

# **BIOLOGICAL REMOVAL OF ARSENIC IN A MULTI-STAGE ENGINEERED WETLANDS TREATING A SUITE OF HEAVY METALS**

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## **ABSTRACT**

A biological engineered wetlands treatment system designed to treat high concentrations of heavy metals built for Teck Cominco in Trail, BC has treated leachate since 1998. Leachate is collected from an historical landfill (now capped) and arsenic storage areas. Metal loadings have averaged more than 350 ppm Zn, 85 ppm As and 5 ppm Cd with peaks of 790 ppm Zn, 355 ppm As and 25 ppm Cd. Total volume of water treated is more than 11 million liters.

The system has consistently removed As, reducing the initially high concentrations to 0.5 ppm or lower year round. During periods of extensive changes and reconstruction the operating characteristics of the system changes and As removal efficiency drops demonstrating the importance of maintaining a consistent environment for the bacteria responsible for As removal.

A pilot-scale arsenic research project has been constructed in Trail. As well as the field program a multifaceted research project has been developed to examine the removal process under controlled laboratory conditions at the Royal Military College in Kingston, Ontario (As speciation) and at CANMET research facilities in Ottawa, Ontario (microbiological studies). Preliminary results from this research program are presented.

## **INTRODUCTION**

The element arsenic is classified as a metalloid and is well known for its toxicity. It has been used in medicines, the cosmetic industry and agriculture (insecticide, rodenticide herbicide, and desiccant). Current uses also include, doping of solid-state materials, laser materials, wood preserving and bronzing.

Arsenic is ubiquitous in the natural environment with background concentrations in Canada of 2 ppb in surface water and 50 ppb in ground water. Health Canada guidelines are set at 25 ppb for

drinking water compared to more stringent USEPA standards of 10 ppb. Arsenic in waters can result from natural weathering processes or from anthropogenic sources. It is found in coal and the by-products of coal combustion. Arsenic is associated with other metals in the earth's crust and mining and smelting activities can release this bound As.

There are many physical and chemical processes that have been developed for the removal of As both from drinking water and from acid rock drainage. Drinking water removal processes include: iron co-precipitation, alum co-precipitation, lime precipitation, activated alumina removal, ion exchange, reverse osmosis, electro dialysis and removal using activated carbon (USEPA 2001). Many different techniques and technologies are used when soil or groundwater contaminated by soil have been developed. Common methods used include: excavation, capping, soil solidification/stabilization (Miller 1996), electrokinesis (USEPA 1995), soil flushing using combined pump and treat (USEPA 1995), in situ redox manipulation, (Palmer 1992; USEPA 1991), reactive barriers, and containment (barrier walls).

Bioremediation researchers and other experts have patented many different systems that remove As including the AsRT technology (developed by Nikolaidis at the University of Connecticut) that removes As by the use of iron filings contained in permeable barrier walls or by introducing iron filings into the sand where As contaminated water flows through. The patent application for this technology describes research where concentrations of As ranging from 45 to 8500 ppb (0.045 – 8.5 ppm) are reduced by 97% by biological oxidation of the iron. Applied Biosciences has developed a procedure for As removal from water (BasR™) using ferric salts and pH adjustment in bioreactors where the metabolic processes of sulphate reducing bacteria generate H<sub>2</sub>S and eventually As<sub>2</sub>S<sub>5</sub> (Applied Biosciences 2001). Their patented system for biological removal of metals, ABMet™, has been constructed to treat ARD drainage that contains As in a full-scale year-round operation. Jack Adams of Bioremediation and Bioprocess Consulting, LLC reports removal of high concentrations of As (as high as 355 ppm in spike events has been demonstrated) using specialized SRB, *Pseudomonas* sp., and other bacterial lines. Arsenic concentrations treated in laboratory, pilot-scale and full-scale application range from 0.5 mg/L to 45 mg/L all treated to below 2 ppb.

