-EM procedure was conducted as per Brouwer and Murphy (1994), except for the fact that 6M HCl was used, instead of 2M HCl, as specified in the protocol. Before starting the extraction, a sulphide antioxidant buffer solution (SAOB) was prepared which contained 2M NaOH (to convert H_2S into S^{2-}), 0.1M ascorbic acid (to prevent S^{2-} oxidation), and 0.1M EDTA (to complex metals that may catalyze the S^{2-} oxidation) (Arowolo and Cresser, 1991). Samples of 1.25g wet reactive mixture were then placed in 50 mL scintillation vials. A smaller vial with 2.5 mL SAOB was placed inside the scintillation vial, and 25mL of 6M HCl was added to the reactive mixture, after which the 50 mL vial was immediately closed to prevent the loss of H_2S. The assembled vials were then placed on a rotary shaker and agitated at 150 rpm for 1h. After agitation, the scintillation vials were opened and the inner vial was removed, its content mixed and analyzed for sulphides using methylene blue Standard Method (APHA, 1998). The supernatant from the 50 mL scintillation vial was then filtered using 0.45 μm membranes and analyzed for metals by flame AAS (Perkin Elmer, model AAnalyst 200).

5.3.4 Solid mineralogy

A differential scanning calorimetry and thermogravimetric analysis (DSC-TGA), in addition to X-ray diffraction (XRD), and scanning electron microscopy equipped with

X-ray energy dispersion (SEM-EDS) microanalysis were used for evaluation of spent mixture mineralogy.

The DSC-TGA was carried out using a TA-SDT-Q600 apparatus (TA Instruments), which allows recording of weight loss and heat flow during thermal treatment of the sample. Thermal behavior of wet samples (27-34 mg) was studied under a N_2 atmosphere (100 mL/min), at a rate of 10°C/min (up to 600°C) then 20°C/min (600-1200°C).

Morphological features of metal precipitates were observed with a SEM (Hitachi S-3500N), equipped with an EDS X-ray microanalysis detector (Oxford Link-Isis 300). The X-ray diffraction analysis used a D8 Advance diffractometer (Bruker AXS), equipped with a Cu source and a scintillation counter. The analyses were step-scanned from 5 to 60°C (2θ) using a step of 0.005°C (2θ) at 1°C divergence slit and 1 s/step integration time. Due to the poor crystalline state of the minerals, samples were passed several times which allowed the software to optimize the signal to noise ratio by adding signals from each pass.

Prior to the SEM and XRD analyses, the samples were dried (at 40°C for 48h), desegregated with a roller and manually homogenized. The SEM analyses were performed on polished surfaces, which were prepared under vacuum and used 2-4 g of dried samples embedded in an epoxy resin (Epoxycure resin, Buehler, Canada) and mounted on plastic stubs. The surface was then polished with diamond paper, diamond suspension and aluminium powder with decreasing grit size down to 0.02 μm to produce a high gloss surface. The observations were performed at an accelerating potential of 20 kV, a probe current of 80 μA, and a vacuum pressure of 25 kPa.

5.4 Results and discussion

Results from sampling and analysis on a weekly basis over a 11-month period are presented in the companion paper and showed the effectiveness of column bioreactors for increasing the pH and alkalinity of contaminated water and removing sulphate and metals (60-82% for Fe and up to 99.9% for Cd, Ni, and Zn) from a highly contaminated AMD at both 7.3d and 10d HRTs (Neculita *et al.*, 2008; this journal). Furthermore, a final analysis of treated effluent, carried out at week 60, indicated that the columns were still effective for AMD treatment at both HRTs.

5.4.1 Physicochemical and microbiological analyses

Physicochemical and microbiological parameters indicated similar trends between the top layers, as well as between the bottom layers of all reactors (Table 5.1). The pH was slightly higher (7.2-7.8) in the 10d HRT columns compared to the 7.3d HRT columns (6.8-7.3), with low (0.2-0.5 units) or no differences between the pH in the top and bottom layers of the bioreactors. The bottom layers, however, had up to 3% higher humidity, due to the vertical downward flow. Volatile solids (VS), determined at 550°C, which are generally used as an indication of organic matter content, showed higher values in the top layers compared to the bottom layers. Among the reactors, the lowest VS values (32.0-34.5%) were found in reactor 1 and the highest (42.4-50.8%) in reactor 2, which were both operated at a 10d HRT (Table 5.1). There is no clear explanation for this discrepancy. Results also show that volatile solids values are not well correlated with total carbon, which was the lowest in the top layers of the reactors. The release of water from the (oxy) hydroxide minerals at temperatures as high as those used during the volatile solids analysis (550°C) could account for this difference. Moreover, in the bottom layers, both organic and inorganic carbon concentrations were higher than in the top layers. The organic carbon in the bottom layers could originate from leaching of the top layers, while the source of inorganic carbon could be either the initial TIC from the reactive mixture composition or TIC generated by SRB activity.

Table 5.1 Physicochemical and microbiological characterization of spent reactive mixtures

Column number	HRT	Sample location	pH	Moisture	Volatile solids	TC	TOC	TIC	S total	S sulphates	THAB x 10^6	SRB x 10^3
				(%)	(%)	(%)	(%)	(%)	(%)	(%)	Cell./100mL	Cell./100mL
1	10d	Top	7.60±0.12	52.6±1.2	34.5±13.8	17.9±0.2	17.0±0.4	0.9±0.3	1.0	1.0	3.0	3.0
		Bottom	7.80±0.03	53.3±4.0	32.0±4.1	20.9±0.3	19.4±0.2	1.5±0.2	1.1	0.6	0.3	1.7
2	10d	Top	7.24±0.02	52.6±0.9	50.8±3.7	22.1±0.2	21.5±0.5	0.6±0.7	1.0	0.8	2.8	5.0
		Bottom	7.52±0.05	54.5±0.4	42.4±0.0	20.1±0.2	18.8±0.2	1.3±0.0	0.9	0.7	0.8	2.3
3	7.3d	Top	6.89±0.43	48.1±1.8	45.0±14.0	23.2±0.1	22.5±0.2	0.7±0.3	0.3	0.2	3.0	5.0
		Bottom	7.34±0.06	51.8±1.3	41.9±7.7	25.5±0.2	23.7±0.4	1.7±0.3	1.2	0.6	0.5	3.0
4	7.3d	Top	6.88±0.01	50.9±4.9	41.1±3.4	19.1±0.3	17.3±0.4	1.7±0.4	0.8	0.6	5.0	1.3
		Bottom	6.85±0.05	53.9±2.6	42.4±3.9	19.5±0.1	18.0±0.5	1.5±0.5	1.1	0.3	0.5	1.7

Results are expressed as mean ± standard deviation from n=4 (2 columns, in duplicate).

Columns #1 and #2 were operated at a 10d HRT; columns #3 and #4 were operated at a 7.3d HRT.

SRB – sulphate-reducing bacteria.

TAHB – total anaerobic heterotrophic bacteria.

It is noteworthy that the reactive mixture contained calcium carbonate (2%) and leaf compost (20%, w/w, dry weight), with a very high content of TIC (14.3%) (Neculita and Zagury, 2008).

Elemental analyses also showed low concentrations of S_{total} (0.3-1.2%) and $S_{sulphates}$ (0.2-1%) in the top and bottom layers of all columns. Moreover, $S_{sulphides}$ (calculated as the difference between S_{total} and $S_{sulphates}$) was up to 0.8% which suggests that metals in sulphide minerals accounted for up to 0.8% (w/w) (or 15% of total metals recovered in spent reactive mixtures).

Microbiological characterization indicated that the spent reactive mixtures from the top layers had at least one order of magnitude more THAB (10^5 cell./100mL) relative to the bottom layers, while the SRB gave comparable counts in the top and bottom layers (10^3 cell./100mL) (Table 5.1). As expected, due to the very high contaminant load in the AMD used to feed the columns, metal concentrations determined with all four methods (A, B, C, and D) were very high (Table 5.2). In the top layers of the columns, lower concentrations of Fe and Mn, as well as higher concentrations of Cd, Ni, and Zn were found relative to the bottom layers, regardless of the digestion method. Relative standard deviations (RSD) were high and varied in the range 2-25% for Fe and Mn, 1-61% Ni and Zn, and 1-126% for Cd.

The scattering of results was not unexpected, given the heterogeneous state of the samples which were collected from reactive mixtures containing several organic materials (e.g. chips and sawdust of maple wood, composted poultry manure, leaf compost) and inorganic materials (e.g. sand, creek sediment). The highest metal concentrations were found using digestion method B and the lowest concentrations were determined with digestion method D, which was in fact the method used in the AVS-EM procedure. The Fe and Mn concentrations determined with method B were twice as high in the bottom layers, whereas Cd, Ni, and Zn concentrations were up to 50-times higher in the top layers (Table 5.2).

Table 5.2 Total concentrations of Fe, Mn, Cd, Ni, and Zn in spent reactive mixtures using four digestion methods

Method	Column number	Sample location	Fe	Mn	Cd	Ni	Zn
			(g/kg)				
A	1	Top	14.1±2.0	0.18±0.02	8.09±1.66	1.74±0.03	2.90±0.59
		Bottom	30.1±0.3	0.31±0.00	0.05±0.00	0.06±0.01	0.15±0.01
	2	Top	11.0±1.6	0.15±0.01	10.4±1.2	0.54±0.29	1.77±1.03
		Bottom	30.3±1.5	0.30±0.01	0.06±0.01	0.09±0.03	0.15±0.04
	3	Top	11.6±1.8	0.14±0.01	7.67±0.52	0.36±0.01	1.14±0.09
		Bottom	28.7±2.7	0.22±0.02	0.15±0.06	0.06±0.00	0.15±0.03
	4	Top	31.6±1.0	0.32±0.02	0.00±0.00	4.07±0.62	3.05±0.21
		Bottom	30.7±1.0	0.37±0.01	0.03±0.04	0.06±0.02	0.12±0.01
B	1	Top	35.7±2.1	0.44±0.03	13.3±1.3	2.37±0.23	2.53±0.01
		Bottom	51.8±6.6	0.61±0.09	0.26±0.08	0.82±0.02	0.51±0.12
	2	Top	30.9±1.7	0.47±0.01	10.2±5.9	5.19±1.97	13.2±3.9
		Bottom	57.8±2.3	0.67±0.05	0.33±0.18	0.89±0.00	0.45±0.01
	3	Top	19.9±3.9	0.35±0.03	6.77±0.09	1.87±0.12	4.07±2.37
		Bottom	50.8±2.4	0.53±0.02	0.38±0.19	0.86±0.03	0.51±0.14
	4	Top	31.8±5.9	0.49±0.06	8.33±0.46	1.80±0.11	5.79±1.34
		Bottom	52.7±4.0	0.70±0.05	0.26±0.10	0.85±0.06	0.45±0.06
C	1	Top	25.0	0.37	3.96	4.23	6.78
		Bottom	32.9	0.44	< 0.01	0.02	0.10
	2	Top	22.2	0.30	5.29	2.73	5.77
		Bottom	31.3	0.55	< 0.01	0.03	0.09
	3	Top	23.2	0.36	1.88	0.25	0.99
		Bottom	30.7	0.33	< 0.01	0.02	0.09
	4	Top	22.9	0.35	0.42	3.50	4.89
		Bottom	29.3	0.33	< 0.01	0.02	0.10
D	1	Top	6.88±0.26	0.07±0.01	6.41±1.31	1.56±0.27	3.87±0.78
		Bottom	18.4±0.1	0.17±0.01	0.05±0.07	0.04±0.02	0.10±0.04
	2	Top	5.91±0.27	0.06±0.00	10.4±2.5	1.73±0.21	9.56±2.19
		Bottom	20.3±0.5	0.21±0.03	0.10±0.10	0.05±0.03	0.18±0.11
	3	Top	5.71±0.42	0.06±0.02	10.4±2.9	1.39±0.33	3.18±0.30
		Bottom	19.5±1.0	0.14±0.00	0.00±0.00	0.03±0.01	0.07±0.00
	4	Top	5.57±0.56	0.08±0.01	10.2±3.1	1.43±0.43	5.97±1.25
		Bottom	20.3±2.8	0.22±0.03	0.13±0.14	0.05±0.03	0.13±0.06

A – Total concentration was determined with a HNO_3, $HClO_4$, and HF mixture.

