NIEA Northern Ireland Environment Agency | An Agency within the Department of Agriculture, Environment and Rural Affairs | SBRI Government challenges Ideas from business. Innovative solutions

COMPETITION: Mobuoy Road Waste Remediation SBRI
Reference: SBRI_DA_313_009

SBRI End of Phase 1 Report Form

NOTE: The Authority reserves the right to amend this form and/or issue additional guidance notes on how it should be completed during the duration of the project.

This Report is the contractor's opportunity:-

- to describe the work undertaken during the project, what outputs were obtained and why these are relevant to the objectives of the Competition

- to explain and prove expenditure; and

- to develop a comprehensive report for contractor's to share with their stakeholders and those that may help further commercialisation pursuant to the terms of the contract.

The Authority may use the Report as part of the assessment for any Phase 2; it is therefore important that contractors complete the form as completely as possible.

The Report will be considered to be confidential and commercially sensitive by the Authority and its contents (other than the response to Section 5) will not be disclosed to third parties other than in accordance with the terms of the contract.

The Report must be submitted via **MobuoyRoadSBRI@sibni.org** within 14 days of the completion, or termination, date. The contractor is reminded that completion of this report is a contractual obligation and forms part of the payment terms. The report should be completed by the lead contractor, with input from any sub-contractors or project partners as appropriate. Please answer, wherever possible, on behalf of the business units, divisions, or companies which were involved in the work. If this is not possible (as a result of merger or acquisition, for example), please specify the organisation to which your answers refer.

Please answer the questions fully, but keep your answers succinct and no longer than necessary to provide a clear explanation. When describing technical solutions, please regard your audience as being someone familiar with the technology, but not an expert. The report may be done in narrative alone, however diagrams or pictures may be annexed to the Report where these aid clarity Please limit your response to a total of **ten sides of A4** plus **an additional limit of ten sides for any supporting diagrams or pictures**. (Please keep to a maximum limit of 5MB per email when submitting supporting information).
Because the true impact of an R&D project often takes several years to emerge, InnovateUK and the Authority may approach you for up to six years after project completion to follow up on the questions in this report. Your co-operation with any such follow up work is greatly valued.

1. Details

Registered Company Name: Queens's University Belfast

Registered Address: University Road

Report Author: [redacted]

Telephone Number: [redacted]

E-mail Address: [redacted]

Project Reference: SBRI

Total Contract Cost: (£s) [redacted]

Start Date: 3/10/2016 End Date: 30/04/2017

2. At the outset of the project what were your aims and objectives?

The aim of this project was a proposal for a new and innovative technology (a Bio-Electrochemical System – BES) that will manage the risk at the Mobuoy road site by breaking the pollutant linkage at the groundwater pathway before contamination reaches the River Faughan. This technology would enhance and monitor the natural biodegradation process at the Mobuoy site providing an innovative, technically and economically viable sustainable risk management solution. The innovative technology is a form of Enhanced Monitored Natural Attenuation. The Attenuation processes enhanced by the novel and innovative use of an engineered Bio-Electrochemical System, or BES that uses upcycled biochar and graphite as a high surface area electrodes to trap and degrade organic (Total Organic Carbon, Total Petroleum Hydrocarbons) and inorganic contaminants. In order to address the aim of the project a list of 8 objectives were set:

1. Access to site and recovery of representative leachate / groundwater.
2. Microbial and chemical analysis of representative leachate/ groundwater which will give a clear understanding of the initial conditions (chemical and microbial) that the bench test will be based upon.
3. Setup of laboratory scale BESs. Our approach was to degrade contaminants in groundwater and no to produce recoverable amounts of energy. The power produced was used as a real time biosensor of how the microbial ecology of the system operates.
4. Monitoring electrical properties / output of BESs. Electrical signals generated by the BES would be continuously logged and benchmarked with regularly sampled leachate from the BESs.
5. Assessment of degradation efficiency of BES.
6. Identification of microbial communities present.
7. Determination of design criteria for Phase Two demonstration project
8. Development of commercialisation / business plan for phase 2 (including identification, prioritisation and selection of project partners)

3. Please provide a summary of the outputs of the project and relate these to the original objectives. How do the outputs address the requirements of this competition? What are the recommendations?