## **Background**

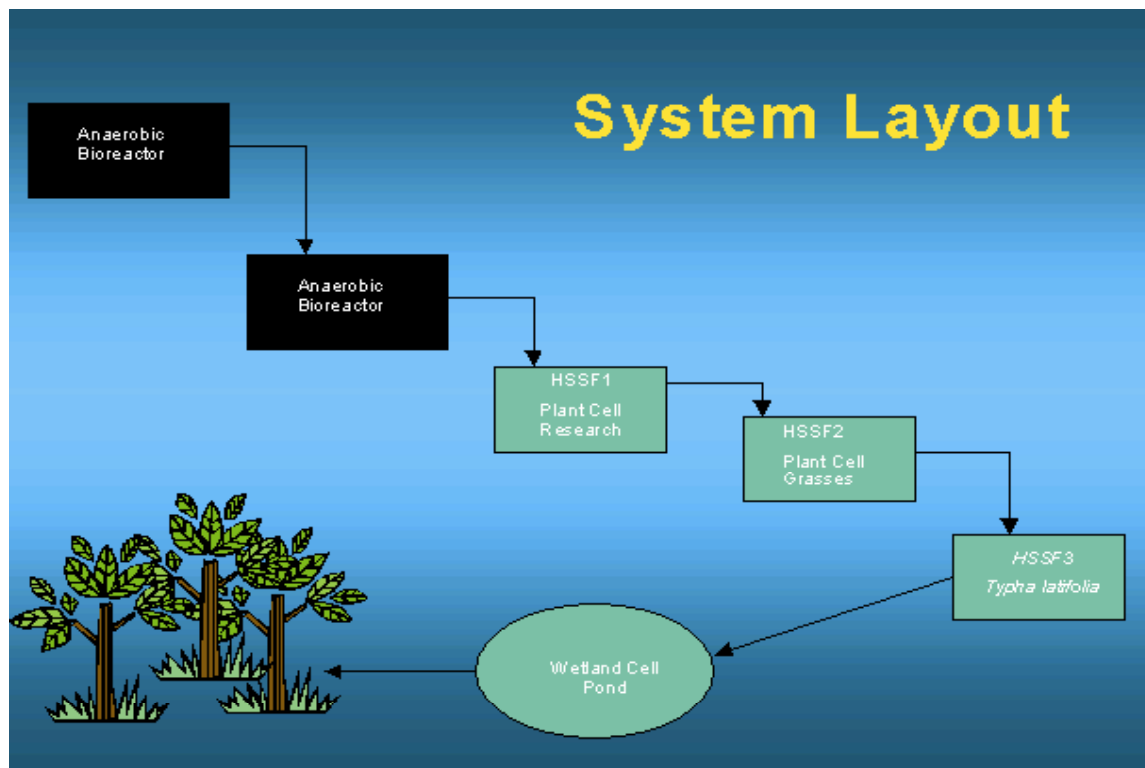
In Trail, BC, smelting has been an ongoing activity since 1897. Historical disposal practices have left legacy issues to be dealt with. To address these legacy issues an engineered collection system was constructed in 1997 to collect seepage from the historical landfill (primary contaminants - Zn, Cd) and arsenic (primary contaminant - As) storage areas.

Nature Works Remediation Corporation was contracted by Teck Cominco to design, build and operate a field scale biological treatment system as a possible alternative to traditional lime-based treatment systems. The first phase (a series of self-contained sub-surface flow wetland cells) was constructed in 1997 to remove high concentrations of Zn, Cd and As from this leachate. The cells were designed and built using typical constructed wetland techniques (Kadlec and Knight, 1996).

The first cell (50 m<sup>2</sup>) included *Brassica*, a species reported to be able to withstand heavy metals and *Helianthus annuus*. The second cell (50 m<sup>2</sup>) contained *Calamagrostis canadensis* and native grasses transplanted from the immediate area. The third treatment cell (300 m<sup>2</sup>) contained *Typha latifolia*. Cells were initially watered (during the 1<sup>st</sup> year) using the local stream while surface and root growth was established. The holding pond built as the last stage retains water for final testing prior to being used for irrigation.

In 1998, a large anaerobic bioreactor (vertical sub-surface flow wetlands) was constructed. The potential to treat 20,000 L per day (13.9 L/min) was used to estimate cell area. The volume sized 'rule of thumb' used in calculation based on metal concentrations is the removal of 0.3 mol/(m<sup>3</sup>d) of metal where the volume component is the total volume neglecting the pore space and moisture content (Dvorak et al. 1991, Hedin et al. 1989, Gusek and Wildeman 1997). The area based 'rule of thumb' of between 10 m<sup>2</sup>min/L and 20 m<sup>2</sup>min/L (pH dependent) was used (Gusek and Wildeman 1997). The composition of biomass used was 60% kraft pulp mill biosolids, 35% sand and 5% cow manure.