B – Total concentration was determined with a HNO_3, $HClO_4$, HF, and HCl mixture.

C – Total concentration was determined with a HNO_3, Br_2, HCl, and HF mixture.

D – Total concentration was determined with 6M HCl.

Results are expressed as mean ± standard deviation from n=4 (2 columns, in duplicate), except for method C, in which metal concentrations are directly provided in % (w/w) by ICP-AES. Columns #1 and #2 were operated at a 10d HRT; columns #3 and #4 were operated at a 7.3d HRT.

Among the metals, Fe concentrations were the highest (100-fold or more) and varied from 19.9 g/kg to 35.7 g/kg in the top layers and from 50.8 g/kg to 57.8 g/kg in the bottom layers. It is noteworthy that the source of iron was the AMD feed, in which Fe concentrations were up to 50-fold higher compared to the other metals (Mn, Cd, Ni, and Zn).

Overall, results from elemental analyses indicated that Cd, Ni, and Zn were concentrated in the top layers and eventually removed from the AMD by adsorption or bound to organic matter, whereas Fe and Mn were concentrated in the bottom layers and eventually removed as sulphide minerals, in addition to (oxy)hydroxides and carbonates.

5.4.2 Stability of metal precipitates and potential mobility

As previously specified, the spent reactive mixtures from both top and bottom layers of all reactors contained very high concentrations of metals. Results from the AVS-EM procedure also indicated a high excess of metals relative to volatile sulphides and a molar ratio of EM to AVS >1, which based on AVS-EM theory (Yu *et al.*, 2001) suggests a high mobility of metals under acidic conditions (Table 5.3). These results are consistent with elemental analyses, which indicated high total metal concentrations (up to 5.8% for Fe) and low $S_{sulphides}$ (up to 0.8%) in spent reactive mixtures. The mobile fraction of Fe (as determined using AVS-EM procedure) was 35-39% in the bottom layers, and 18-29% in the top layers. For Mn, the mobile fraction was comparable to Fe (26-32%) in the bottom layers, while in the top layers it was less than that for Fe (13-17%). The potential for metal mobility was also confirmed by results from the SEP procedure (Table 5.4), which are presented in terms of absolute concentrations to allow the direct comparison between studies (Bacon and Davidson, 2008).

Based on partitioning data (sum of metal concentrations from five extracted fractions), the ranking of metal mobility was similar between the top layers, as well as between the bottom layers of all reactors for Fe, Mn, Ni, and Zn, whereas Cd behaved differently for

the two HRTs of 7.3d and 10d (Table 5.4). A ratio between the total metal concentrations in the bottom and top layers showed relatively low values for Fe (1.2-3.7) and Mn (1.3-2.6). For Cd, Ni, and Zn, however, the concentration ratio between the top and bottom layers was significantly higher (4-166), with the lowest variation for Zn (10-26) and the highest variation for Cd (50-166). Moreover, for Cd this ratio was twice as high in the 10d HRT columns (100-166) compared to the 7.3d HRT columns (50-80).

Table 5.3 Metal and sulphide concentrations in reactive mixtures using a simultaneous determination of acid volatile sulphides and extracted metals (AVS-EM)

Column number	Sample location	Fe	Mn	Cd	Ni	Zn	H_2S+HS^-
		mmol/kg (%)	mmol/kg (%)	mmol/kg (%)	mmol/kg (%)	mmol/kg (%)	mmol/kg
1	Top	123.1±4.7 (19.6±3.8)	1.2±0.1 (15.1±9.4)	57.0±11.6 (48.2±20.4)	26.5±4.6 (65.6±17.4)	59.2±11.9 (67.1±13.5)	0.03±0.02
	Bottom	328.6±2.1 (35.4±0.6)	3.1±0.2 (27.9±6.2)	0.5±0.6 (21±122)	0.7±0.4 (5.2±56.3)	1.6±0.5 (20.5±33.9)	0.03±0.01
2	Top	105.7±4.8 (19.1±4.6)	1.1±0.0 (12.8±2.0)	92.9±22.0 (103±23.6)	29.5±3.5 (33.4±12.0)	146.2±33.5 (72.3±22.9)	0.05±0.02
	Bottom	364.0±8.4 (35.2±2.3)	3.8±0.6 (30.6±15.7)	0.9±0.9 (29.5±100)	0.8±0.4 (5.1±56.6)	2.8±1.7 (41.5±60.7)	0.07±0.00
3	Top	102.2±7.4 (28.6±7.3)	1.1±0.3 (17.0±25.2)	92.6±25.6 (154±28)	23.6±5.7 (74.3±24.1)	48.7±4.6 (78.3±9.4)	0.00±0.00
	Bottom	349.1±17.3 (38.3±5.0)	2.5±0.0 (26.0±1.5)	0.0±0.0 (0.9±47.1)	0.4±0.1 (2.9±25.7)	1.1±0.0 (13.6±2.3)	0.04±0.01
4	Top	99.7±10.1 (17.5±10.1)	1.4±0.2 (16.1±14.7)	91.0±27.8 (123±31)	24.4±7.3 (79.5±30.0)	91.3±19.1 (103±21)	0.00±0.00
	Bottom	363.6±49.7 (38.5±13.7)	4.1±0.5 (32.1±11.9)	1.2±1.2 (49.7±106)	0.9±0.5 (6.0±56.8)	2.0±1.0 (29.2±48.3)	0.05±0.00

Results (mmol/kg) are expressed as mean ± standard deviation from n=4 (2 columns, in duplicate)

Results presented in parenthesis (%) are expressed as mean (EM) to mean (total concentration as determined with method B) ratio ± RSD

RSD – relative standard deviation (standard deviation to mean ratio, %)

Columns #1 and #2 were operated at a 10d HRT; columns #3 and #4 were operated at a 7.3d HRT.

Based on the ranking of metals in the reactive mixtures from the top and bottom layers the results indicated that:

1. In the top and bottom layers, Fe had the smallest soluble and exchangeable fraction, as well as the lowest carbonate bound fraction, with concentrations varying from 0.5 to 30 mg/kg. The precipitation of insoluble ferric iron minerals at the top, and the generation of insoluble ferrous sulphides at the bottom of the columns, could explain these low mobile fractions. However, Mn, Cd, Ni, and Zn concentrations were higher in the soluble and exchangeable fraction, whereas the carbonate bound fraction was low for all metals.
2. In the top layers, Fe and Mn were mainly recovered in the residual fraction, Cd and Zn in the oxidisable/organic matter bound fraction and Ni in the reducible fraction.
3. In the bottom layers, metals were mainly found in the reducible fraction (Fe and Zn in all columns, and Ni in the 10d HRT columns), in the residual fraction (Mn, Ni in the 7.3d HRT columns) and in the oxidisable/organic matter bound fraction (Cd).

In summary, the lowest potential mobility was found for Mn, which was mainly recovered in the residual fraction from the top (46-74%) and bottom (42-44%) layers of all columns. A low potential mobility was also found for Fe in the top layers which had a higher residual fraction (44-77%) than in the bottom layers which had a higher reducible fraction (35-39%). Ni and Zn were divided between reducible, oxidisable/organic matter bound or residual fractions, whereas Cd was recovered (72-97%) only from the oxidisable/organic matter bound fraction.

The results from both chemical extractions (SEP and AVS-EM) and elemental analysis were therefore consistent and indicated a low fraction of metals removed as sulphides. Moreover, Fe and Mn were found in a more stable chemical form (residual fraction) relative to Cd, Ni or Zn, which seemed weakly bound (oxidisable/reducible fractions) and had higher potential mobility. However, all metals were stable in the reactive mixtures during bioreactor operation, which effectively treated a highly contaminated AMD over a 15-month period.

Table 5.4 Metal (Fe, Mn, Cd, Ni, and Zn) fractionation in reactive mixtures using a sequential extraction procedure (SEP)