1 Access to site and recovery of representative leachate / groundwater

Based on information available on the Mobuoy Website we selected a likely sampling location. A site walkover took place in November 2016, with representatives of SIB, QUB, and other SBRI project members. A sample selection point was suggested to the contractor (Causeway Geotech) on site, however it appears that it was difficult to recover the desired volume and an alternative Borehole was found (BH4). 80L of leachate was recovered and delivered to QUB by Causeway Geotech. This sample made up the experimental work reported as Run 1-3 in the results shown in the Appendix. A second visit to site was carried out with Causeway Geotech in January 2017 – More leachate was recovered from BH2 closer to the buried wastes above the road, and a smaller quantity from close to the leachate collection pond, totalling approximately 80L. This sample made up the experimental work reported as Run 4-6 in the results shown in the Appendix

2 Microbial and chemical analysis of representative leachate/ groundwater
The site is very large with differing sources & varying levels of contamination: 2 field campaigns took place to obtain representative samples from different points in the site. The groundwater exhibits high values of Total Organic Carbon, Ammonium, Manganese and Nickel, and low values of Lead, Chromium, Arsenic and Zinc. The microbial analysis is covered in detail within point 6 of this section.
A new method of Total Petroleum Hydrocarbon analysis (GCxGC FID) was undertaken for this project in order to try to identify potential sources of contamination and to identify breakdown products produced by microbial degradation. A statistical method known as principle component analysis (PCA) was used to compare the raw data generated by the 2D analysis and compare this to a known library of anthropogenic and natural sources of contamination (Figure 1). The PCA is plotted on 2 axes- the x axis or Principle Component 1 (PC1) highlights carbon number with lighter petrols on the left and heavier crudes and lube oils on the right. The second component PC2 on the y axis is probably related to the presence of highly recalcitrant compounds, with gasworks contaminated groundwater which has easily degraded polar phenols polar plotting high on the axis with lube oil and highly degraded crudes plotting low on the axis. The 2 Mobuoy samples fall centrally within PCA and do not relate to a petroleum source but rather aromatic and aliphatic compounds that result from hydrolysis and fermentation of municipal waste products. Due to low levels of Total Petroleum hydrocarbons we also undertook a worst case scenario test of a highly contaminated gasworks groundwater that contained very high levels of aliphatic and aromatic hydrocarbons at around 1500 ppm (Table 1)

3 Setup of laboratory scale BES.

Application of an in situ technology for wastewater clean-up requires knowledge of chemical and physical parameters of the groundwater contaminants at the treatment site. To simplify the design of the remediation process for an in situ clean-up process a short bench-scale treatability study was performed using contaminated wastewater from the site. For the purposes of this project, identical graphite and Teflon chambers or cells were constructed. We ran a series of six lab scale experiments with each series (or run) containing 5 cells to monitor and enhance the biodegradation processes with Mobuoy leachate / groundwater (Figure. 2). Cells 1 (Biochar BES) and 3 (BES) intended to compare power output when the anodic surface area is enhanced by a conductive activated carbon, produced by the reactivation of mineral coal based, used activated carbon at temperatures above 850 °C. A comparison of Cells 1 (Biochar BES) and 2 (Biochar control) is taken to isolate effects of contaminant decay due to activated carbon sorption (Biochar control) vs sorption and enhanced degradation (Biochar BES). A comparison of cells 2 (Biochar control) and 4 (Glass control) identifies the sorption capacity of the graphite chambers when glass beads are used as substrate in cell 4. Cell 5 was a Teflon blank chamber with no graphite or biochar present. Each active cell was connected with a high precision data-logging voltmeter and a variable resistor box. The leachate inlet and outlet were held in non-reactive Tedlar bags connected with specialist tubing that helped control the flow rates from our peristaltic pumps. The experiments were carried out under the same conditions (duration, resistor configuration) to check the repeatability of the results.

4 Monitoring electrical properties / output of BES.

Measuring the electrical properties / output of BES is a novel method used to monitor the biodegradation processes. The rate of the current production can be used as a proxy for monitoring rates of microbial activity. Many studies have proven that the exponential phase of biofilm growth matches the exponential rates of current production measured in the BES. This enables a real-time view of the process. The thickness of the microbial community in the anode controlled by the amount of energy available for cell growth, which, in turn, depends on the voltage between the anode and cathode, the concentration and type of electron donor, mass transport limitations, and incubation time. The current yield is proportional to the biofilm thickness, which in turn depends on the concentration and type of electron donor. Thus, changes in the concentration or type of electron donor will affect the current production very rapidly, and these changes can be detected almost instantly.

In the appendix, some representative results are presented (Figures 3 & 4). During the remediation process within the BES cells, the change of the electrical responses over time was recorded to test the viability of the approach. The cell voltage was measured across a multi resistor setup and datalogged with a series of voltmeters. The output current can then be calculated with Ohms law as $I = V/R$, where current (I) is in amps, voltage (V) is in volts, and resistance (R) is in ohms. In Fig. 2, the calculated power and current outputs during the measurements across a wide range of resistance options are presented. The electrical responses showed drop and offset in the measured current production after 5 days treatment, followed by approximately stable measurements over the next days; this indicates that the maximum growth and electro-activity of the microbial community occurred in the first week. The readings of voltage as a function of total time are shown in Figure 3. It can be seen that the voltage increases in the beginning of the wastewater injection, and after a certain number of days the voltage is decreasing again. This decrease is an indication of ion exchange between anode and cathode and reduction of the organic load of the wastewater until the stabilization of the system and the maximum removal of the organic load that can be achieved. This suggests that the BES systems are rapidly colonised with both degrading and electroactive microbes. This is in agreement with microbial findings in section 6 which suggests that Biochar BES has a less diverse population which is more focused towards degradation and electroactive activity.