During 1998, the system treated leachate for 8 weeks and in 1999 treatment commenced in late spring and continued until the end of October with a two-week shutdown in late August to repair short circuiting in the plug flow bioreactor design. In total the system operated for 18 weeks in 1999.



**Figure 1:** Diagram showing lay out of final configuration of complete system installed in Trail BC for treatment of high concentrations of heavy metal contaminated leachate.

To improve Zn removal, a second larger anaerobic bioreactor containing limestone was constructed upstream of the existing system in 2002. This reactor helped to achieve higher pH levels (7.2 to 7.6) for optimal Zn removal. While this bioreactor was the second one constructed, it is considered the first 1<sup>st</sup> bioreactor, as it is first in the treatment series. The original bioreactor is denoted as the 2<sup>nd</sup> bioreactor as it follows in treatment sequence the 1<sup>st</sup> bioreactor. As well, the system was fully winterized for year-round operation. This construction work delayed spring startup and treatment started June 8, 2000. The system then ran continuously January 20, 2001 (261 days) when a frozen input pipe necessitated a system shutdown. In the spring repairs were made and the system has operated continuously since May 1<sup>st</sup> 2001 to present.

## **Sampling**

Regular sampling has taken place since the system was constructed. Originally, samples were weekly, but once the system became operational year-round sampling frequency increased to three times weekly during summer months and weekly during spring and summer and bi-weekly during winter operations. Grab sampling were taken at the input and output of each cell in the system – resulting in six samples weekly until completion of the second bioreactor and winterizing. Following this construction activity samples were taken from sampling ports built into the system with grab samples taken only from the final holding pond for a total of 8 samples per week. More than 200 assays of As concentrations throughout the system are available from initial construction through to the end of December 2003.

Samples were assayed for metal concentration using ICP-MS, and results reported for As, Cd, Zn (Table 1). Samples were also tested for suspended solids; P, SO<sub>4</sub> and NH<sub>3</sub>/N as well as Fe, Mn and Sb. Prior to ICP-MS assay samples were tested for pH and dissolved oxygen. Over the course of two summers extensive sampling of above ground plant material was completed and tissue assays completed using ICP-MS.

Flow rates were initially monitored manually using a stopwatch and measured container and adjustments made as required. Currently, a digital timer is used to control pump operations and flow is monitored by an accumulating digital flow meter installed at the central sump.

## **Results**

In 1998, the first anaerobic bioreactor was constructed and operated for 8 weeks in conjunction with the three plant-based treatment cells for the initial trial. In 1999, the system was started in early spring and operated until November (total 261 days). Winterization was completed in 2000 and the system operated without interruption until January of 2001 when a frozen pipe stopped flow from January 27 to April 8. A major re-construction project in 2002 to replace the original temporary anaerobic bioreactor with a permanent redesigned one subsequently reduced operating time to 295 days for that year. Since then the system has operated continuously and the total volume treated and the total moles of contaminating metals removed is shown (Table 1).

**Table 1:** Operating days, total flow and moles of metal removed (As, Cd and Zn) during operating lifespan of biological treatment system.

<b>Year</b>	<b>Operating Days</b>	<b>Total Flow</b>	<b>Moles As Removed</b>	<b>Moles Cd Removed</b>	<b>Moles Zn Removed</b>	<b>Total Moles</b>
1999	126	1,606,279	1,636	64	4,325	6,025
2000	261	2,824,000	1,899	173	20,698	22,770
2001	305	3,776,806	4,918	153	16,676	21,747
2002	295	2,111,026	2,495	79	10,155	12,729
2003	345	3,010,257	4,313	93	12,129	16,535
<b>Total</b>	<b>1332</b>	<b>13,328,368</b>	<b>15,261</b>	<b>565</b>	<b>63,983</b>	<b>79,806</b>