Column number	Sample location	Fraction	Fe	Mn	Cd	Ni	Zn
			(g/kg)	(mg/kg)			
1	Top	F1	< 0.01	46.0±2.7	94.1±34.9	1260±32	212±40
		F2	< 0.01	1.61±0.93	8.06±6.90	58.9±6.7	22.9±0.9
		F3	5.42±0.85	57.4±0.2	58.2±8.1	1540±286	4 460±98
		F4	8.35±0.23	39.0±6.4	5900±1070	373±46	1 560±2130
		F5	10.7±2.7	123±33	63.3±7.9	22.0±8.4	150±0
		Sum	24.4±1.0	267±47	6130±1070	3260±291	6 400±2130
	Bottom	F1	< 0.01	55.0±0.2	1.44±0.01	6.46±0.37	4.32±5.43
		F2	< 0.01	0.96±0.00	1.44±0.01	10.3±0.3	2.39±2.70
		F3	10.6±3.7	107±14	2.15±0.01	32.3±8.3	87.6±27.8
		F4	9.97±0.45	32.6±0.4	9.33±0.04	10.0±1.0	48.9±36.7
		F5	9.81±0.32	148±1	46.9±52.3	11.5±5.0	101±26
		Sum	30.4±3.7	344±15	61.3±68.1	70.6±12.7	244±62
2	Top	F1	0.02±0.01	33.4±0.8	497±136	182±11	583±28
		F2	< 0.01	0.93±0.06	94.2±4.1	12.1±1.4	26.6±2.5
		F3	0.90±0.22	1.76±0.60	150±10	56.0±0.2	756±40
		F4	1.41±0.27	2.15±2.10	3950±600	35.1±0.8	1 830±73
		F5	7.78±1.35	106±11	280±13	6.55±6.40	100±9
		Sum	10.1±0.5	144±28	4970±610	292±12	3 290±90
	Bottom	F1	< 0.01	61.2±3.4	1.36±0.07	6.85±1.63	3.72±3.40
		F2	< 0.01	0.91±0.05	1.36±0.07	10.7±0.9	2.70±1.15
		F3	14.6±2.9	101±5	2.05±0.10	27.8±21.6	93.6±52.4
		F4	11.4±0.6	46.7±16.8	21.5±27.6	11.6±14.6	40.7±22.3
		F5	11.2±0.2	160±36	3.75±1.15	17.1±14.5	68.5±14.2
		Sum	37.2±3.0	369±47	30.0±27.7	74.1±30.8	209±62
3	Top	F1	0.03±0.04	42.8±16.2	263±197	646±605	346±208
		F2	< 0.01	0.83±0.01	43.8±42.6	19.6±11.9	18.9±9.8
		F3	2.35±0.14	6.85±3.48	72.1±6.2	262±181	1 160±360
		F4	2.27±0.02	9.69±7.56	7450±3820	179±175	1 230±290
		F5	7.54±1.89	114±45	123±73	27.7±5.1	88.8±19.6
		Sum	12.2±0.4	174±62	7950±3830	1130±660	2 840±508
	Bottom	F1	< 0.01	34.8±2.5	1.30±0.04	6.26±0.13	11.3±9.2
		F2	< 0.01	0.86±0.02	1.30±0.04	8.87±0.55	4.09±1.42
		F3	10.9±1.7	65.7±3.2	1.95±0.05	6.58±7.51	63.9±21.1
		F4	11.0±0.5	27.5±205	93±106	3.20±2.66	31.8±17.9
		F5	7.04±1.60	99.4±35.5	2.70±0.07	18.4±5.2	61.3±5.5
		Sum	28.9±1.8	228±40	100±106	43.3±15.2	172±33
4	Top	F1	< 0.01	49.0±10.5	176±119	678±178	168±40
		F2	< 0.01	1.09±0.25	40.1±23.3	26.9±24.5	18.3±3.2
		F3	3.03±0.56	21.0±12.3	57.5±26.0	833±367	1 040±1420
		F4	5.10±0.40	15.4±0.5	9610±1800	166±49	746±986
		F5	7.49±1.48	112±0	57.6±4.6	19.0±5.4	102±50
		Sum	15.6±0.7	199±16	9940±1800	1720±410	2 080±1760
	Bottom	F1	< 0.01	55.1±21.4	1.42±0.10	7.37±2.17	8.56±4.41
		F2	< 0.01	0.94±0.06	1.42±0.10	8.96±0.61	6.11±0.25
		F3	12.3±1.2	83.8±6.3	2.46±0.33	12.6±4.1	98.4±44.7
		F4	10.2±0.6	25.3±4.3	191±252	2.90±2.19	42.4±30.6
		F5	8.74±0.11	120±9	2.95±0.20	23.5±0.2	61.4±3.5
		Sum	31.2±1.3	286±28	199±252	55.3±5.9	217±56

Results are expressed as mean ± standard deviation from n=4 (2 columns, in duplicate)
Columns #1 and #2 were operated at a 10d HRT; columns #3 and #4 were operated at a 7.3d HRT.

5.4.3 Solid mineralogy

Although elemental analysis and chemical extractions provided data on metal concentrations, as well as on their stability and potential mobility from spent reactive mixtures, they provided limited insight into metal removal mechanisms. Mineralogical non-destructive analyses can be used to identify the mineralogy of metal precipitates and to evaluate mechanisms of metal removal.

5.4.3.1 X-ray diffraction

Due to a high organic content and sample heterogeneity, the X-ray diffraction technique allowed only qualitative characterization of the spent reactive mixtures. Also, due to a high detection limit of the method (1-5%, depending on mineral crystallinity) the XRD peaks in samples from the top and bottom layers of all columns indicated little difference in mineral composition (Figure 5.1). The identified peaks correspond mainly to silicate (quartz, albite, and muscovite), (oxy)hydroxide (goethite: α-FeO(OH) and lepidocrocite: y-FeO(OH)), carbonate (calcite and oxamite: $(NH_2)_2(CO_2)_2H_2O$), sulphate (gypsum), urea (from reactive mixture constitution), and monosulphide (makinawite and greigite) minerals.

These findings are not in agreement with previously reported results, where an XRD analysis did not detect any peak in solids recovered from the inlet of a column filled with a mixture of calcite, municipal compost, and river sediment, and which was used for the treatment of AMD over a 158-day period (Gibert *et al.*, 2005). However, they are comparable with results from a study performed on substrates (mixtures of mushroom compost, animal manure, and barley mash wastes) collected from the inlet and outlet pipes of a field-scale wetland which treated AMD for over 2 years (Machemer *et al.*, 1993). Moreover, the XRD analysis detected lower contents of calcite in the top layers compared to the bottom layers, due to the acidic pH of the AMD reactor feed (pH 2.9-5.7).

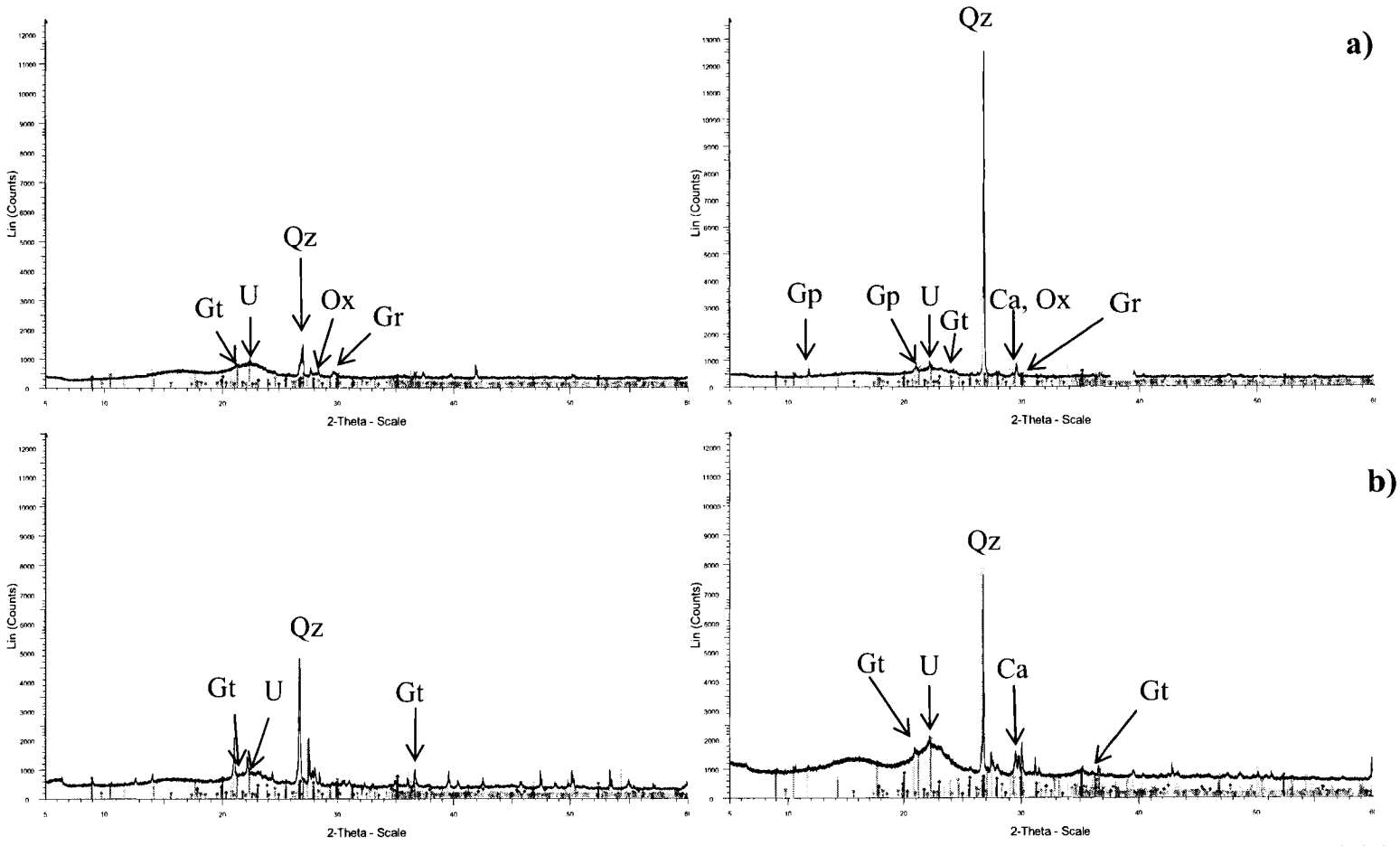

Figure 5.1 Results from X-ray diffraction (XRD) analysis on reactive mixtures collected in top (left) and bottom (right) layers from columns operated at (a) 10d HRT and (b) 7.3d HRT. (Qz-quartz, Gt-goethite, U–urea, Ca–calcite, Ox–oxamite, Gp–gypsum, Gr-greigite)

Slightly lower concentrations of iron (oxy)hydroxides (goethite and lepidocrocite) were also found in the top layers relative to the bottom layers in the 10d HRT column, while in the 7.3d HRT column, these minerals had comparable values in the top and bottom layers.

Although the X-ray diffraction technique provided some information about the chemical form of the minerals present in the spent reactive mixtures, its high detection limit mainly allowed the identification of iron precipitates as (oxy)hydroxides (goethite and lepidocrocite) and sulphides (greigite and makinawite) but not of the minerals containing Mn, Cd, Ni, and Zn. These results can be explained by the total Fe concentration, which was the highest of all metals (up to 5.8%, w/w). A higher inorganic carbon content in the bottom layers relative to top layers is also supported by the chemical analyses.

5.4.3.2 Thermogravimetry and differential scanning calorimetry

Thermal behavior of wet reactive mixture samples from the top layers of two reactors (one for each 7.3d and 10d HRT) was characterized by three endotherms at approximately 100°C, 360°C, and 730°C (Figure 5.2). At the beginning, with increasing temperatures up to 100°C when the first endotherm was observed, the weight loss was 53% (7.3d HRT columns) and 35% (10d HRT columns) (Figure 5.1). The evaporation of adsorbed water and structural OH which were rapidly lost at a large rate of thermal transformation could explain the weight loss. With increasing temperature, additional weight was lost (13% for both columns) at slower rates, to yield a second endotherm at around 360°C. The degassing of CO_2 and H_2O derived from organic matter oxidation could explain this trend, based on the fact that total volatile solids determined at 550°C are generally used as a measure of organic matter content in soils (Karam *et al.*, 1993). As the temperature further increased, less (1.7%) weight was lost to finally reach a third endotherm around 730°C. This last transformation was attributed to SO_3 volatilization (Kim and Kim, 2003).

