

Multi-stage Biological Treatment System for Removal of Heavy Metal Contaminants

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ABSTRACT

A biological treatment system designed to passively remove high concentrations of metals from leachate collected from a landfill near a large integrated zinc lead smelter has been in operation intermittently for the past five years including winter time operations. High metal removal efficiencies in the system were achieved. After 729 days of operation and treating over 8 million L of water the original anaerobic bioreactor constructed in 1997 was taken apart and rebuilt. The deconstruction of the bioreactor allowed systematic sampling at all depths of the biological matrix. Samples were analyzed for metal, S and TOC concentrations; as well bacterial populations were determined. Results of this sampling procedure are examined and compared at various depths within layers and between layers using one-way ANOVA and linear regressions. Metal concentrations were positively correlated with sulphur and negatively with total organic carbon. This is in concurrence with a model of biogenic precipitation of metal sulphides. *Keywords: biological metal removal, anaerobic bioreactor, zinc, cadmium, dissimilatory arsenate reduction.*

INTRODUCTION

A historical vegetation-capped landfill, near a zinc lead smelter in Trail BC, produces a leachate that contains metals. A seepage collection system was built in 1997 to funnel the seepage into sumps from which it was pumped to a lime-based Effluent Treatment Plant until the prototype biologically based treatment system was completed. In 2003, the landfill was covered with an engineered cap.

Nature Works Remediation Corporation was contracted by Teck Cominco to design build and operate a field scale biological treatment system as an 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 (primary contaminants) from this leachate. The cells were designed and built using typical constructed wetland techniques described by Kadlec and Knight (1). The first cell (50 m²) included *Brassica*, a species reported to be able to withstand heavy metals and *Helianthus annuus*. The second cell, (50 m²), contained *Calamagrostis canadensis* and native grasses transplanted from the immediate area. The third treatment cell, (300 m²) contained *Typha latifolia*. Cells were initially watered (1st year) using the local stream while root growth was established. The holding pond built as the last stage retains water for final testing before 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/(m³d) of metal where the volume component is the total volume neglecting the pore space and moisture content (Dvorak et al. (2); Hedin et al (3); Gusek and Wildman (4)). The area based 'rule of thumb' is estimated to be between 10 m²min/L and 20 m²min/L by Gusek and Wildman (4). The composition of biomass used was 60% kraft pulp mill biosolids, 35% sand and 5% cow manure. The cell was designed to be temporary and RPE – a waterproof tarpaulin material – was used as a liner.

In an attempt to increase contact time and to prevent failure in the plug flow design the cell was constructed in 3 layers separated by RPE placed to direct flow through gaps at opposite ends. This design attempted to force the flow in a serpentine pattern up through the cell. The final layer was covered with non-woven geotextile and a 10 cm layer of sand (Figure 1). The output manifold, protected from sediment by a stone gabion, was placed in this final sand layer to act as a filter. The original cell was constructed to allow 1m of free water to stand on the surface to provide for winter protection and to ensure that the sedimentary surface remained as anaerobic as possible. A second anaerobic bioreactor was constructed upstream from the first in 2000. This new cell was designed as a permanent cell and constructed accordingly, lined with 60 mm HDPE liner material.