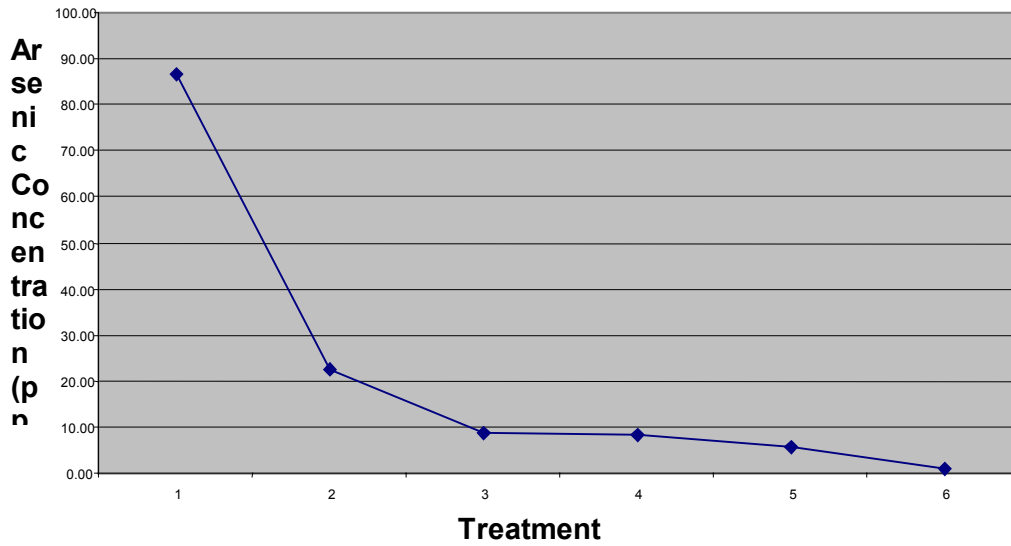
Arsenic has been removed from the system since it was first constructed with a steady decline in concentration evident as water is treated by the various cells from input to the final holding pond (Table 2; Figure 1). During initial trial (summer of 1998) when 500,000 liters of leachate were treated, starting As concentrations of 46 ppm were reduced at the output to 0.56 ppm – a reduction of 96.7 %. Table 2 incorporates all assays from 1999 to 2003 including the period during 2000 when the system experienced considerable disruption (winterization and the construction of a new upstream bioreactor) and during 2002 when the 2<sup>nd</sup> anaerobic bioreactor was removed and reconstructed. Immediately following periods of disruptions and when the system was re-started in the spring following latent periods (1999 and 2001) higher concentrations of As were observed in the final holding pond. Oxidation and subsequent mobilization of the already precipitated As is the likely cause of this increase when the system is disrupted. Following lengthy periods of dormancy the bacterial populations may decline and require time to re-build. This may be responsible for the reduced As treatment efficiency following dormancy periods.

**Table 2:** Mean concentrations of As (ppm) at the input to the system and at the input to each of the subsequent stages from June 16<sup>th</sup> 1999 through December 31<sup>st</sup> 2003.

	<b>Input</b>	<b>2<sup>nd</sup> An.</b>	<b>1<sup>st</sup> Plant</b>	<b>2<sup>nd</sup> Plant</b>	<b>3<sup>rd</sup> Plant</b>	<b>Final</b>
Mean	83.74	22.06	8.46	8.01	5.76	1.02
St. Dev.	55.88	22.63	10.81	16.82	11.57	2.08

Even with the disruption in operations, the system removed arsenic consistently with an overall removal efficiency of 98.8%. The two anaerobic bioreactors removed 90.0% of the total As that entered the first bioreactor. The third plant cell consists of a large deep *Typha* cell that was designed as a polishing cell. It is effective at As removal and sequestration with a removal efficiency of 82.8%. The most rapid reduction in As appears to take place in the first anaerobic cell where 73.9% of the As entering the cell is sequestered (Figure 1).

### Arsenic Removal in Five Cell Biological Treatment System



**Figure 1:** Arsenic removal in five-cell biological treatment system showing input Arsenic concentration (ppm) at each of the five stages and concentration in the final holding pond. Stage 1 is the input to the system, stage 2 is the output from the 1<sup>st</sup> anaerobic bioreactor, stage 3 is the output from the second anaerobic bioreactor, stage 4 is the output from the 1<sup>st</sup> plant cell, stage 5 is the output from the second plant cell and stage 6 is the output from the final plant cell of fully treated water in the final holding pond.