5 Assessment of the degradation efficiency of the Biochar BES

The experimental setup was designed in order to determine whether the Biochar BES was more efficient than a standard BES and more efficient than Biochar as a sorption agent. The Biochar BES out performed both the BES and the Biochar in reduction of contaminants across the board.

The Biochar BES was quickly populated by a microbial community as both the electrical output (Figures 3 & 4) and PCA of microbial populations suggest (Figure 13). Within the PCA plots principle component 1 of the unweighted unifrac is dominated by the presence of the Biochar (Both the Biochar BES and Biochar control plot to the left of the x axis). Principle component 2 (y axis) was dominated by Electrogenic microbial activity with the Biochar BES and the Glass BES occupying the upper part of the plot with the non electrogenic controls at the lower end. This suggests that the Biochar BES is dominated by microbial communities that work well with the biochar and have electrogenic properties.

Manganese was highlighted as constituent from the first sampling round that was reduced by between 99 and 93%. The system is designed to promote an anaerobic environment and as such was easily removed. (Figure 7)

Total Organic Carbon was consistently more efficiently removed by the Biochar BES than the other systems with removal rates of up to 75% (Figure 6)

Total Petroleum Hydrocarbons were present in small quantities ug/L rather than mg/L (i.e. generally 1000 times less than the Total Organic Carbon concentration). Figure 9 shows a representative aromatic fraction. Run 1-3 and 4-6 contained a small quantity of light aromatic hydrocarbons which were quickly and effectively degraded by the Biochar BES, however the results of the PCA analysis suggests that these hydrocarbons may be degradation productions or metabolites from the anaerobic breakdown of the Total Organic Carbon load which is dominated by municipal waste rather than a petroleum hydrocarbon source.

Worst Case Scenario

We ran the same experiment with a highly contaminated gasworks contaminated groundwater (dominated by light PAHs and BTEX compounds). We achieved very high removal rates (**up to 99%** Table 1) for the majority of aliphatic and aromatic fractions. The only drop in removal was with the heavy aromatic fraction (C35-44) which accounted for only 0.56% of the total contaminant load.

Contaminants that were not affected by the Biochar BES

It was expected that Ammonia would be degraded by anammox processes in the anaerobic environment of the BES systems – This has proven not to be the case over the short time frame for these tests. The systems as present did not reduce ammonia –but within a larger scale 2 chamber demonstration as opposed to the one chamber approach here, ammonia would be quite easily oxidised in the aerobic cathode chamber

As expected conservative non-toxic cations such as Sodium, Potassium, Magnesium & Calcium did not take part in any major reactions within the system.

6 Identification of microbial communities present.

A thorough knowledge of the microbes active in BES is crucial to characterize the degradation of pollutants by these systems. Analysis of the sequencing of variable regions of the bacterial 16S rRNA gene allows us to unveil the bacterial communities present at the site. With this in mind, sampling of the microbial communities was performed in two stages: 1) at T0, 2.5 L of input water were sampled; 2) at the end of the run, biofilm and 4 cm^3 of material (biochar/glass beads) were sampled from inside the chambers. The water at T0 was vacuum-filtered through a 0.45 µm membrane to collect the microbial cells. Microbial DNA was purified from the membranes/biofilm/activated carbon/glass beads using the MOBIO PowerWater ® DNA Isolation Kit and the DNA was quantified using the QuantiFluor® dsDNA System. To ensure enough material was collected and that the purity of the DNA was up to good standards a universal 16S PCR was performed before 16S rRNA sequencing. All DNA was sequencing-ready (clean of contaminants that inhibit sequencing reactions). The amplicon sequencing of the V4 variable region of the 16S rRNA gene was performed using the Illumina MiSeq platform. Analysis of the amplicons obtained was performed using a standardized QIIME pipeline. In Fig. 10, the phyla, family and genera of the input water of the first BES run is represented. The bacterial community of the first sample shows promising potential for the degradation of contaminants: more than 75% of the bacterial population is composed of Proteobacteria. In the literature, degradation of organic compounds and metals is often attributed to members of this phylum. Furthermore, at the family/genus level we found bacterial members that have been described to carry out sulphur oxidation in oil contaminated sites (Sulfurivucum) and degradation of oils (Campylobacteraceae) or benzene/nitroaromatic compounds (Comamonadaceae), for example. The results from the worst case BES experiment using highly contaminated groundwater also show an enrichment of Proteobacteria in the chambers, particularly Pseudomonas/Pseudomonodales, Comamonadaceae Caulobacteraceae and Dechloromonas, allowing us to hypothesise that we were seeing an adaptive fitness of the bacterial community structure to the conditions we were testing (Fig. 10). Further statistical analyses are ongoing, and a comparative bacterial study between different chamber environments is being carried out for the six runs performed in this study. We show in this report alpha and beta diversity results (Fig. 12 and 13) from the worst case BES experiment (groundwater with BTEX and PAHs). Rarefaction plots show that the sequencing depth being used in the BES studies are adequate as observed taxonomic units curves reach a plateau in all samples (Fig. 12). It is also worth noting that those cells with biochar are much less diverse than cells with glass beads or even the control sample (Fig. 12). Furthermore, it also showed that cells with biochar were distinctly different from others in qualitative bacterial composition (Fig. 13). Quantitively, biochar cell 1, which had an activated BES, showed more similarities to cells with glass beads exposing an occurring selection of bacterial fitness and abundance (selection for electrogenic properties) (Fig. 13).