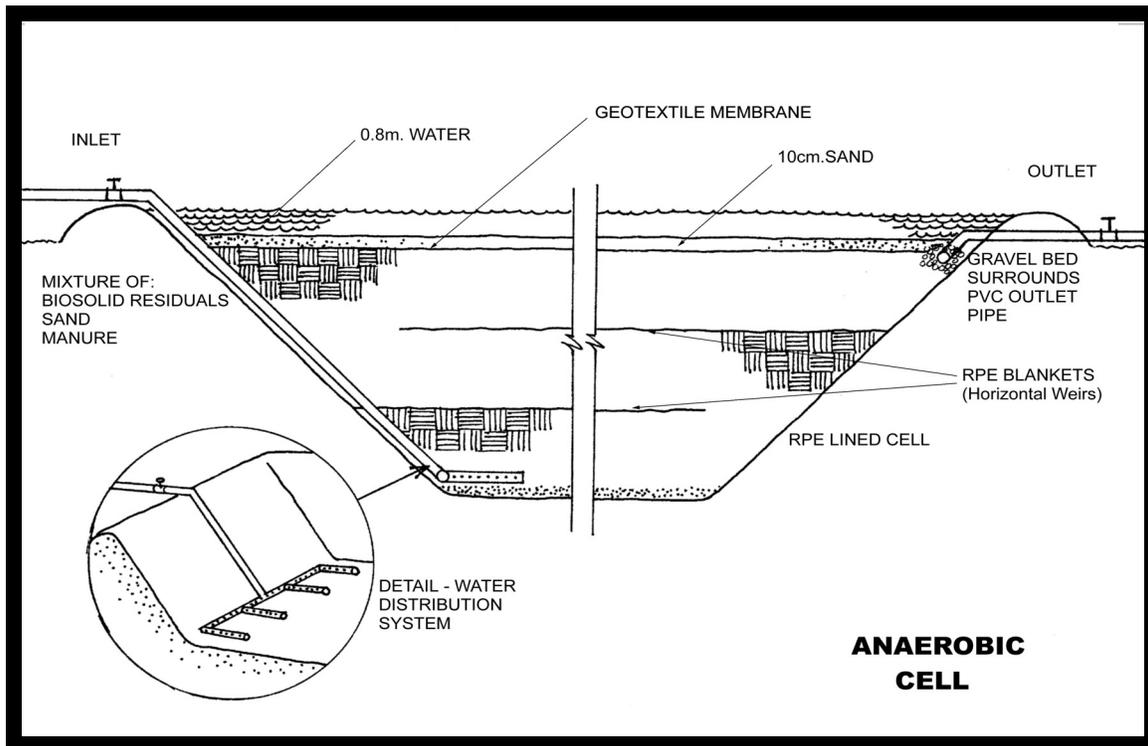


Figure 1 - Schematic of original cell design showing layers constructed to control water flow through biological matrix. The original cell design included a layer of freestanding water on the surface to control anaerobic conditions and assist in over-wintering protection.

OPERATIONAL LIFETIME

During the first year (1998) the initial anaerobic bioreactor cell operated for an 8-week period at the end of the summer growing period and results of metal assays completed on the effluent showed that it was successfully removing metals according to design expectations. Following the successful operations during this 8-week trial period, the system was prepared for a full summer (18 weeks) of operations in 1999. Following winterization of the entire system and construction of the up stream anaerobic bioreactor it operated from June 8, 2000 to Jan. 20, 2001 (261 days) when a frozen input pipe necessitated a system shut down until spring when repairs could be made. It has operated continuously since May 1st 2001. Data will be reported from the two trial periods, the 261 day operating period and the period 01/05/01 to 08/04/02 – for a total of 729 days.

Operational parameters and metal concentrations

An extensive program of regular sampling was established with samples taken from 8 points in the system (input to the system and output from each of the five cells and the

final holding pond). Assays include metal levels (total and dissolved) for Zn, As, Cd, and Fe by ICP-MS, as well as levels of NO₃ and SO₄. Additional field measurements include temperature, pH, and dissolved oxygen. Flow rates were originally measured using a battery powered impellor type accumulating flow meter or manually using stopwatch and measured container. Weather data were collected for two years (hours of sunlight, evaporation pan measurements, rainfall accumulation) to determine evaporation dynamics (data not presented).

Microbiological sampling

Four samples were taken from the two anaerobic cells by driving 2.5 cm diameter PVC pipes into the cell at depths up to 1.5m. The pipes were then removed, sectioned into 20cm lengths, sealed with saran wrap and placed in storage at 4°C until they could be processed. Four liquid samples were taken from the lower cells (two plant cells, *Typha* cell and final holding pond). Four bulk biosolids samples were taken from the surface of the first anaerobic cell and three were taken from the surface of the second anaerobic cell.

The sulphate reducing bacteria (SRB) were grown in a modified Postgate (5) medium C in 20 mL serum bottles. Preparation of the medium and dilution of the samples for enumeration is described in Benner et al. (6). The serum bottles were incubated in the anaerobic chamber for 30 days. Positive growth of SRB was indicated by the precipitation of Fe-sulphides. The iron reducing bacteria (IRB) medium and dilution procedure is described in Mattes et al. (7). After 30 days the serum bottles were injected with a 0.1% ferrozine solution according to Sorensen (8). A positive result was indicated by a purple complex formed between ferrozine and ferrous iron. The procedure for the enumeration of fermentative bacteria is described in Hulsof et al. (9). The numbers of SRB and IRB were calculated from an MPN table developed by Cochran (10).

Metals removed during operation

The mean concentration of Zn in the input effluent during the operating period was 355 ppm, for As it was 69.5 and 5.1 for Cd (Table I). The total input metal concentration is 430 ppm, while the output metal concentration of the complete system is 16.4 ppm for a net removal efficiency of 96.2%. A reduction of each metal's concentrations is evident in each of the five cells that comprise the total treatment system.