Censoring data for the summer of 2000 (when construction disrupted the system) provides better treatment results with mean As concentrations in the final holding pond of 0.55 ppm (Table 3) versus 1.02 ppm (Table 2); an difference in As concentration of 0.47 ppm. Although the difference is very small in absolute terms, the increase in As concentration is 85%, an increase that illustrates that long-term uninterrupted operations increases operational efficiency of this biological treatment system. Differences are also seen in the mean concentration of As in individual cells with higher concentrations in the input to the first plant cell and lower concentrations in the inputs to all others. The higher mean concentration of As in the holding pond for periods when the system is disrupted compared to the total operating period are significant at the 99% level of confidence using a one-way ANOVA.

**Table 3:** Mean concentrations of As (ppm) at the input to the system and at the input to each of the subsequent stages from June 16<sup>th</sup> 1999 through December 31<sup>st</sup> 2003 with data from the construction periods (July and August 2000) censored.

	Input	2 <sup>nd</sup> An.	1 <sup>st</sup> Plant	2 <sup>nd</sup> Plant	3 <sup>rd</sup> Plant	Final
Mean	95.69	21.60	8.92	3.42	2.62	0.55
St. Dev.	57.53	23.78	11.63	18.21	12.41	0.73

Arsenic removal through biological processes is clearly demonstrated in the system. At the time of writing the system in Trail had completed its second winter of operations without a freezing incident and the removal efficiencies remained high throughout the year. Our present research focuses on understanding the removal mechanisms and the stability of the precipitated As compounds

### **Biological Arsenic Removal Research Consortium**

Biological arsenic removal is currently receiving considerable attention from investigators in many parts of the world. As far as we can determine a few of them have isolated some species of bacteria that play an important role but these are relatively rare and hard to find in a biological system that appears to be removing As. Therefore, our efforts at setting up a research program have been enthusiastically embraced by many other researchers.

### **System Construction:**

Throughout the summer of 2002 Nature Works embarked on two major projects simultaneously – the reconstruction of the wetlands treatment system to improve operations and to improve winter time performance; the design and construction of an entirely new research system that will investigate the arsenic removal process.

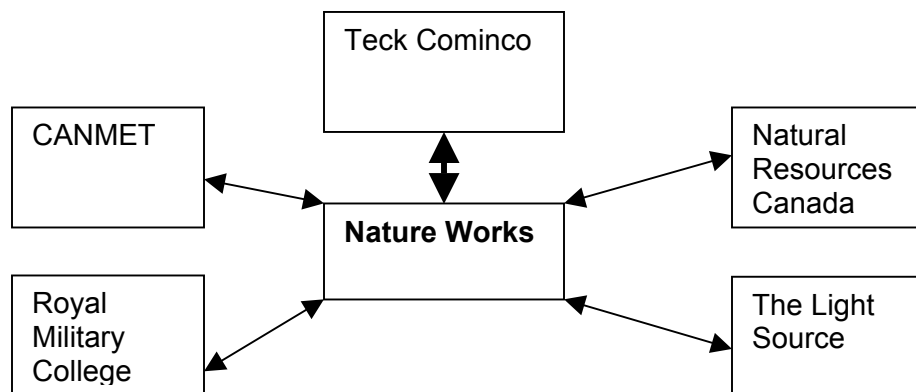
The reconstruction of the wetlands system focused on the second anaerobic bioreactor. This large cell had been initially constructed in 1998 when the system was considered temporary. Less robust materials were used in construction (RPE liner rather than 60 ml polyethylene) and our design for improving flow through in the plug flow reactors we used had been improved. The original cell material was removed and stored in a lined temporary storage area while a new liner was installed in the original cell. During removal it was possible to systematically sample the biological substrate, by dividing the surface area into nine quadrats and each quadrat into three levels. Since we had four separated treatment layers in our original design the sampling procedure gave us 108 sample points. The samples were assayed for metal and sulphur using ICP-MS and for total organic carbon by Rock-Eval pyrolysis as described by Lafargue et al (1998).

Building the new dedicated research system was necessary because As was being removed in the larger system and there is no known process that satisfactorily explains the dynamics of the removal. To reduce confounding variables, a new smaller scale system was constructed that operates using only water collected from the arsenic sump in an attempt to elucidate the removal mechanisms. Arsenic sump water has mean concentrations of 26.1 ppm As, 0.1 ppm Cd and 4.1 ppm Zn and a SO<sub>4</sub> concentration of 561ppm.