7 Determination of design criteria for Phase Two demonstration project

From the data collated to date we can design a larger insitu Biochar BES within a Permeable reactive Barrier (PRB) system such as the SEREBAR / Portadown PRBs (Novel biological full scale groundwater systems which [redacted] helped to design & emplace) based on the following design parameters

1) Type of Bio-Electrical system to use in a demonstration project. The BES biochar system is more efficient that either a sorption only approach or a BES chamber approach. A larger scale system would be based on a 2 chamber (anode – cathode) approach to allow for enhanced aerobic oxidation in the cathode for compounds such as ammonia that weren't degraded in the anode chamber
2) Type of Biochar to use (We found that the source of the biochar is of critical importance with respect to quality of system output)
3) Confidence in the rapid development of microbial communities that select for both Biochar and BES – We are confident that indigenous microbial communities present in leachate and contaminated groundwaters would rapidly select for the environments designed for the Biochar BES System

8 Development of commercialisation / business plan for phase 2 (including identification, prioritisation and selection of project partners)

Our plan of a phase 2 application is well under way. To this end we have already submitted an application to Innovate UK that proposes to use the BES technology BES technology for an Ex-Situ Technology in conjunction with CDEnviro. We envision using the same partners along with Ashfield Solutions using the February Innovate application (COMET) as a blueprint for an insitu demonstrator approach. We are also working with other Mobuoy Road research teams [redacted] to combine our approaches into an effective landfill leachate treatment system.

4. Describe any changes to the original application. What was the reason for these changes? Please include any circumstances that aided or impeded the progress of the project and the actions taken to overcome them.

No changes to the original application

5. Please provide a brief, public facing description of the project objectives, work completed and the most significant outcomes of your work. The Authority reserves the right to amend the description before publication if necessary, but will consult you about any changes.

Rainwater that sinks through the soil eventually fills up the spaces between the grains and becomes groundwater. Groundwater flows downhill and feeds rivers and springs. When waste material is put on land, the rain water soaks through and leaches out contaminants. These contaminants can form a groundwater plume that can reach rivers and springs. A contaminated plume can quickly use up dissolved oxygen in groundwater becoming anaerobic (a term used to describe a without oxygen state). Uncontaminated groundwater usually has plenty of oxygen and is termed as aerobic (i.e. with oxygen).

Naturally occurring microbes can degrade contaminants at the edge of a groundwater plume and this process is known as natural attenuation. The microbes in the centre of a plume can slowly breakdown some contaminants without the need for oxygen and this process produces electrons as a by-product. Some microbes can help pass these electrons from the centre of the anaerobic groundwater plume to the aerobic fringe. This movement behaves like a biological battery with the anaerobic plume being the battery anode that creates the electrons by degrading contaminants which then get passed to the battery cathode or the oxygen rich aerobic groundwater at the edge of the plume. This project aims to speed up this natural breakdown process by providing a place to capture the anaerobic contaminants onto a charcoal called Biochar. This Biochar also acts like an electrode passing electrons to the aerobic groundwater. We can monitor and optimise this process by measuring the voltage and current produced by this 'bio-battery' or Bio-Electrochemical System (BES).

This study has carried out a series of trials looking at a range of lab-scale designs for BES designs that capture and breakdown some of the contaminants in the groundwater plumes. We looked in detail at the contaminant's chemistry, microbiology and electrical outputs from the BES. We found that the BES was able to degrade a range of contaminants found within the groundwaters retrieved from the site. We found that a BES-Biochar set up was more effective than the single Biochar setup and a standard BES configuration. The data from this stage of the project has confirmed that a BES-Biochar system can work at a lab-scale and that, using the lessons learnt within this project, a larger scale on-site demonstration project is necessary

6. Describe the innovative aspects of the work including any new findings or techniques.

A field based BES and the monitoring of electrical properties of the system provides the potential to indirectly observe the biodegradation and microbial processes occurring in situ. As shown in Fig. 4, the electrical response of the system can be used as management agent and assistance associated with execution of the sampling and analysis plan. The non-invasive data acquisition and the spatially continuous properties of the system present opportunities to explore biodegradation processes outside of the laboratory, at spatial scales that are not feasible or non-economically viable with chemical or microbiological techniques.