The majority of the metal removal process is seen to take place in the first two anaerobic bioreactors. Comparing concentrations at input and output of these two cells combined shows 92.8% of the As, 98% of the Cd and 84.4% of the Zn has been removed. The remaining plant cells further reduce metal concentrations. Some of this is sequestered in plants and testing of above ground biomass has been completed and

results reported previously by Mattes et al. (7). However, no plants tested approached the hyperaccumulation standard of > 1% total metals by dry weight determined by Baker and Brooks (11) and Baker et al. (12).

Table I - Metal concentrations (ppm) and percentage removal during two operating periods (08/05/00 to 20/01/01 (n=56) and 01/05/01 to 08/04/02 (n=72).

| Sample Point | As. Conc. | % Removed | Cd. Conc. | % Removed | Zn Conc. | % Removed |
|---------------------|------------------|------------------|------------------|------------------|-----------------|------------------|
| Input | 69.5 | | 5.1 | | 355 | |
| Anaerobic 1 | 18.7 | 73.1 | 2.1 | 58.8 | 177 | 50.0 |
| Anaerobic 2 | 8.2 | 56.1 | 0.4 | 81.0 | 101 | 43.4 |
| Cell 1 | 5.0 | 39.0 | 0.1 | 75.0 | 55.4 | 45.2 |
| Cell 2 | 4.7 | 1.5 | 0.1 | 0.00 | 46.8 | 15.5 |
| Cell 3 | 1.5 | 68.1 | 0.04 | 60.0 | 19.8 | 57.7 |
| Final Pond | 1.3 | 13.3 | 0.03 | 25.0 | 15.1 | 23.7 |
| Total % | | 98.1 | | 99.4 | | 95.7 |

DECONSTRUCTING THE ANAEROBIC BIOREACTOR

In 2002 the original bioreactor was re-built using appropriate lining material. The reconstructed cell included design changes incorporated and proven in operations of the upstream (second constructed) bioreactor.

Little is known about the potential life expectancy of these anaerobic bioreactors. It has been suggested by Gusek and Wildeman (4) that wetlands could have a potential lifespan of 20 + years. The lifespan is determined by the presence of available carbon that can be used by bacteria and potential for plugging or short-circuiting. The opportunity to sample an anaerobic cell of this size at all levels of the cell provides useful information that could be used in modeling life expectancy of bioreactors that rely on SRB for the metal removal process.

Sampling during deconstruction

During cap removal, 25 samples were taken uniformly over the surface from the top layer of the cell. Once the cap was removed, further samples were taken from each subsequent layer systematically. The cell was roughly gridded into nine quadrats. The top layer was designated A (Figure 1). Each of the nine quadrats was sampled at three levels – at the top of the layer (immediately below the non-woven geotextile) in the middle of the layer, and at the bottom of the layer (immediately above the RPE separation). Samples were placed in labeled 250 ml sample bottles and capped. The B and C layers were sampled in the same way. Samples were stored in a cool place until

delivery to the assay lab. The solid samples were analyzed for metals and sulphur by ICP-MS and for total organic carbon (TOC) by Rock-Eval-6 pyrolysis as described by Lafargue et al. (13).

Rock-Eval-6 is an automated commercial two-step pyrolysis and oxidation instrument manufactured by Vinci Technologies of France. This method provides not only TOC (as %), but also many other parameters such as hydrogen index, oxygen index, %PC (pyrolizable carbon), %RC (residual carbon) and S2 carbon (from thermal cracking) that may provide useful insights to a cell's life expectancy. In this paper we focus mainly on the relationship of TOC to metals and sulphur. Soluble metal ions are reduced to insoluble metal sulphides using carbon as the energy source and deposited in the cell. By comparing TOC to metals and to sulphur concentrations it may be possible to elucidate the relationship between carbon consumption by bacteria and metal deposition as sulphides using statistical procedures.

As cell material was being removed it was also sampled for anaerobic bacterial analysis by insertion and capping of lengths of PVC pipe, as described previously. Extensive sampling to determine bacterial levels had been completed in the past but samples were only taken from the surface layers previously by Mattes et al. (7).

RESULTS

Baseline data for anaerobic bioreactor

Prior to deconstruction calculations were completed on total metal loading of the three main metal contaminants - As, Cd and Zn. In total the system had operated for 729 days treating 8,200,000 liters and removing a combined total of 16,000 moles of As, Cd and Zn (Table II).