### Establishing a Network of Scientific Resources:

Issues related to the biological removal of arsenic are complicated and require expert attention in both the field and laboratory. Teck Cominco supported the initial endeavour by supplying funds that paid for rebuilding the existing anaerobic digester and for the design and construction of an entirely new research system to assist in determining the mechanism of biological arsenic removal.

Other researchers and institutions were interested in the idea of studying the dynamics and organisms underlying the biological removal process and they have offered support in the form of research facilities, graduate student assistance and a willingness to share results. The laboratory work is being carried out in four centers across Canada, using a consortium of research expertise that is working through and together with Nature Works in order to answer questions related to biological arsenic removal.



The work that will be completed at each of the laboratories is as follows:

1. Natural Resources Canada – Scientific Authority Dr. F. Goodarzi, Co-ordinator, Environmental Studies, Institute of Sedimentary and Petroleum Geology, Geological Survey, Calgary, Alberta, Canada agreed to analyze 138 samples of the previously existing anaerobic digester (reconstructed in 2002) for metal content and carbon analysis (Rock-Eval 6). The results will be reported at the Metallurgical Conference in Hamilton (Duncan et al., August 2004). Samples from the deconstructed bioreactor will be examined by an electron microscope to determine the form of arsenic compounds present using elemental mapping to show what other elements are in close proximity to As. While fluorescent microscopy will be used to examine the form of carbon present in close proximity to the As that is present.
2. A MSc. graduate student examined aspects of the system under the direction of Dr. Ken Reimer, Director, Environmental Sciences Group at Royal Military College in Kingston, Ontario. Research was completed using bench-scale systems, modeled on the Trail

system using Celgar pulp mill biosolids delivered from Trail. The work looked at three factors:

- The gases that are emitted from an operating system
  - The question of whether or not the removal process is bacterially moderated or bacterially facilitated (i.e. do bacteria actually reduce the As or do they change the micro environment so that As is reduced)
  - The long-term stability of the As compounds that are formed.
3. Detailed examination of the bacterial processes involved in As removal is currently underway at the CANMET labs in Ottawa under the direction of Dr. Doug Gould. Dr. Gould has been working on aspects of our system in Trail since 2000. Initial results have previously been reported (Mattes et al, 2002). Column tests were completed to determine if removal in Trail might be site specific as a result of activity of specialized bacteria that had evolved over time in that environment as a consequence of long-term exposure. Additional tests were performed to determine if trace elements such as Mn might be a contributing factor to As removal. After each trial run the material in the columns is sectioned and distributed for specific analyses. One sub-sample is used for ICP-MS analysis to determine metal concentrations; a second sub-sample will be used for SEM work as previously described. A third sub-sample will be examined by staff of the Light Source using beam time on the Synchrotron at the Argonne National Research Lab in Chicago. A final sub-sample will be used for bacterial analysis. Cultures of the material will be made and the bacteria grown under anaerobic conditions. Following this, bacterial speciation using DNA sequencing techniques will be completed.
  4. Dr. Jeffrey Cutler of the Light Source in Saskatoon will supervise the synchrotron work on original samples from the deconstructed bioreactor and on samples taken from the field system in Trail and from the bench scale reactors constructed in Kingston. The oxidation state of the As in the samples will be determined using XANES and, if the concentrations are high enough As speciation will be determined using EXAFS data.
  5. Nature Works will operate its two systems in Trail – the larger wetlands based system that has demonstrated highly effective arsenic removal rates and the specially constructed smaller system designed to specifically examine leachate from the arsenic sump only. This will provide ongoing operational data, a source for inoculums for other research sites and provide samples for both metal and bacteriological analyses.

### **Preliminary Results**

Reconstructing the bioreactor enabled us to assay a system that had been functioning effectively for several years. When the assays were completed it was clearly demonstrated that there was a positive relationship between the deposition of As in the cell layers and the presence of sulphur. The findings will be presented in August at the International Metallurgical Conference in Hamilton, Ontario (Duncan et al, 2004). For As and sulphur there is a strong highly significant linear regression correlation between the presence of sulphur and As (Table 4). The correlation is

positive and is significant at > 99% confidence. Similarly we can demonstrate that there is a negative linear regression between the presence of As and the presence of total organic carbon (TOC) that is significant at > 99% confidence level when all four layers are examined and significant at the 95% level of confidence when the biosolid layers only are examined. The original bioreactor had a filtering layer on top of three layers of biosolids that were separated by waterproof liner material and each of the three layers were examined separately and combined for linear regressions between sulphur and the primary metals of concern: Zn, Cd, and As.