One of the most surprising findings was the effectiveness of the Biochar BES on the removal of metals from leachate / groundwater, for example Manganese and Nickel, which was unexpected. We understand the mechanism of metal removal by bio-precipitation due to a forced change in redox environment but in this case the Biochar BES consistently outperformed the other controls (sorption only and BES only) suggesting a BES enhanced mechanism for metal removal.

We are studying this in more detail and hope that it will form the basis of a research publication once all the data has been fully collated, synthesised and evaluated

7. Please give a description of how funds were spent with reference to the original budget and explain any significant variations.

Funds were spent in line with the original budget. The only change from the original plan was based around Objective 1 - Access to site and recovery of representative leachate / groundwater.

Here we used the contractor (Causeway Geotech) who was nominated by the NIEA in order to access the site. This facility was arranged in conjunction with the other SBRI Mobuoy road contractors (AFBI. ByrneLooby) in order to provide best value for money.

Laboratory setup and monitoring:

Bench scale experiments a set in triplicate for repeatability. alongside a microbiological control, a biochar sorption control and 'glass blank' or Teflon control chamber with no biochar or graphite present. Budget Spent as suggested.

Microbiological analysis:
Spent as suggested awaiting final invoices .

Chemical Analysis (subcontract):
Spent as suggested awaiting final invoices

Project Staff:

[redacted]

8. Describe any potential long-term collaborations/partnerships entered into. Please list the company and the role they played in the project.

We have entered into the following collaborations

1) Innovate UK application with CDEnviron submitted February 2017. CDEnviro were a company that was approached as part of WP8 Development of commercialisation / business plan for phase 2 (including identification, prioritisation and selection of project partners). The commerical needs of CDEnviro were not for a bespoke insitu method to treat landfill leachate, however there was an opportunity to apply the technology to exsitu treatment of wash / waste waters which formed the basis of the Innovate UK application
2) University of Sao Paolo / Embrappi / Volta Fria landfill – [redacted] is one of the applicants for this project. We have identified a Brazilian brownfield / landfill site that the technology could be used on. Embrappi are the Brazilian equivalent of Innovate UK who are searching for Brazilian project partners who could deploy the technology. We attempted to put together a team for an April 2017 Innovate UK / Embrappi submission but were unable to do so within such a short time frame. We hope to do so at the next available opportunity.
3) AFBI – [redacted] AFBI are also SBRI Mobuoy funding beneficiaries. We coordinated sampling rounds with AFBI and shared expertise/ experience. We intend to submit an industrial NERC CASE innovation application for a PhD project by July 2017 that could combine our technologies into a single system for landfills
4) Ashfield Solutions Ltd is an independent environmental liability advisory service. Through its work and contacts it is able to access sites where this groundwater remediation approach could be applicable. This access and its technical and commercial approach to contaminated land management will allow the project team to identify and integrate the Biochar BES approach into further schemes throughout Northern Ireland and beyond. As part of its services to clients, Ashfield is well versed in identifying appropriate and sustainable remedial approaches for its clients and central to this work is its ability to financially quantify the time-bound implications of a remedial works. As the project moves on, this commercial and feasibility aspect of Ashfield's input will place the project team in an informed position in terms of practicalities and financial viabilities. For this stage of the project, Ashfield has provided technical review services and the sourcing of suitable Biochar. As Ashfield has clients who generate charcoal through the pyrolysis of wastes, the next logical step would be to develop those processes so that suitable Biochar can be generated from waste materials.

9. Please describe how your company has gained from this project. What new business opportunities have been created? Do you expect your company to grow as a result of this project?

As well as the collaboration directly related to this project in section 8 we have gained from the project in the following ways:

1) There will be the opportunity to present the findings of this work at international conferences (i.e. AQUACONSOIL 2017, Lyon France)
2) There will be the opportunity to publish the findings of this work in scientific journals of international standing
3) New industry/ academic collaboration not related to the science in this project focusing on the Circular Economy has already developed from the previous collaboration with CDE highlighted in section 8. Examples of the new opportunities beyond this research area are: Recovery of valuable material from road sweepings (Sweepings Symposium 2017) and an application to the EU funded Renewable Engine project (Lead by SouthWest College)

10. Describe the potential for exploiting the work. Please identify any new IP which has been filed or for which filing is anticipated.