Table II - Operating days, total flow and moles of metal removed (As, Cd and Zn) during operating lifespan of de constructed anaerobic bioreactor.

| Year | Operating Days | Total Flow | Moles As Removed | Moles Cd Removed | Moles Zn Removed | Total Moles |
|-------------|-----------------------|-------------------|-------------------------|-------------------------|-------------------------|--------------------|
| 1999 | 126 | 1,606,279 | 1329 | 41 | 2835 | 4206 |
| 2000 | 261 | 2,824,000 | -47 | 41 | 5343 | 5336 |
| 2001 | 342 | 3,776,806 | 1079 | 136 | 5513 | 6646 |
| Total | 729 | 8,207,085 | 2361 | 136 | 13692 | 16188 |

Initial assays were weekly for the first 18 weeks of operations changing to three times weekly during the summer months (startup to the end of October) for the second and third year of operation. During winter operations samples were taken weekly.

Microbiology of the system

Most of the metal removal mechanisms in the engineered wetland system are microbially-mediated. Three functional groups of bacteria were SRB, fermentative bacteria and IRB. SRB are critical to the operation of the anaerobic bioreactor cells as they generate alkalinity and cause metals to precipitate. Fermentative bacteria produce substrates (electron donors and carbon source) for the SRB. SRB are obligate anaerobes and can use low molecular weight carbon compounds and hydrogen as electron donors. Most IRB are facultative and can use ferric iron as a terminal electron acceptor in the absence of oxygen.

The main metal removal mechanism for zinc, cadmium and lead will be the precipitation of these metals as their sulfides. Some removal of these metals may also occur by precipitation as carbonates and adsorption to the cation exchange complex of the pulp mill solids. The composted solids have a cation exchange capacity of 25 meq/100g. Arsenic as an oxy-anion will behave differently than the other metals. Some strains of SRB can reduce both sulfate and arsenate to produce the mineral orpiment (As_2S_3) as shown by Newman et al. (14). The solubility of orpiment is controlled by both the sulfide concentration and the pH. Because the cells also contain calcium carbonate some of the arsenic may also precipitate as calcium arsenate. Preliminary mineralogical work indicates that most of the arsenic is present in the cells as amorphous arsenic sulfides. Jong and Parry (15) described the possibility of arsenic sulphide precipitation or the concomitant removal of arsenic with the Zn and iron sulphides in their bench scale upflow anaerobic packed bed reactors.

Table III - Microbial populations in the anaerobic and plant cells

| Location | Microbial Populations, #/g or #/ml* (Average values) | | |
|------------------------|--|----------------------------|------------------------|
| | Fermentative Bacteria | Sulphate Reducing Bacteria | Iron Reducing Bacteria |
| First cell: surface | 1.3×10^4 | 1.3×10^6 | 5.1×10^4 |
| First cell: 5-20 cm | 2.2×10^6 | 7.4×10^5 | 6.3×10^4 |
| Second cell: surface | 6.4×10^4 | 8.2×10^6 | 2.1×10^6 |
| Second cell: 5-20 cm | 1.1×10^6 | 1.7×10^7 | 4.1×10^6 |
| Plant cell #1 outlet * | n.d. | 3.3×10^5 | 7.9×10^3 |
| Plant cell #2 outlet * | n.d. | 3.1×10^4 | 7.9×10^4 |
| Typha cell outlet * | n.d. | 3.3×10^5 | 7.9×10^3 |

n.d. - not done.

Appreciable numbers of all three groups of bacteria were found in the surface of the upper cell (Table III), which indicates that the operation of the cell was satisfactory. High numbers of SRBs found in the surface layer also indicate that the entire cell was anaerobic. The second cell had bacterial numbers that were on average an order of magnitude higher than the first cell (Table III). The higher numbers in the second cell

may be due to the fact that it has been in operation for two years longer than the first cell and at least a year is required for bacterial populations to reach optimal numbers. Anaerobic bacteria (SRB and IRB) were also detected in the plant cells and the Typha cell, which indicates that these cells also have anaerobic zones (Table III). It is likely that metal removed in the plant cells may occur by both filtration (removal of suspended solids) and also by precipitation as metal sulphides.

Bacterial samples during deconstruction

During deconstruction one sample was taken from the second (B) layer while four were taken from the bottom (C) layer of the cell for enumeration of SRB's. The top cap layer and A layer had previously been sampled and results reported by Mattes et al. (7). When compared to the new samples taken during deconstruction, bacterial populations were not different in the cap layer or the top biosolid (A) layer. Enumeration using the most probable number technique (MPN) showed a population of 3.5×10^6 bacteria/gram in the second (B) layer whereas in the bottom (C) layer the values ranged from 4.9×10^5 to 1.3×10^7 with a mean MPN of 7.2×10^6 . The wide range of values is indicative of the heterogeneity of the matrix.