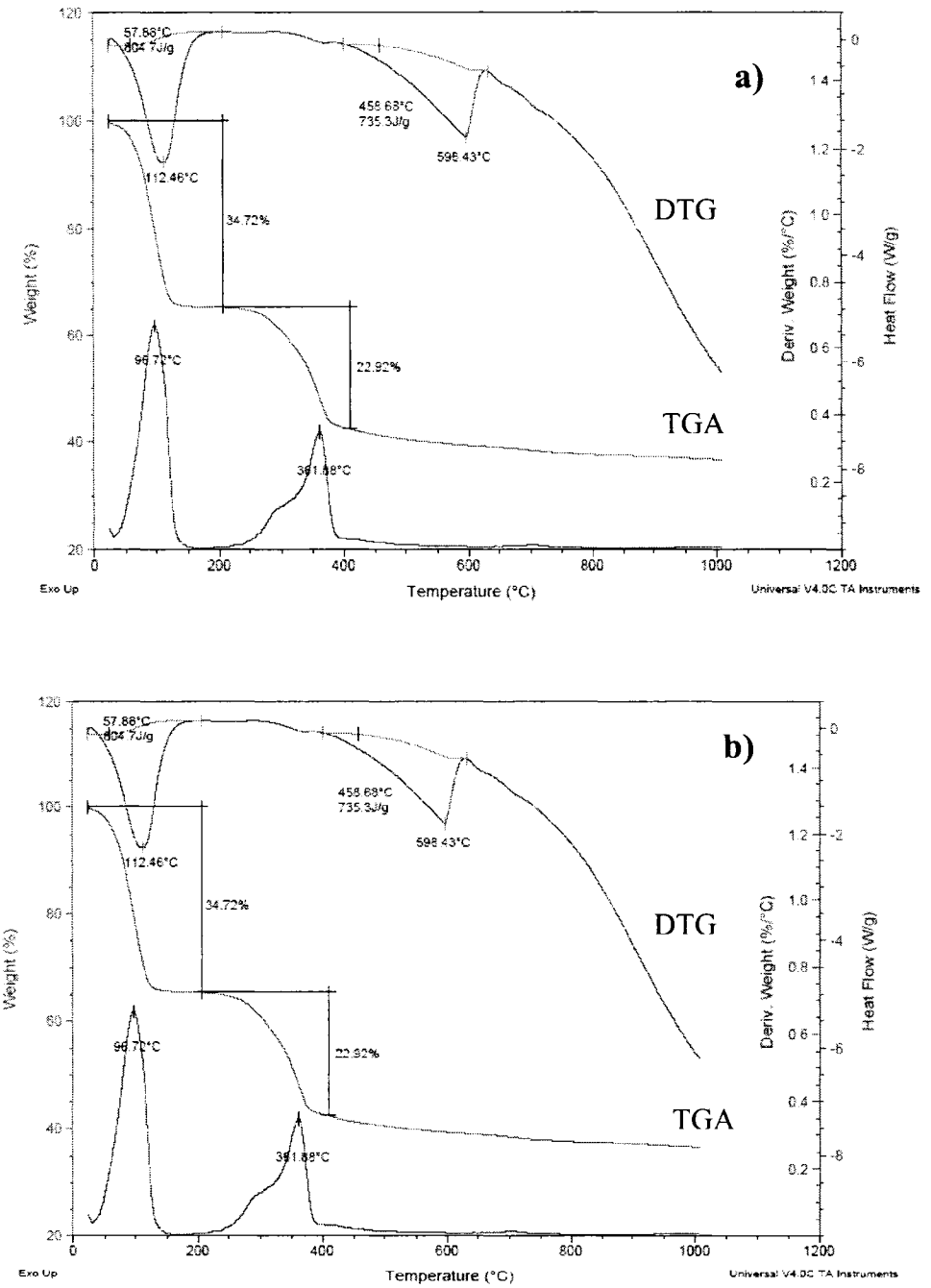

Figure 5.2 Differential scanning calorimetry and thermogravimetric analysis (DSC-TGA) on reactive mixtures from columns operated at (a) 10d HRT and (b) 7.3d HRT.

The study of thermal behavior of spent reactive mixtures thus provides information about the high organic matter content which still characterized the reactive mixtures after 11 months of operation. The study also provides insight into oxidation and volatilization of sulphur compounds.

5.4.3.3 Scanning Electron Microscopy and X-ray microanalysis

As already discussed in the introduction, SEM is a proven technique to study the morphology and mineralogical composition of amorphous solids such as metal minerals precipitated in spent reactive mixtures from AMD passive treatment systems (Machemer *et al.*, 1193; Herbert *et al.*, 1998; Neculita *et al.*, 2006). A lower limit of detection (0.2%) compared to XRD allows a closer study of the chemical form of metal precipitates, especially of those unidentified during the XRD analysis.

Indeed, the SEM images from samples of reactive mixtures showed abundant precipitates of a broad size (2-10μm) and irregular spatial distribution (Figures 5.3 to 5.6). According to elemental mapping and EDS analysis, the granular precipitates could contain sulphides which would be entrenched both in organic fibbers and around the edge of alveoli from the cellular texture of maple wood. Sulphides detected in the bottom layer of the 10d HRT column contained Fe associated with S in stoechiometric ratios similar to pyrrhotite ($Fe_{1-x}S$) with inclusion of Zn, Ni, and sometimes Cu (Figure 5.3), while in the top layer of the same column, the presence of CdS and ZnS was confirmed (Figure 5.4).

In the bottom layer of the 7.3d HRT column, in addition to what could be metal sulphides such as chalcopyrite ($CuFeS_2$), some carbonates were also observed (Figure 5.5), while in the top layer, the metal precipitates contained iron (oxy)hydroxide minerals with S, Ni and Zn in the structure (Figure 5.6). The morphology and size-distribution of the metal sulphides is comparable to previously reported results (Machemer and Wildeman, 1992; Herbert *et al.*, 1998).

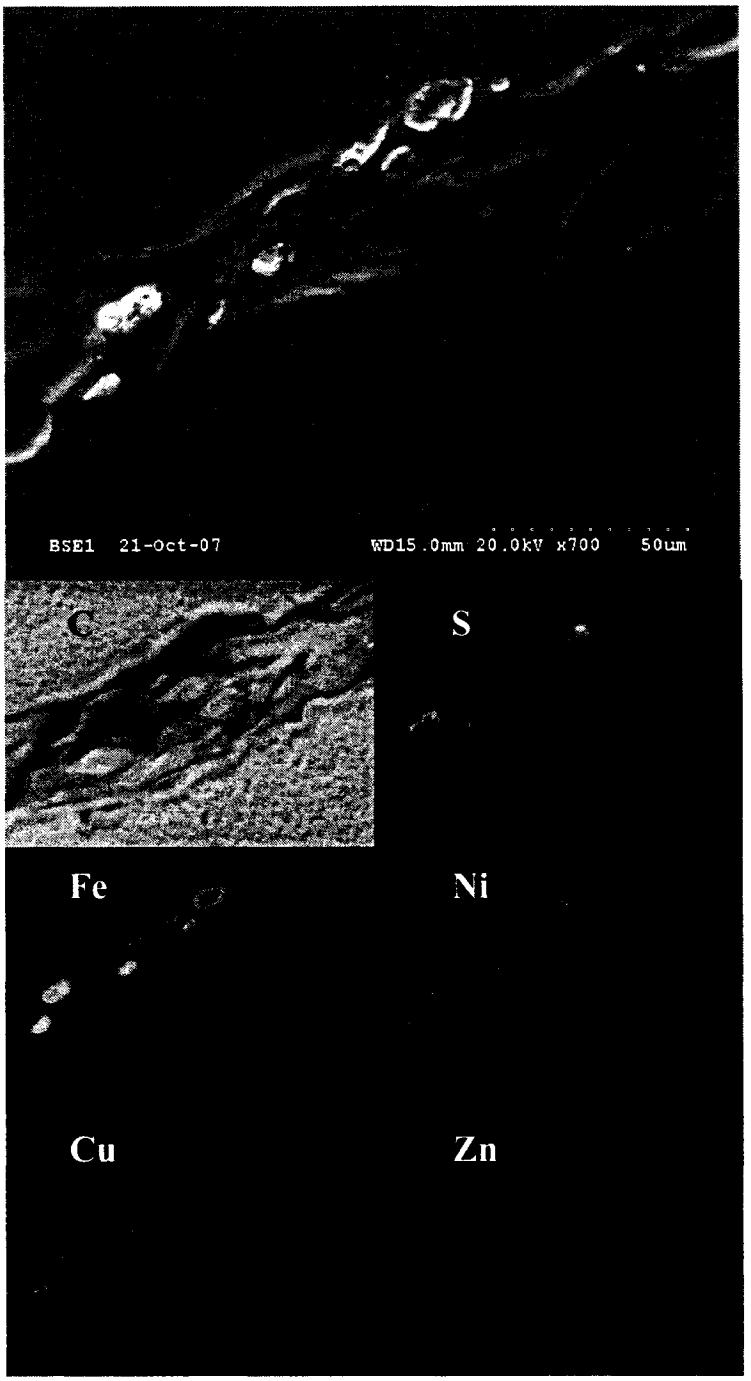

Figure 5.3 SEM-BSE image and elemental mapping for C, S, Fe, Ni, Cu, and Zn on reactive mixture from the bottom of a column operated at 10d HRT. Sulphide precipitation with a Fe:S ratio corresponding to pyrrhotite and the presence of other metals (Ni, Cu, Zn) in the structure was observed.