We believe that there isn't particular IP identified at this state as the technology is being transferred and much of the scientific literature around Bio electrochemical systems is already in the public domain within published scientific literature

However the potential for exploitation comes from 2 areas

1) Time (first) to market with the technology
2) Developing a new process at the demonstration stage that utilises the technology which can then be patented (this may arise from the Biochar BES ability to remove metals from groundwater).

Appendix

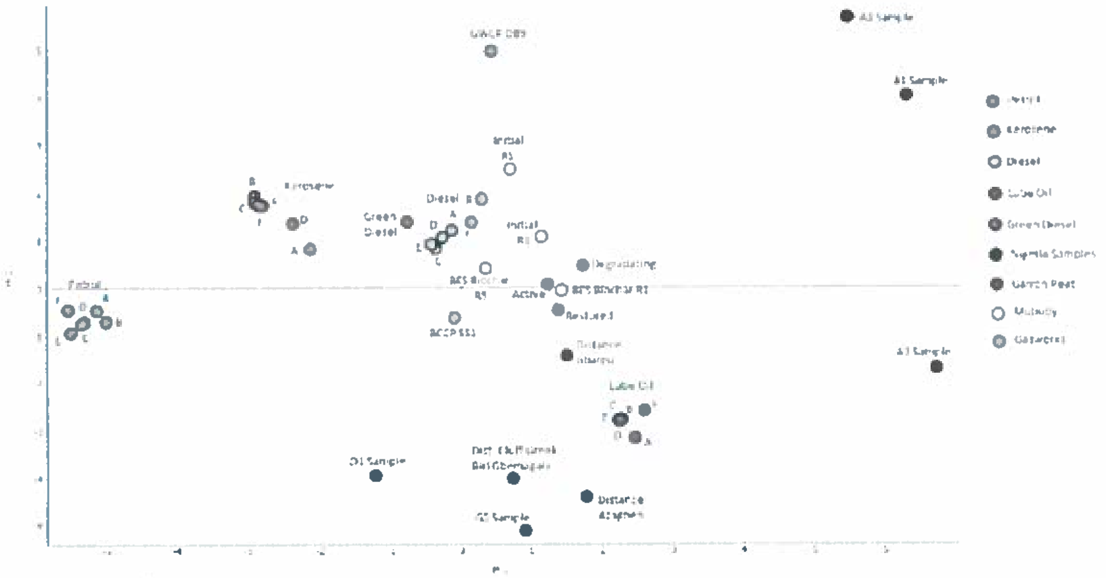

Figure 1 Principal Component Analysis of Total Petroleum Hydrocarbons by GCGC FID. PC1 (x axis) is dominated by carbon number PC2 (y axis) is dominated by ease of degradation. The initial Run 1 (R1) is comparable to non-petroleum organic matter (degrading organic matter from landfill) the initial Run 5 is from closer to the source area but still does not have a dominant petroleum signature.

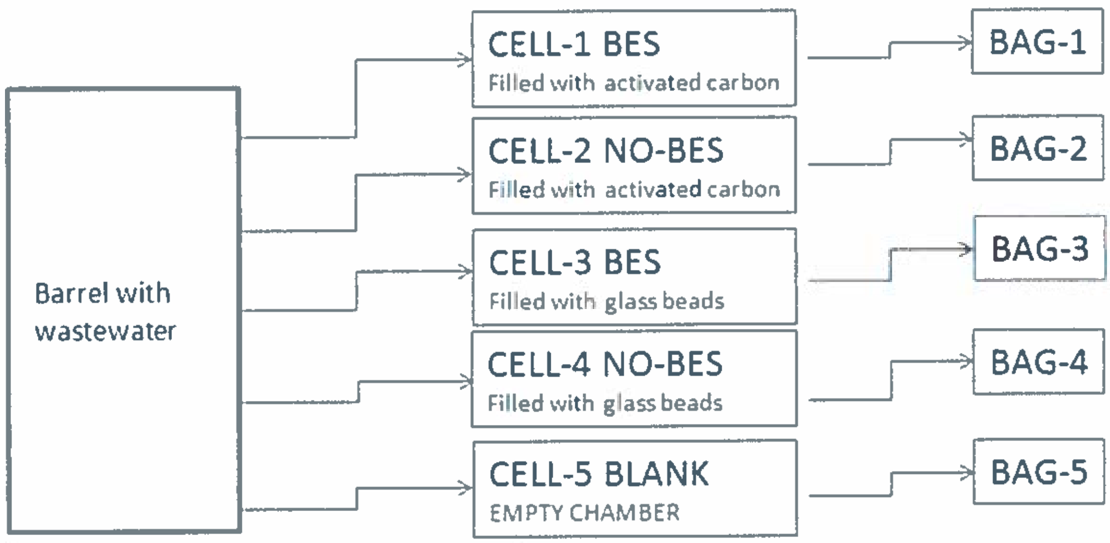

Figure 2 Schematic configuration of experimental setup.