Metal content in the deconstructed cell

In the original construction a 10 cm layer of sand was placed on top of the three layers of the active biological matrix. However, during the second year of operations short circuiting developed with effluent moving along the outside walls of the cell and traveling directly to the filtering layer. This became apparent when rhodamine dye was added to the input of the system to determine hydraulic residence time and the dye quickly appeared on the surface of the cell. Repairs included adding a layer of 60 mm HDPE liner that covered the sand filter and that lapped over the sides of the existing cell leaving an open area in the middle of the cell. The entire cell was then capped with a biosolids matrix resulting in another treatment area which comprises the cap layer referred to in this paper.

The added biosolid cap was therefore active for a shorter time period and metals found there are the result of both *in situ* bacterial treatment and metal sulphide migration from lower layers. Sampling attempted to exclude the sand only layer when sampling the Cap layer. However, some extremely low TOC values found in the Cap layer indicate that in some cases the sand layer was inadvertently sampled. These samples were excluded from further statistical treatment as were two samples with anomalous very high TOC values.

Total concentrations of those metals that can form sulphides given the pH conditions of our system include Sb, As, Bi, Co, Cd, Cu, Fe, Pb, Hg, Ni, Ag, Sn, and Zn. The total of all these metal combined is presented for the four layers (Cap, A, B and C) that comprised the cell matrix (Table IV). The main inflow contaminants in the system

are As, Cd and Zn which taken together comprise 34% of the total potential metal sulphide concentrations in the cell matrix with Fe accounting for over 60%. Almost no Fe was present in the inflow but Fe was present in the sand added to improve the hydraulic conductivity of the bioreactors. Dissolved Fe shows a marked increase in concentration entering to the second anaerobic bioreactor as compared to the collected seepage – an increase from 0.1 to 14.5 ppm Fe in the first bioreactor. The dissolved iron was likely due to the activities of IRB, which were present in both of the anaerobic cells in relatively high numbers. As well, the outflow of the second anaerobic bioreactor shows decreased dissolved Fe as compared to its inflow (from 14.5 to 5.0 ppm Fe). Iron sulfides will form only when the iron is in the Fe^{+2} oxidation state. Therefore, Fe was included in the total metal sulphide calculations as under the conditions in the bioreactor it could easily precipitate as a sulphide. Metal concentrations assayed in each level in each layer were analyzed for statistical significance using the oneway ANOVA test for variance. Part of this procedure used the quadrat samples as replicates across the three sample layers and across the three layers plus the capping material (Table IV).

The bioreactor system was designed to remove metals by formation of insoluble sulphides. The partially treated effluent enters the cell through the bottom (C) layer and exits through an exit pipe that is buried in the capping layer and it is expected that higher concentrations of sulphides will be seen in the lowest layer. Mean metal sulphides concentrations (Table IV) confirm this assumption with significantly higher concentrations in the bottom (C) layer when compared to A, B, or Cap layers. Significant differences are also seen when the three biosolid layers are examined together as well as when all four layers are combined (Table IV).

The reactor is functioning as expected with highest metal sulphides concentrations seen in the C layer and progressively lower amounts seen in layers A and B. The high concentration in the Cap layer is expected since the layer was originally designed as a filtering mechanism and to treat water short circuiting directly from the C layer that bypassed the A and B layers.

Linear regressions support the model of declining concentrations of TOC and increasing concentrations of S as metal concentrations increase (Table V). The R^2 values when the four layers combined or biosolids only are examined are not high and reflect at best, a weak relationship with the model but the correlations are significant at the 99% level of confidence except when As and TOC in the biosolid layers alone regressions are examined where the significance level drops to 95%. The highest R^2 value (0.20) is seen for Cd and S in the four layers combined.

Table IV - Comparison of total potential metal sulphides concentrations in the deconstructed anaerobic bioreactor showing mean metal concentration (ppm) across all layers, standard deviation, and results of one-way ANOVA tests across all four layers and between each of the four layers. A separate ANOVA for the three combined biosolid layers (A, B & C) is included.

| Layer | Cap | A | B | C | One-way | ANOVA |
|-----------------------------|-------|-------|-------|-------|-------------|--------------|
| | | | | | Results | P |
| | | | | | F | |
| Mean Metal Sulphides | 20106 | 23554 | 23350 | 26791 | 4.04 | 0.0096* |
| St. Dev. | 11475 | 1586 | 2548 | 6684 | | |
| Cap to A | | | | | 2.22 | 0.14 |
| Cap to B | | | | | 1.88 | 0.18 |
| Cap to C | | | | | 5.66 | 0.02** |
| A to B | | | | | 0.12 | 0.74 |
| A to C | | | | | 4.93 | 0.03** |
| B to C | | | | | 5.20 | 0.02** |
| Biosolid Layers Only | | | | | 4.69 | 0.01* |

* Significant at the 99% confidence level

** Significant at the 95% confidence level

When looking at the metals and S linear regressions it is likely that the high concentrations of sulphides found in the capping layer affect the analysis when the four layers combined (i.e. the entire cell) are examined. The designed filtering action results in higher than expected concentrations of metals present in this layer. Although Fe is considered as being present as sulphide for purposes of this report it is not certain that all the Fe found in the system is present as sulphides. Since the highest metal concentration seen in the system is Fe by including it in the analysis it strongly affects the results. Examining the R^2 values for the cap layer offers support for this as the results show values of 0.66, 0.58, and 0.73 for As, Cd and Zn respectively and only 0.29 when all sulphides are considered.