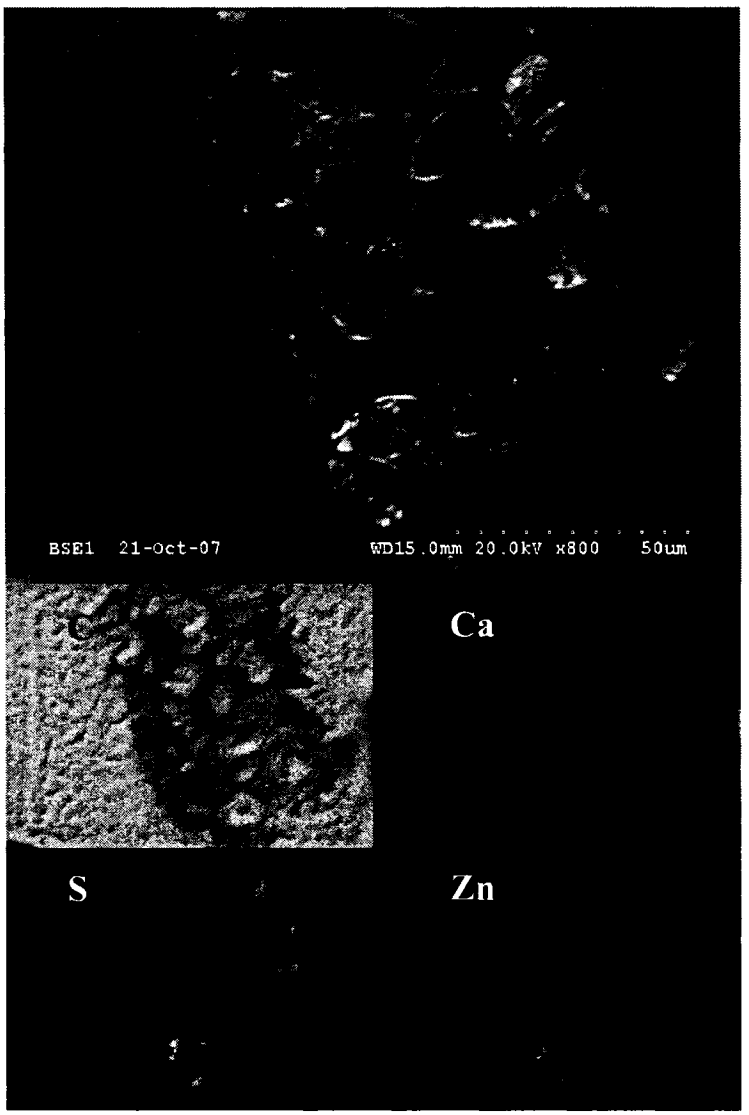

Figure 5.4 SEM-BSE image and elemental mapping for C, Ca, S, and Zn on reactive mixture from the top of a column operated at 10d HRT. Sulphide precipitation was observed with a metal to sulphur ratio corresponding to ZnS and CdS, as well as the presence of other metals (Fe, Cu) in the structure.

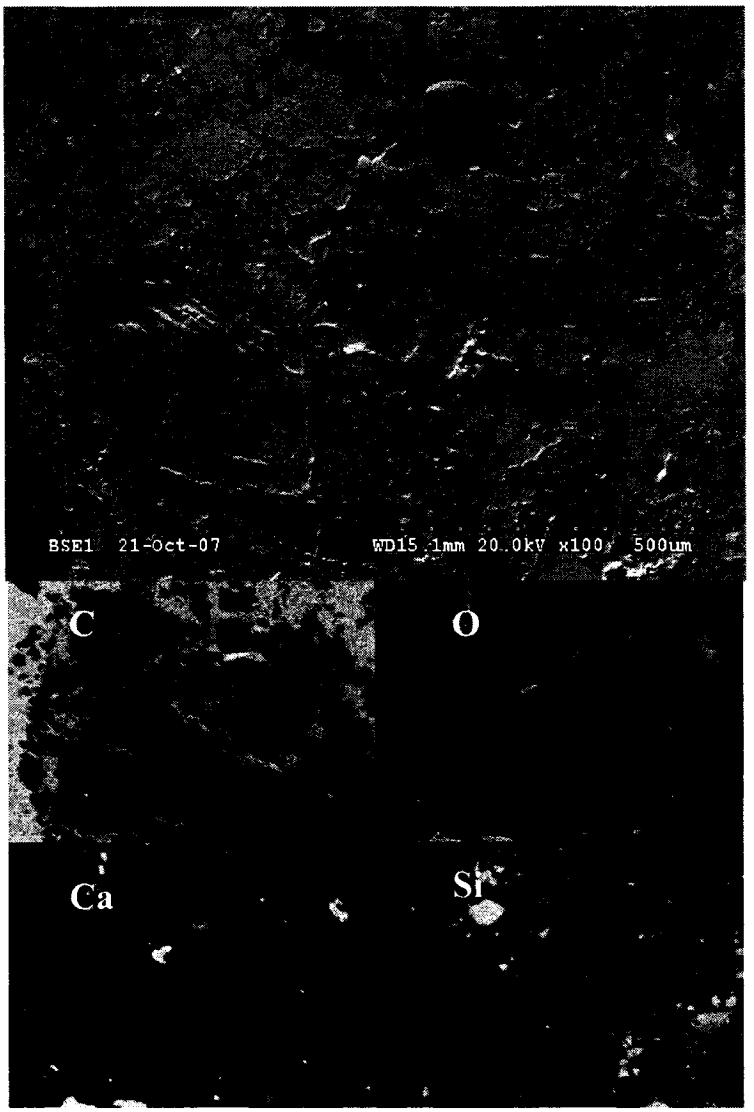

Figure 5.5 SEM-BSE image and elemental mapping for C, O, Ca, and Si on reactive mixture from the bottom of a column operated at 7.3d HRT. Sulphur (as sulphate) was mainly associated with O_2, Ca, and Al.

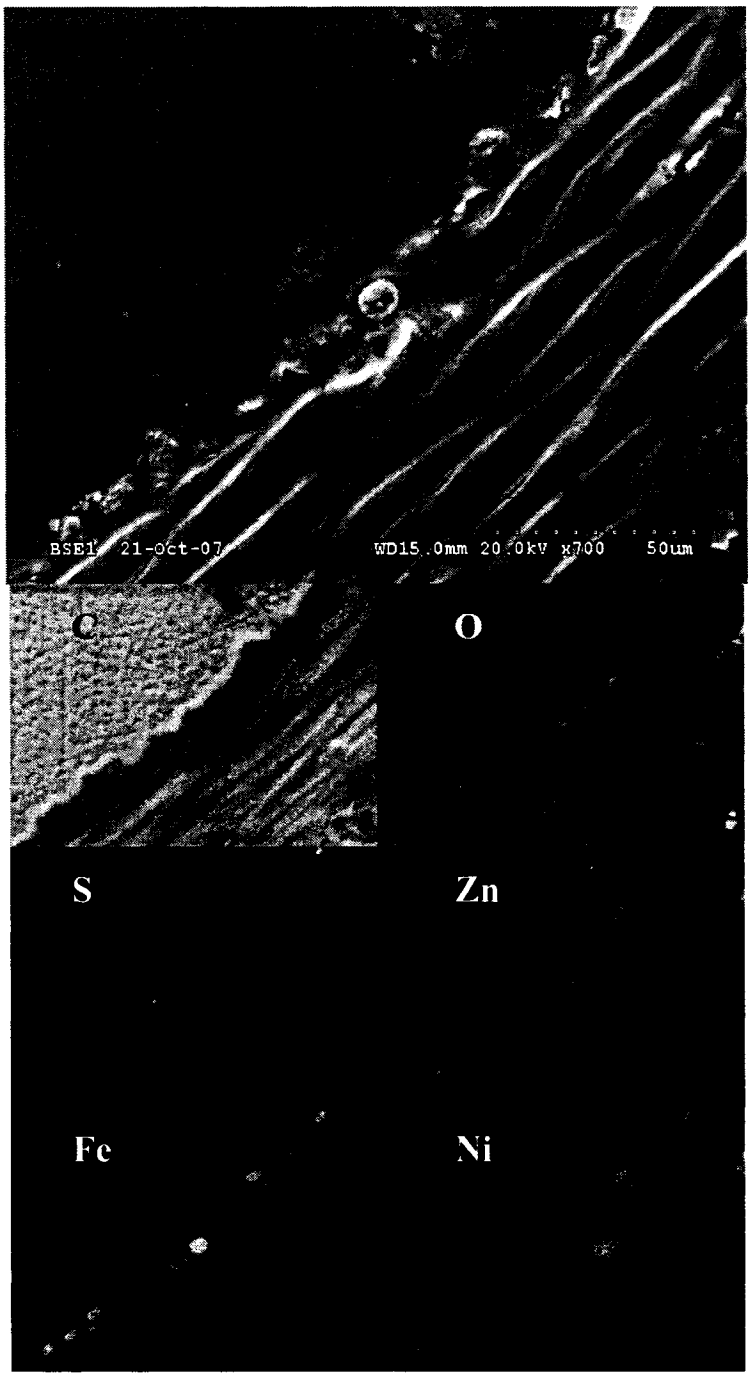

Figure 5.6 SEM-BSE image and elemental mapping for C, O, S, Zn, Fe, and Ni on reactive mixture from the top of a column operated at 7.3d HRT. Iron (oxy)hydroxide and the presence of S, Ni, and Zn in the structure were observed.

Thus, in addition to (oxy)hydroxide and carbonate minerals, and complementary to the XRD analyses, the SEM-EDS technique allowed observing what was interpreted as other metal sulphides (containing Cd, Ni, and Zn) which were unidentified by XRD analyses. Moreover, although the SEM-EDS technique did not provide quantitative data on the chemical form of the metal minerals, it confirmed the results from chemical extractions (AVS-EM) on the presence of metal sulphides in spent reactive mixtures from column bioreactors (Table 5.5).

5.5 Conclusions

Consistent with the high metal concentrations in the AMD drainage feed, as well as with elevated metal removal measured in the treated effluent (Neculita *et al.*, 2008, this journal), the physicochemical analyses indicated high concentrations of metals (Fe, Mn, Cd, Ni, and Zn) in the top and bottom layers of all columns. Moreover, the concentrations of Fe (50.8-57.8 g/kg) and Mn (0.5-0.7 g/kg) were up to twice as high in the bottom layers, whereas the concentrations of Cd (6.8-13.3 g/kg), Ni (1.8-5.2 g/kg), and Zn (2.5-13.2 g/kg) were up to 50-times higher in the top layers. Chemical extractions (SEP and AVS-EM) and elemental analysis gave consistent results, which indicated a low fraction of metals removed as sulphides (up to 15% of total metals recovered in spent reactive mixtures). Moreover, Fe and Mn were found in a more stable chemical form (residual fraction was 42-74% for Mn and 30-77% for Fe) relative to Cd, Ni or Zn, which seemed more weakly bound (oxidisable/reducible fractions) and showed higher potential mobility. Besides identifying (oxy)hydroxide and carbonate minerals, the mineralogical analyses identified metal sulphides containing Fe, Cd, Ni, and Zn.

In summary, highly contaminated AMD was effectively treated in column bioreactors operated at HRTs of 7.3d and 10d over a 15 month period.