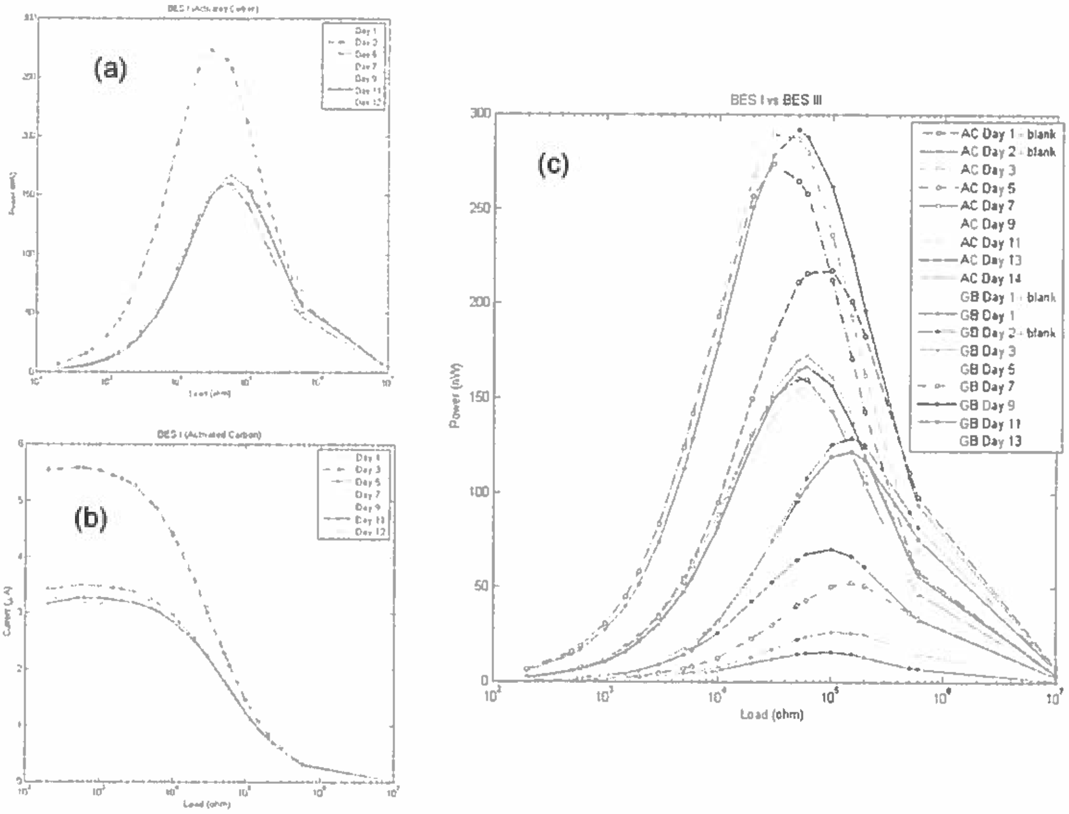

Figure 3 (a) Power curve during the treatment process in BES 1 filled with activated carbon, **(b)** current curve during the treatment process in BES 1 filled with activated carbon, **(c)** power curve between BES 1 filled with Biochar (AC) and BES 3 filled with glass beads (GB) during 5th run.

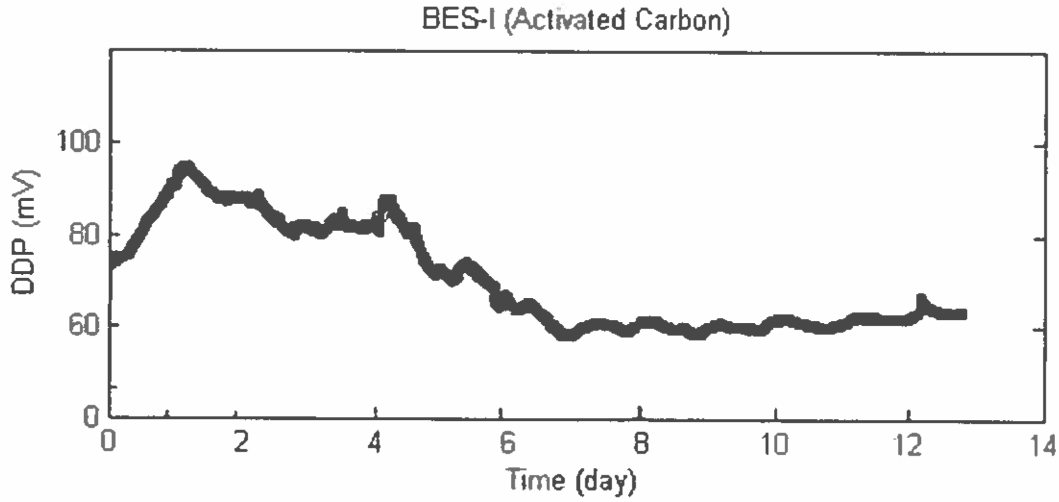

Figure 4 Voltage measurements across the resistor box that used as a real time sensor of how the system operates.