When the four layers combined are taken together the affect is less pronounced with R^2 values of 0.19, 0.20 and 0.11 for As Cd and Zn respectively and 0.11 for all sulphides taken together. When the biosolid layers alone are looked at the regressions for metals and S are 0.18 for As, 0.25 for Cd, 0.25 for Zn and 0.06 for all sulphides.

Examining the individual layers offers additional insights into the operations of the cell. For the bottom or C layer much higher statistically significant correlations are evident for As Cd and Zn indicating a moderate adherence to the model when regressions for S and metals are examined. For total sulphides, however, the regression is much lower and not significant. When the B and A layers are examined the regressions are neither strong nor significant. In the B layer the As, Cd and total sulphide correlations are negative but the R^2 values are so low and the P values so high that the model is not undermined. Two possible reasons for this are that the sulphides from this area migrated upwards to the

filtering area and/or that the short circuit meant that there was a greatly reduced flow of effluent through these layers and therefore a correspondingly lower metal removal. Mean values of sulphides show lower mean concentrations of more than 3000 ppm in the A and B layers when compared to the C layer (Table IV).

Table V - Single linear regressions on deconstructed four-layer anaerobic cell. Individual metals, As, Cd, Zn were regressed against S and TOC. Single regressions were also completed on total potential metal sulphides against both S and TOC. The same regressions were completed on each of the four layers separately, on three biosolid layers together and on four layers combined. Signs in parenthesis indicate either a positive or negative correlation. *Abbreviations: Var. is variable; Sul. is Sulphides.*

| 1 st Var. | 2 nd . Var. | N | R ² | P | 1 st Var. | 2 nd . Var. | n | R ² | P |
|-----------------------------|---------------------------|----|----------------|---------------------|-----------------------------|---------------------------|----|----------------|---------------------|
| Four layers Combined | | | | | Biosolid Layers Only | | | | |
| As | S (+) | 92 | 0.19 | 0.0000 ^a | As | S (+) | 75 | 0.18 | 0.0001 ^a |
| As | TOC (-) | 92 | 0.09 | 0.0021 ^a | As | TOC (-) | 75 | 0.07 | 0.0157 ^b |
| Cd | S (+) | 92 | 0.20 | 0.0000 ^a | Cd | S (+) | 75 | 0.25 | 0.0000 ^a |
| Cd | TOC (-) | 92 | 0.15 | 0.0002 ^a | Cd | TOC (-) | 75 | 0.09 | 0.0056 ^a |
| Zn | S (+) | 92 | 0.11 | 0.0006 ^a | Zn | S (+) | 75 | 0.25 | 0.0000 ^a |
| Zn | TOC (-) | 92 | 0.16 | 0.0000 ^a | Zn | TOC (-) | 75 | 0.10 | 0.0037 ^a |
| Sul. | S (+) | 92 | 0.11 | 0.0006 ^a | Sul. | S (+) | 75 | 0.06 | 0.0000 ^a |
| Sul. | TOC (-) | 92 | 0.08 | 0.0037 ^a | Sul. | TOC (-) | 75 | 0.21 | 0.0000 ^a |
| C Layer | | | | | B Layer | | | | |
| As | S (+) | 25 | 0.45 | 0.0002 ^a | As | S (-) | 23 | 0.01 | 0.88 |
| As | TOC (-) | 25 | 0.09 | 0.1172 | As | TOC (-) | 23 | 0.02 | 0.48 |
| Cd | S (+) | 25 | 0.36 | 0.0011 ^a | Cd | S (-) | 23 | 0.14 | 0.06 ^c |
| Cd | TOC (-) | 25 | 0.10 | 0.1296 | Cd | TOC (-) | 23 | 0.02 | 0.47 |
| Zn | S (+) | 25 | 0.46 | 0.0001 ^a | Zn | S (+) | 23 | 0.22 | 0.02 ^b |
| Zn | TOC (-) | 25 | 0.10 | 0.1221 | Zn | TOC (-) | 23 | 0.09 | 0.13 |
| Sul. | S (+) | 25 | 0.08 | 0.1623 | Sul. | S (-) | 23 | 0.02 | 0.41 |
| Sul. | TOC (-) | 25 | 0.39 | 0.0006 ^a | Sul. | TOC (-) | 23 | 0.35 | 0.0016 ^a |
| A Layer | | | | | Cap Layer | | | | |
| As | S (+) | 23 | 0.01 | 0.7027 | As | S (+) | 16 | 0.66 | 0.0001 ^a |
| As | TOC (-) | 23 | 0.41 | 0.0006 ^a | As | TOC (-) | 16 | 0.43 | 0.0041 ^a |
| Cd | S (+) | 23 | 0.05 | 0.2740 | Cd | S (+) | 16 | 0.58 | 0.0003 ^a |
| Cd | TOC (-) | 23 | 0.45 | 0.0002 ^a | Cd | TOC (-) | 16 | 0.32 | 0.0170 ^b |
| Zn | S (+) | 23 | .009 | 0.1412 | Zn | S (+) | 16 | 0.73 | 0.0000 ^a |
| Zn | TOC (-) | 23 | 0.42 | 0.0004 ^a | Zn | TOC (-) | 16 | 0.47 | 0.0024 ^a |
| Sul. | S (+) | 23 | 0.07 | 0.1988 | Sul. | S (+) | 16 | 0.29 | 0.0229 ^b |
| Sul. | TOC (-) | 23 | 0.01 | 0.2313 | Sul. | TOC (-) | 16 | 0.21 | 0.0602 ^c |