Table 5.5 Chemical form of metals determined with different techniques on spent reactive mixtures from top and bottom layers of bioreactors

Approach	Technique	Chemical form	Metals				
			Fe	Mn	Cd	Ni	Zn
Chemical analyses	Digestion	- metal total concentration	- up to 2-times higher in the bottom layers		- up to 50-times higher in the top layers relative to bottom layers		
	Elemental analyses	- sulphates and sulphides - carbonates and bicarbonates	- 0.2-1.2% total sulphur with 0.2-1.0% in sulphates form and up to 0.8% in sulphides form - 0.6-1.7% in the top layers and 1.3-1.7% in the bottom layers				
	SEP	- soluble and exchangeable - carbonate bound - reducible/bound to Fe-Mn oxides - oxidisable/bound to organic matter - residual	≤ 0.2% < 0.1% 9-39% 14-38% 30-77%	16-25% ≤ 0.6% 1-31 2-15 42-74%	1-10% 0.1-5% 1-7% 72-97% 0.6-15%	9-62% 2-20% 19-48% 5-16% 1-43%	2-18% 0.4-3% 23-70% 19-56% 2-41%
	AVS-EM	- sulphides - metals	EM to AVS molar ratio: >>> 1; low fraction of metals in sulphides form				
Mineralogical analyses	XRD	- (oxy)hydroxides - carbonates - sulphides - sulphates - silicates	- goethite (α-FeO(OH) and lepidocrocite (y-FeO(OH)) - calcite ($CaCO_3$) - makinawite (FeS) and greigite(Fe_3S_4) - gypsum ($CaSO_4 \cdot 2H_2O$) - quartz				
	DSC-TGA	- sulphur compounds	- SO_3 evolving at temperatures around 730°C				
	SEM-EDS	- (oxy)hydroxides - carbonates - sulphides - silicates	- phases consisting mainly of Fe and O, along with S, Ni, and Zn - phases consisting mainly of Ca, O - pyrite, pyrrhotite ($Fe_{1-x}S$), chalcopyrite ($CuFeS_2$), CdS, ZnS, as well as other metals in the structure (Ni, Al, Na) - e.g. $ZrSiO_4$				

SEP- sequential extraction procedure; AVS-EM – acid volatile sulphides and extracted metals; XRD – X-ray diffraction; DSC-TGA – diferential scanning calorimetry and thermogravimetric analysis; SEM-EDS – scanning electron microscopy with X-ray dispersion microanalysis

The study of metal removal mechanisms in spent reactive mixtures after 11 months of bioreactor operation indicated that metals were mainly removed from the AMD by adsorption and other binding mechanisms (e.g. complexation) with organic matter (for Cd, Ni, and Zn), and by precipitation as (oxy)hydroxide minerals (for Fe and Mn). After 15 months, however, the column bioreactors did not lose their capacity for removing metals from the AMD. Moreover, the highly contaminated AMD used in this study represents a possible worst-case scenario in terms of the quality of the feed influent for passive bioreactors. This new insight into the potential mobility of metals under particular conditions could, however, be used for their recovery after decommissioning of the passive bioreactors.

5.6 Acknowledgement

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CHAPITRE VI

CONTRIBUTIONS À L'AVANCEMENT DES CONNAISSANCES ET CONCLUSION

Les travaux de recherche de cette étude portent sur le bioréacteur passif sulfato-réducteur comme moyen de traitement d'un DMA très contaminé. Les principaux résultats et les conclusions issues de l'ensemble du travail sont les suivants:

1. Disponibilité du carbone organique pour les bactéries anaérobies

La caractérisation des quatre matériaux organiques naturels (copeaux et sciure de bois d'érable, fumier de volaille composté et compost de feuilles) en termes de biodégradabilité, couplée à des essais en bioréacteurs type batch de trois mélanges réactifs constitués avec les matériaux caractérisés ont montré leur efficacité pour l'enlèvement du sulfate et des métaux (Fe, Mn, Cd, Ni et Zn entre 91,8 et 99,8%) à partir d'un DMA très contaminé. Des efficacités plus élevées ont été observées dans les réacteurs avec 30% (p/p) de déchets cellulosiques (copeaux et sciure de bois d'érable), qui ont permis de réduire les concentrations en sulfate de 5500 mg/L à < 1 mg/L, par rapport aux réacteurs avec 2-3% déchets cellulosiques où les concentrations finales en sulfate ont été de 2000-2750 mg/L. La caractérisation des matériaux organiques a aussi indiqué que des rapports plus élevées C/N, DOC (Demande Chimique en Oxygène)/SO_4^{2-} et COD (Carbone Organique Dissous)/ SO_4^{2-} sont associés avec de meilleures conditions sulfato-réductrices et d'enlèvement des métaux.

Ces travaux suggèrent que les rapports C/N et COD (carbone organique dissous)/ SO_4^{2-} considérés ensemble représentent les facteurs clefs pour évaluer la biodégradabilité des matériaux organiques naturels en conditions sulfato-réductrices.

2. Qualité et débit du DMA et évolution des paramètres hydrauliques

Les bioréacteurs type colonnes de 3,5 litres, remplis du mélange réactif identifié comme le plus efficace par des tests en batch et suivi durant une période de 11-15 mois à deux TRH (7,3 jours et 10 jours) ont été efficaces pour le traitement d'un DMA très contaminé (10-15 mg/L de Mn, Cd, Ni et Zn et 500 mg/L de Fe). De plus, la qualité de l'effluent traité a été significativement meilleure à un TRH plus long (10 jours). Cependant, la diminution de porosité et de perméabilité du substrat à un TRH plus long (10 jours) suggère que des problèmes hydrauliques pourraient affecter le système de traitement et ainsi limiter l'efficacité durant l'exploitation à long-terme.

Un compromis doit donc être fait pour le dimensionnement d'un bioréacteur passif efficace à long-terme afin de respecter les normes de rejet de l'effluent traité et de limiter les problèmes hydrauliques du mélange réactif.

3. Réglementation sur la qualité de l'effluent traité

L'effluent des bioréacteurs type colonnes, après 7 mois de suivi à un TRH de 10 jours n'était pas toxique pour la truite, mais il l'était pour les daphnies (*Daphnia magna*). La source de toxicité suspectée est le fer, ce qui a été confirmé à la fois par une procédure d'identification de la source de toxicité et les résultats de spéciation et de fractionnement des métaux. Cependant, une aération pendant 2 heures de l'effluent traité s'est avérée suffisante pour rendre l'effluent non-toxique pour *D. magna.*

Le dimensionnement des bioréacteurs passifs pour le traitement d'un DMA très contaminé par le fer doit prévoir un TRH suffisant, en plus d'intégrer une aération post-traitement afin de respecter les exigences de toxicité aigue.

4. Mécanismes d'enlèvement et forme chimique des métaux

Les résultats des analyses physico-chimiques et minéralogiques des mélanges réactifs post-démantèlement de quatre bioréacteurs (en duplicatas pour chaque TRH testé de 7,3

et 10 jours), après 11 mois de suivi ont servi pour identifier les mécanismes d'enlèvement des métaux, évaluer la stabilité des mélanges réactifs à long-terme, ainsi que la mobilité potentielle des métaux.

Les concentrations des métaux ont été élevées dans tous les mélanges réactifs, indépendamment du TRH, ce qui est en accord avec la charge en métaux (Fe, Mn, Cd, Ni et Zn) du DMA traité et les concentrations faibles mesurées dans l'effluent traité. De plus, les concentrations de Fe (50,8-57,8 g/kg) et Mn (0,53-0,70 g/kg) ont été jusqu'à 2 fois plus élevées dans le bas, alors que les concentrations de Cd (6,77-13,3 g/kg), Ni (1,80-5,19 g/kg) et Zn (2,53-13,2 g/kg) étaient jusqu'à 50 fois plus élevées dans le haut des bioréacteurs. Les extractions chimiques et les analyses élémentaires ont donné des résultats concordants, qui indiquent une fraction faible des métaux enlevés sous forme de sulfures (au plus 14% des métaux récupérés des mélanges réactifs). Les analyses minéralogiques ont permis l'identification de sulfures métalliques contenant du Fe, Cd, Ni et Zn, en plus des (oxy) hydroxydes et des carbonates. Donc, les principaux mécanismes d'enlèvement des métaux d'un DMA très contaminé sont l'adsorption et d'autres mécanismes de liaison des métaux par la matière organique (pour Cd, Ni et Zn) et la précipitation des (oxy) hydroxydes (pour Fe et Mn).

Après 15 mois d'exploitation, les bioréacteurs n'ont pas perdu leur capacité d'enlèvement des métaux du DMA. Les métaux sont stables dans le mélange réactif durant l'exploitation des bioréacteurs. De plus, l'augmentation de leur mobilité potentielle dans des mélanges réactifs à la fin de la vie du système est possible. La récupération des métaux par une lixiviation de ces mélanges réactifs pourrait être une alternative économiquement viable. Cette option devrait être évaluée dans le futur.

RECOMMANDATIONS

- Les tests en bioréacteurs de type batch ont suggéré que les rapports C/N et COD (carbone organique dissous)/ SO_4^{2-} considérés ensemble représentent les facteurs clefs pour évaluer la biodégradabilité des matériaux organiques naturels en conditions sulfato-réductrices. De plus, l'étude a confirmé qu'un rapport C/N autour de 10 est approprié pour la décomposition des substrats complexes en conditions anaérobies. D'autres essais seront nécessaires pour trouver les valeurs appropriées du rapport COD/SO_4^{2-} pour un traitement efficace du DMA en bioréacteur passif sulfato-réducteur.

- Deux tests de traceur colorés ont échoué en raison d'une très forte adsorption/absorbtion de la couleur dans le mélange réactif. D'autres traceurs devraient être testés tel que des métaux (ex. Na) afin d'évaluer le temps de résidence hydraulique réel dans le bioréacteur.

- Les bioréacteurs passifs peuvent être utilisés efficacement comme milieu de rétention de certains métaux (ex. Fe, Cd, Ni, Zn) d'un DMA très contaminé. Les métaux sont stables durant le traitement. Cependant, à la fin de la vie du système, la mobilité des métaux peut être augmentée. La faisabilité pratique de la récupération des métaux par lixiviation devrait être testée.

- À 7,3 jours de TRH, la diminution de la porosité et de la perméabilité du mélange réactif étaient moindres et, conséquemment, les risques que l'efficacité du système de traitement à long-terme soit affectée par des problèmes hydrauliques par rapport à 10 jours de TRH sont moindres. Cependant, la qualité de l'effluent traité à 7,3 jours était moins bonne du point de vue physico-chimique et écotoxicologique. De plus, l'identification de la source de toxicité, de même que l'évaluation de la toxicité de l'effluent ont montré que la source de toxicité était le fer. D'autres études

pourraient être réalisées avec des concentrations plus faibles de fer et/ou des TRH plus courts.

- Améliorer les méthodes d'identification des minéraux dans la matrice solide du mélange réactif (ex. trier les matériaux pour concentrer les précipités métalliques).
- Réaliser des travaux additionnels afin d'établir un lien entre les matériaux utilisés dans la composition du mélange réactif et l'évolution des paramètres hydrauliques.