**Table 4:** Showing linear regressions between the increased presence of sulphur as As is deposited in a four layer anaerobic bioreactor and the related reduction of total organic carbon present at the same sampling point in the bioreactor.

1 <sup>st</sup> Var.	2 <sup>nd</sup> . Var.	N	R <sup>2</sup>	P	1 <sup>st</sup> Var.	2 <sup>nd</sup> . Var.	n	R <sup>2</sup>	P
<b>Four layers Combined</b>					<b>Biosolid Layers Only</b>				
As	S (+)	92	0.19	0.0000 <sup>a</sup>	As	S (+)	75	0.18	0.0001 <sup>a</sup>
As	TOC (-)	92	0.09	0.0021 <sup>a</sup>	As	TOC (-)	75	0.07	0.0157 <sup>b</sup>

From the data we can assume that As is being removed and is deposited in close association with sulphur and from the reduction in the total organic carbon we hypothesize that the removal mechanism is biologically-based and is dependent on the presence of specific bacterial processes.

It has been reported in the literature (Newman et al, 1997) that *Desulfotomaculum auripigmentum* can precipitate arsenic trisulphide by reducing As(V) to As (III) and S(VI) to S(-II) at concentrations of 0.1 to 1 mM. This bacteria appears to be one that can produce As<sub>2</sub>S<sub>3</sub> directly, but the process is pH sensitive and is affected by As concentration. There are also reports of the importance of ferrihydrite as a sorbing site for the transformation of As(V) to As(III) at non-toxic concentrations (Lagner and Inskeep 2000) but once again the issue of non-lethal concentration is crucial. In the system in Trail the mean concentrations of 84 ppm (Table 2) is 1.12 mM and the peak concentrations (as high as 200 ppm) is 2.67 mM. If the reducing processes that are reported by these researchers are active in this system, there must certainly be other factors that are playing a role given the maximum mM concentration that they suggest of 1 mM as being lethal for As reducing bacteria. Perhaps the bacteria in the Trail system are different or better adapted due to prolonged exposure to high As concentrations.

Reports by the various consortia partners are important confirmations of our statistical finding that As and S are strongly correlated. CANMET researchers have reported that anaerobic examination of samples from the bioreactor assayed at the Stanford synchrotron shows the presence of amorphous arsenic sulphides (Gould, *personal communication*). Similarly, researchers at RMC examined samples from their lab-scale bioreactors at the Synchrotron at the Argonne National Research Lab and the x-ray absorption near edge spectroscopy (XANES) indicated the possible presence of As<sub>2</sub>S<sub>3</sub> with the spectrum observed similar to As<sub>2</sub>S<sub>3</sub> standards

(Reimer, *personal communication*). Additional samples are prepared and waiting for beam time to confirm this finding.

We are waiting for these reports and for the results of other work that is being completed at the laboratory facilities in Calgary, where samples from the bioreactor will be examined using a scanning electron microscope to provide elemental mapping of the molecules that are closely adjacent to the As present in the samples. The presence of large quantities of S in association with As will provide further proof that the system is forming polysulphides arsenic compounds. Further microscopic examination using fluorescent microscopy will provide details of the type of carbon associated with the As and possibly even images of bacteria.

Other research that is presently underway at CANMET will examine the possibility that bacteria present in the As seep waters that we treat might be an important factor and that other metals, present in trace amounts might be significant. The focus of the research is directed towards understanding and improving the dynamics of the current system with a view to determining the maximum treatment concentrations. Understanding the process will continue to be a focus of the group as well. The final results from the consortium will be available in late summer 2004.

The system at Trail treats industrial strength As contamination unlike others reported in the literature. Although the final As concentrations remain too high to meet new stringent standards, when our technology is combined with that of our collaborator Jack Adams of Bioremediation and Bioprocess Consulting, the final As concentration should be reduced to levels well below 2 ppb.

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