a = statistically significant at the 99% level of confidence

b = statistically significant at the 95% level of confidence

c = statistically significant at the 90% level of confidence

When TOC regressions are examined the correlations are all negative as expected by the model (Table V). But only in the A and Cap layers do the R^2 values for individual metals indicate a moderate to strong relationship with values as high as 0.47 for Zn and TOC in the cap layer and 0.42 in the A layer. The correlations for the regressions in these two layers are significant at the 99% level of confidence except for Cd in the cap layer where the confidence level drops to 95%. In both these layers the R^2 values for total sulphides are much lower, although it is significant at the 90% level for the cap layer. In the B layer the only high R^2 value (0.35) is seen in the total sulphide regression a measurement that is significant at the 99% confidence level.

When TOC measurements are regressed against either individual metals or total sulphides for the combined cell or biosolid layer only analysis, the results show a weak relationship with R^2 values as low as 0.07 for Cd and TOC for biosolids and as high as 0.15 for Cd and TOC when all four layers are taken together. The correlations are all negative as expected and all are significant at the 99% level of confidence but only the regression for total sulphides against the biosolid layers data points indicates a moderately strong relationship with a R^2 value of 0.21.

The strength of the relationship of metals and TOC is less obvious than for metals and S. This can be explained several ways. Firstly, the starting matrix was not homogeneous in starting TOC concentrations so considerable noise would be expected in the data. Secondly, not all the metals being treated by the anaerobic cells remain where the TOC was consumed (e.g., Cap has high metal sulphides but some of the lowest TOC values and is likely filtering sulphides produced elsewhere). Third, the metals adsorbed to TOC would be higher where TOC is higher. Finally, Fe was distributed through the cell as diffuse source in the sand and may not all be converted to sulphides confounding the analysis somewhat.

The major contaminant in the leachate is Zn with concentrations ranging as high as 500 ppm. In the deconstructed anaerobic bioreactor the major metal sulphide concentration in the solids from inputted metals is Zn. So a classification scheme (based on Zn concentration) was developed as follows: 0 – 200, 200 – 400, 400 – 800; 800 – 1600; 1600 – 3200; 3200 – 6400; and 6400 – 12, 800 ppm Zn. Corresponding mean values for As, Cd, S, and carbon (TOC, %PC and %RC) were calculated for the respective groupings and mean values determined (Figure 2). A relationship between decline in TOC and increase in total metal appears evident. The values for %RC and %PC are included with this graph for illustrative purposes and possible future research directions. Similarly, increasing value for S concentration versus metals provide strong visual support of the model. Future analysis and more detailed analysis of the other carbon parameters obtained by Rock-Eval-6 analysis may provide a way to distinguish the available carbon for bacterial processing versus residual carbon available for metal adsorption sites.