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ANNEXE #1

Scan-ICP sur les matériaux organiques naturels utilisés dans la constitution des mélanges réactifs

Éléments	Al	As	Ba	Be	Bi	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Na*	Ni	Pb	S	Sb	Se	Si	Ti	Zn
Unité	(%)																						
CB	0,001	0,001	0,003	0,000	0,000	0,374	0,000	0,000	0,000	0,001	0,006	0,036	0,009	0,000	0,010	0,000	0,000	0,030	0,000	0,000	0,003	0,000	0,002
CBd	0,002	0,001	0,003	0,000	0,000	0,398	0,000	0,000	0,000	0,000	0,007	0,036	0,009	0,000	0,018	0,000	0,000	0,011	0,000	0,002	0,003	0,000	0,001
CF	0,711	0,000	0,011	0,000	0,000	25,3	0,000	0,001	0,017	0,022	1,87	1,97	0,062	0,000	1,09	0,005	0,006	0,484	0,000	0,000	0,420	0,031	0,027
CFd	0,704	0,000	0,011	0,000	0,000	25,5	0,000	0,001	0,016	0,018	1,89	1,96	0,061	0,000	1,09	0,005	0,006	0,464	0,002	0,000	0,353	0,029	0,030
FV	0,282	0,000	0,012	0,000	0,000	5,81	0,000	0,000	0,003	0,009	0,705	0,920	0,064	0,001	0,519	0,002	0,000	1,01	0,000	0,000	0,141	0,010	0,054
FVd	0,277	0,000	0,011	0,000	0,000	5,97	0,000	0,000	0,002	0,009	0,704	0,937	0,066	0,001	0,541	0,002	0,000	1,02	0,000	0,000	0,106	0,010	0,056
SB	0,002	0,001	0,002	0,000	0,000	0,222	0,000	0,000	0,000	0,000	0,004	0,034	0,015	0,000	0,002	0,000	0,000	0,025	0,000	0,001	0,003	0,000	0,002
SBd	0,001	0,001	0,002	0,000	0,000	0,221	0,000	0,000	0,000	0,001	0,003	0,033	0,014	0,000	0,000	0,000	0,000	0,016	0,000	0,001	0,004	0,000	0,001

LDM - limite de détection de la méthode

n/d – not déterminée

CB – copeaux de bois

CF – compost de feuilles

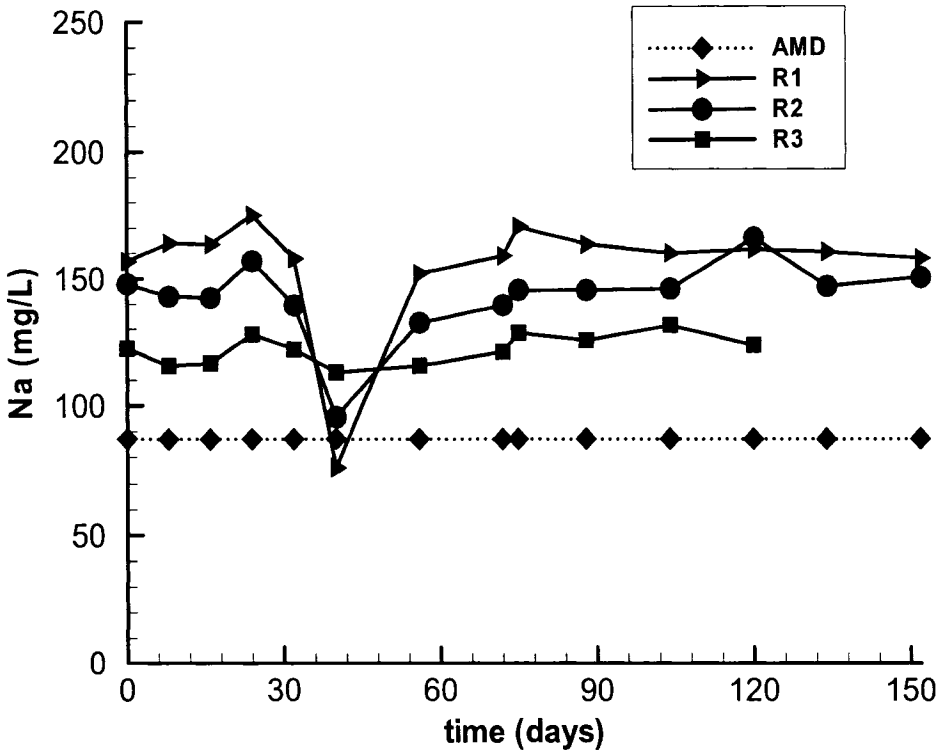
FV – fumier de volaille

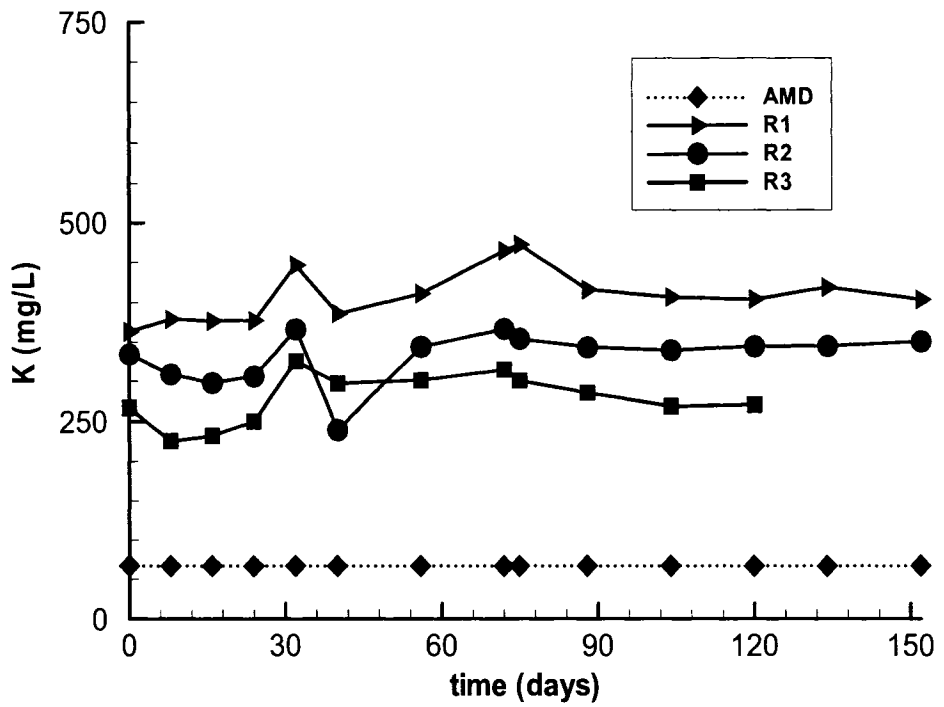
SB – sciure de bois

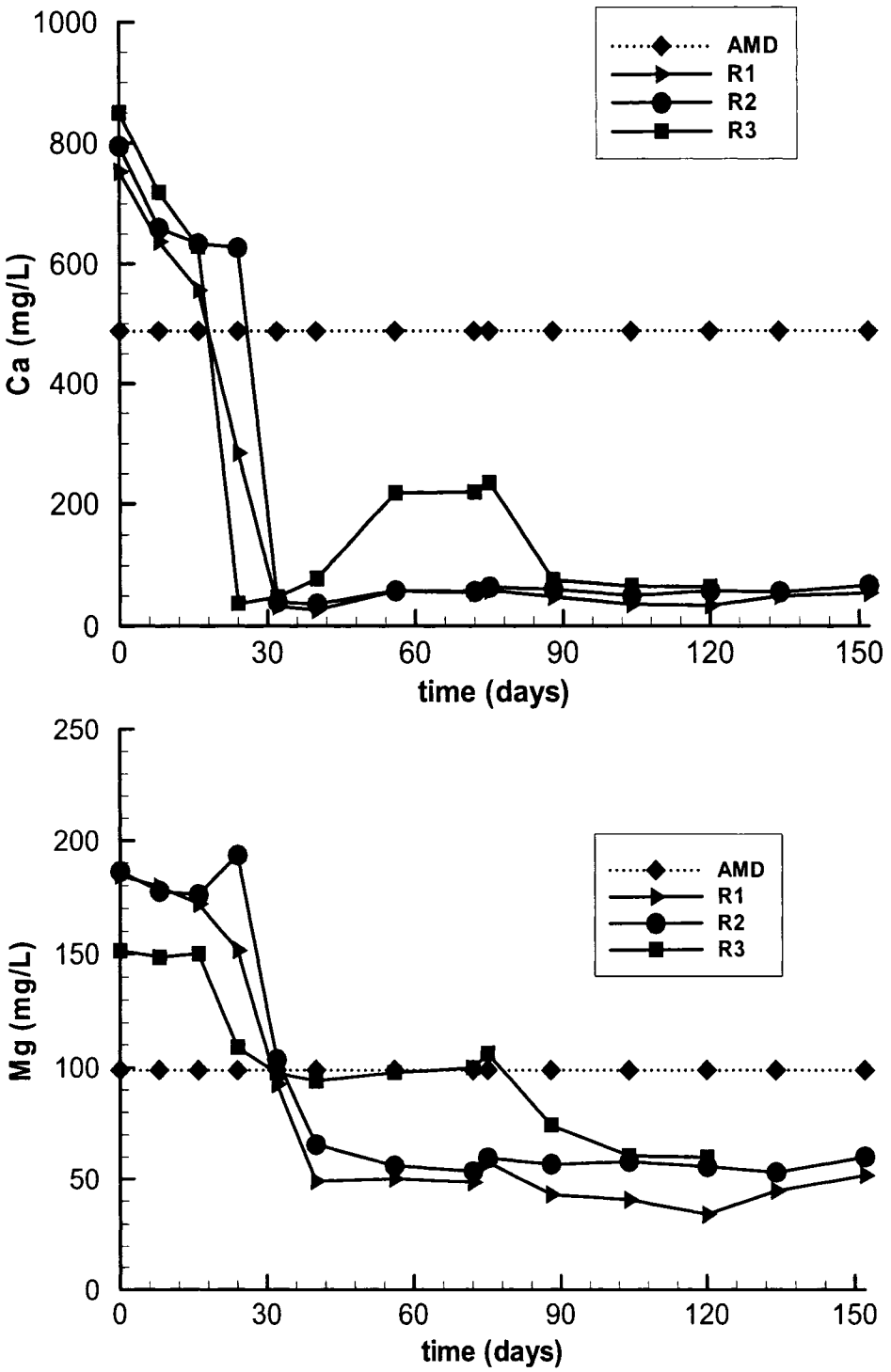
Note. Les analyses ont été réalisées en duplicatas par ICP-AES, suite à une digestion avec un mélange de HNO_3 et H_2O_2.

ANNEXE #2

Évolution des concentrations du Na, K, Ca et Mg durant les tests en bioréacteurs type batch

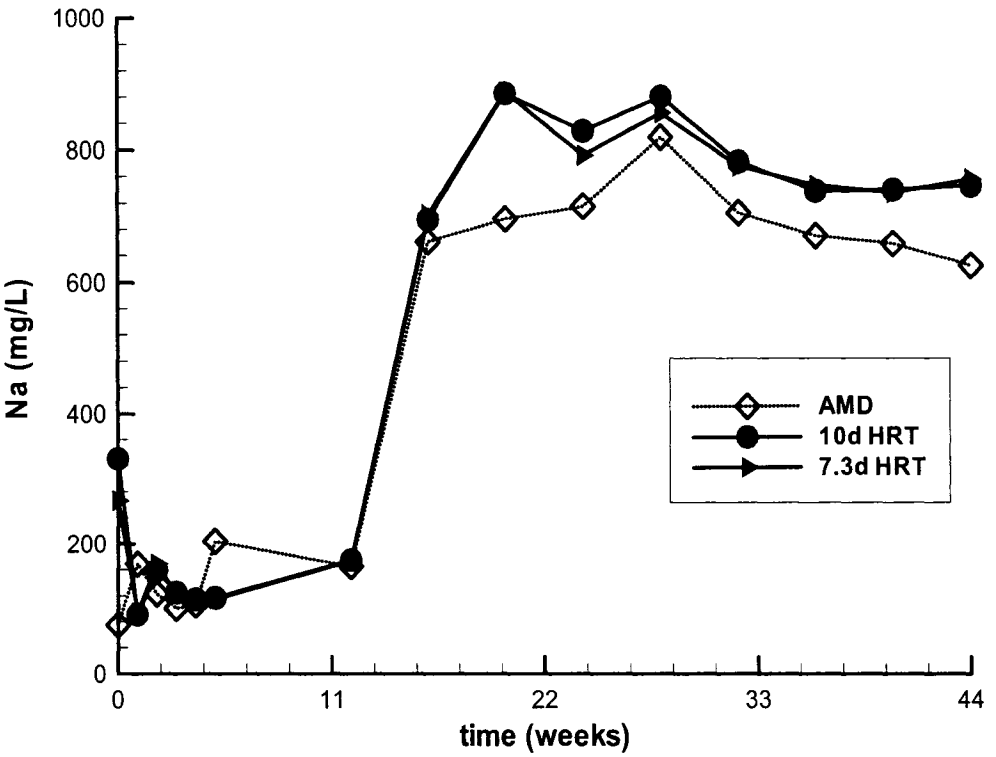

250
200
150
100
50
0
Na (mg/L)
0
30
60
90
120
150
time (days)
AMD
R1
R2
R3

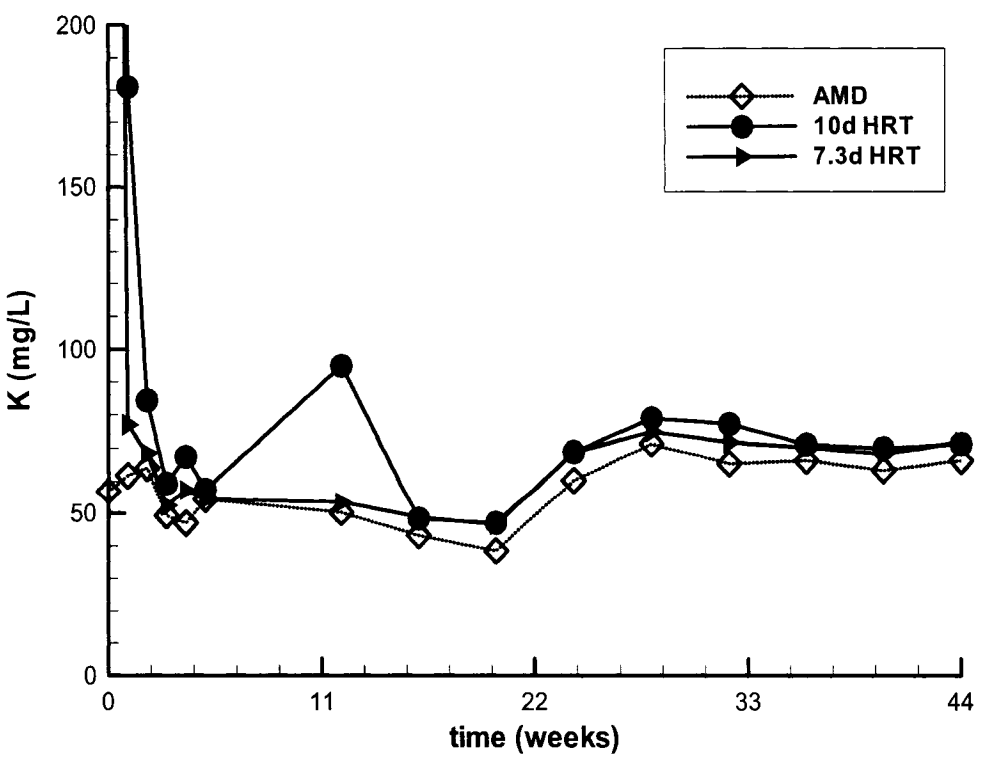

750
500
250
0
K (mg/L)
0
30
60
90
120
150
time (days)
AMD
R1
R2
R3

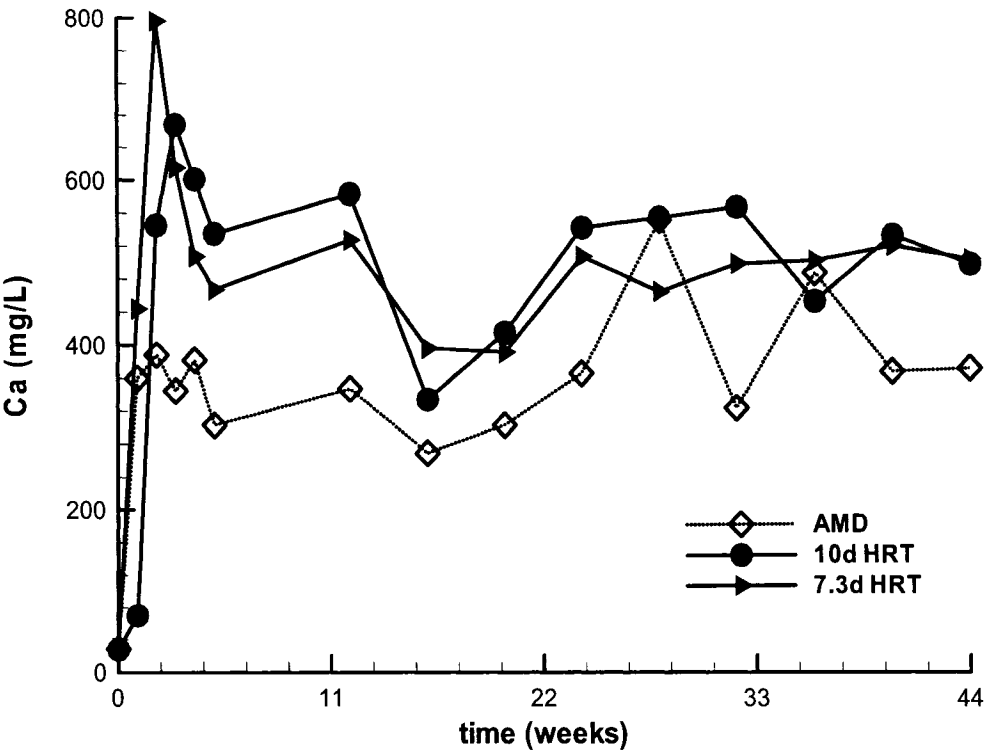

1000
800
600
400
200
0
Ca (mg/L)
0
30
60
90
120
150
time (days)
AMD
R1
R2
R3
250
200
150
100
50
0
Mg (mg/L)
0
30
60
90
120
150
time (days)
AMD
R1
R2
R3

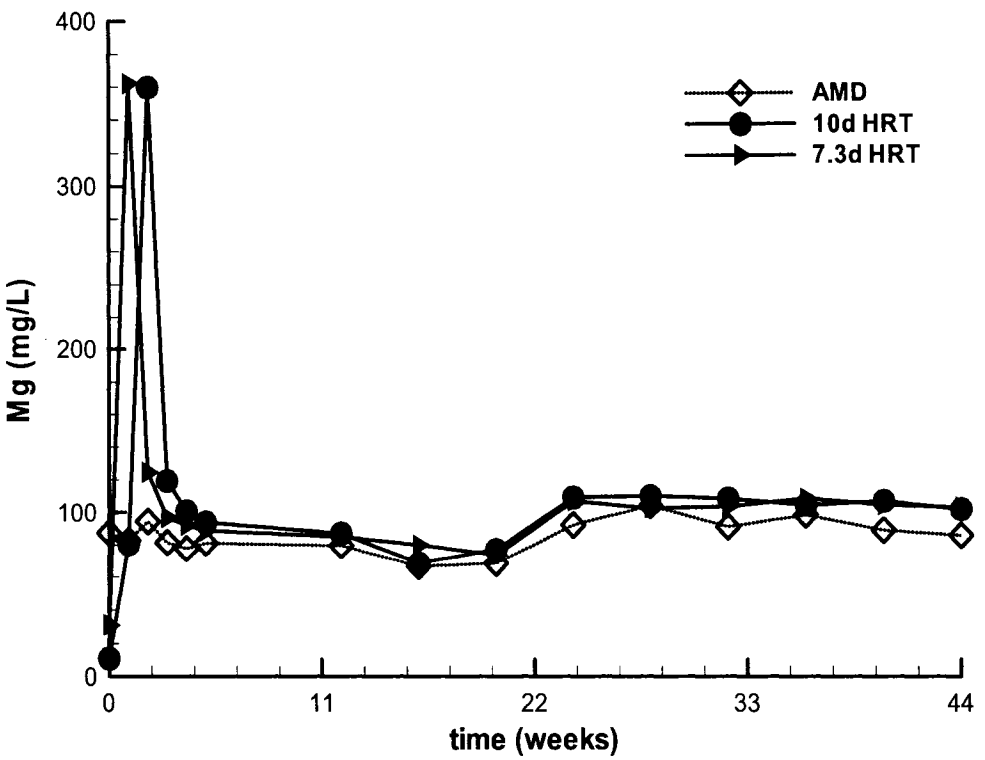
ANNEXE #3

Évolution des concentrations du Na, K, Ca et Mg durant les tests en bioréacteurs type colonnes


1000
800
600
400
200
0
Na (mg/L)
0
11
22
33
44
time (weeks)
AMD
10d HRT
7.3d HRT


200
150
100
50
0
K (mg/L)
0
11
22
33
44
time (weeks)
AMD
10d HRT
7.3d HRT


800
600
400
200
0
Ca (mg/L)
0
11
22
33
44
time (weeks)
AMD
10d HRT
7.3d HRT


400
300
200
100
0
Mg (mg/L)
0
11
22
33
44
time (weeks)
AMD
10d HRT
7.3d HRT

ANNEXES #4, #5, #6 et #7 (sur Cd-Rom)