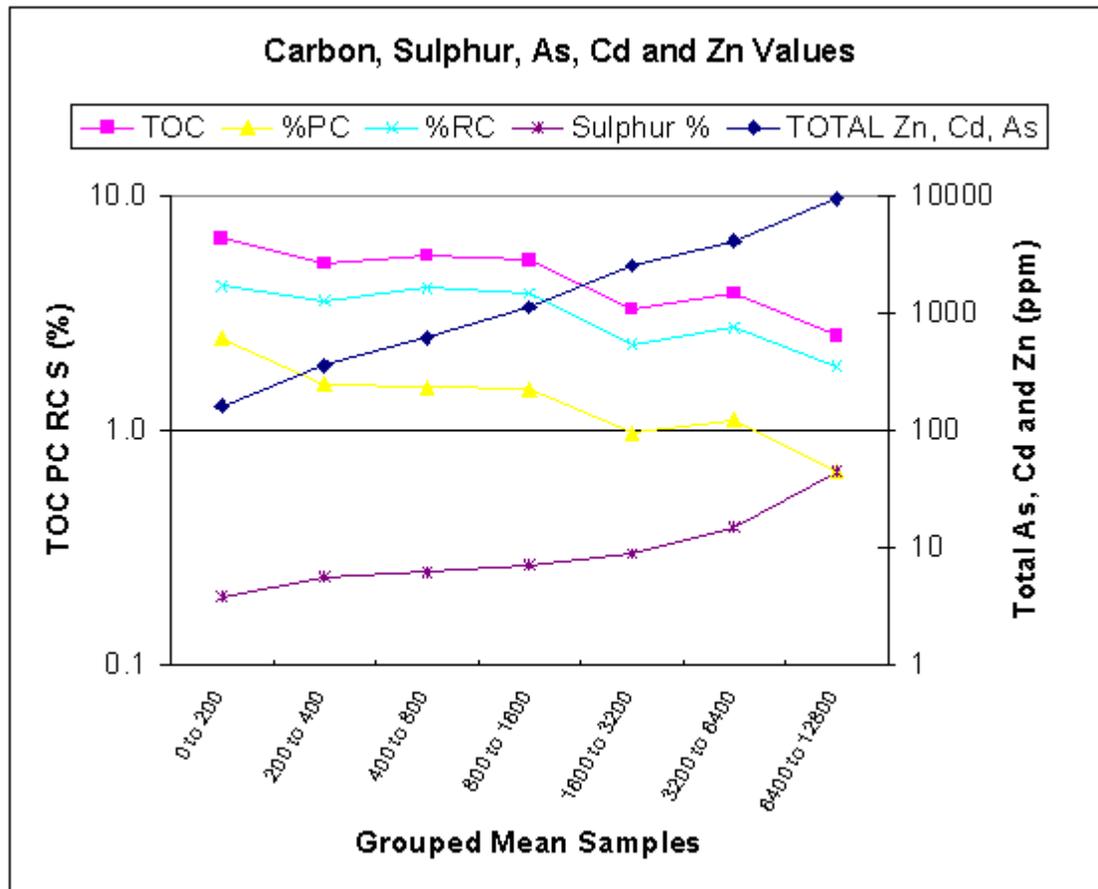


Figure 2 - Showing mean values of TOC, %PC, % RC, Sulphur and combined grouped Zn, Cd and As concentrations.

CONCLUSIONS

After what amounts to two full years of operations (spread over a five year time frame of intermittent operations) more than 8 million L of contaminated water were treated and more than 16,000 moles of the three primary metal contaminants As, Cd and Zn were removed and sequestered in the anaerobic bioreactor. Metals were present at all depths in the bioreactor with statistically significant differences observed between layers when only metal sulphides are examined.

The system is operating as designed with higher concentrations of metals seen in the bottom layer when compared to the higher layers. High concentrations of metals are seen in the Cap (filter) layer as designed. Linear regressions suggest that metals are present as sulphides and that there is some evidence that lower TOC carbon values are correlated with higher metal concentrations. Based on TOC alone, it is not yet possible to

project a time frame when the cell's carbon will be insufficient to allow for bacterial metabolism and sulphide production. Further analysis of all the available carbon data may assist in this regard.

Linear regressions of As versus S are significant suggesting that this metalloid is present in close association with sulphur. Under anaerobic conditions with available carbon and sulphate it is likely forming As_2S_3 (orpiment) as a result of dissimilatory arsenate and sulphate reduction as shown by Newman et al. (16). Synchrotron analysis on a small number of samples confirmed that As is present as a polysulphide. Future work will focus on isolating the bacteria responsible for dissimilatory arsenate and sulphate reduction.

Analysis of microbiological activity in the bioreactor showed high levels of SRB's, IRB's and fermentative bacteria. There were no differences in bacterial populations seen in the cell layers. Bacterial activity in primary and secondary bioreactors is seen as the primarily metal removal mechanism.

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