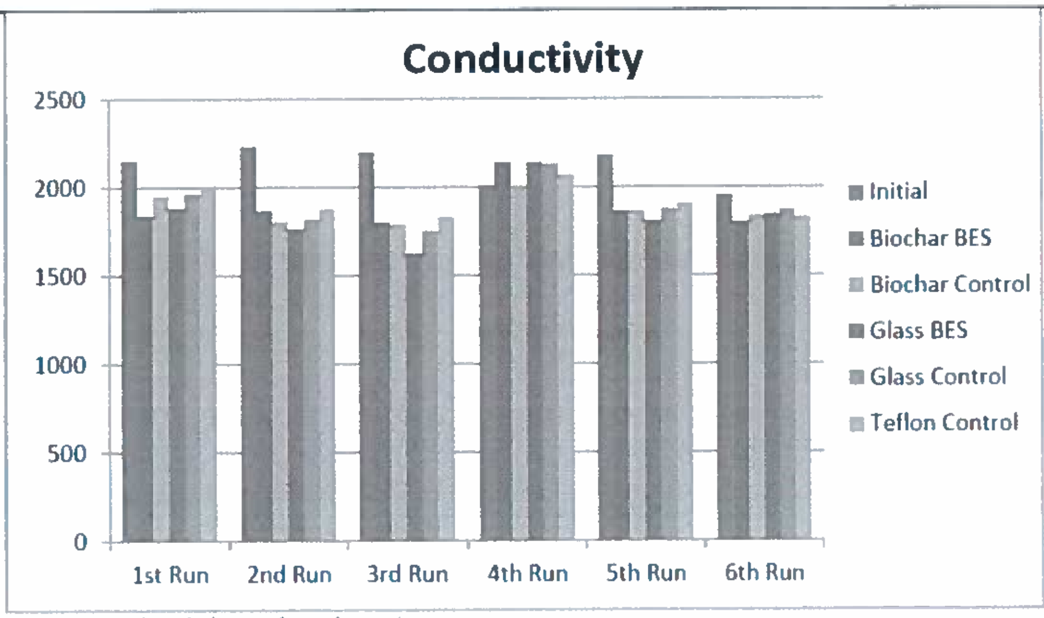

Figure 5 Conductivity values in us/cm.

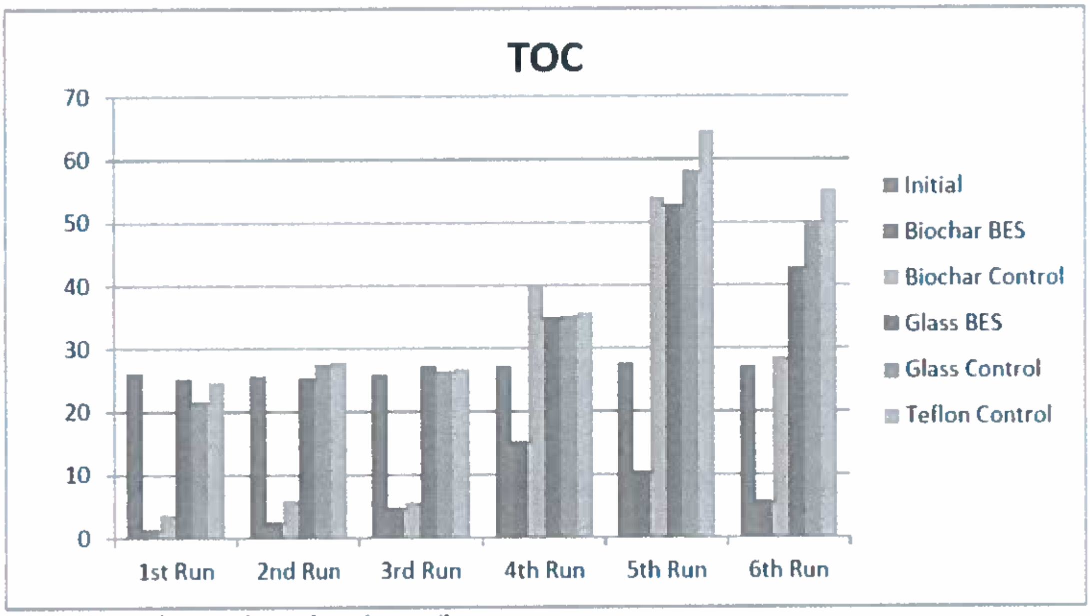

Figure 6 Total organic carbon in mg/l.

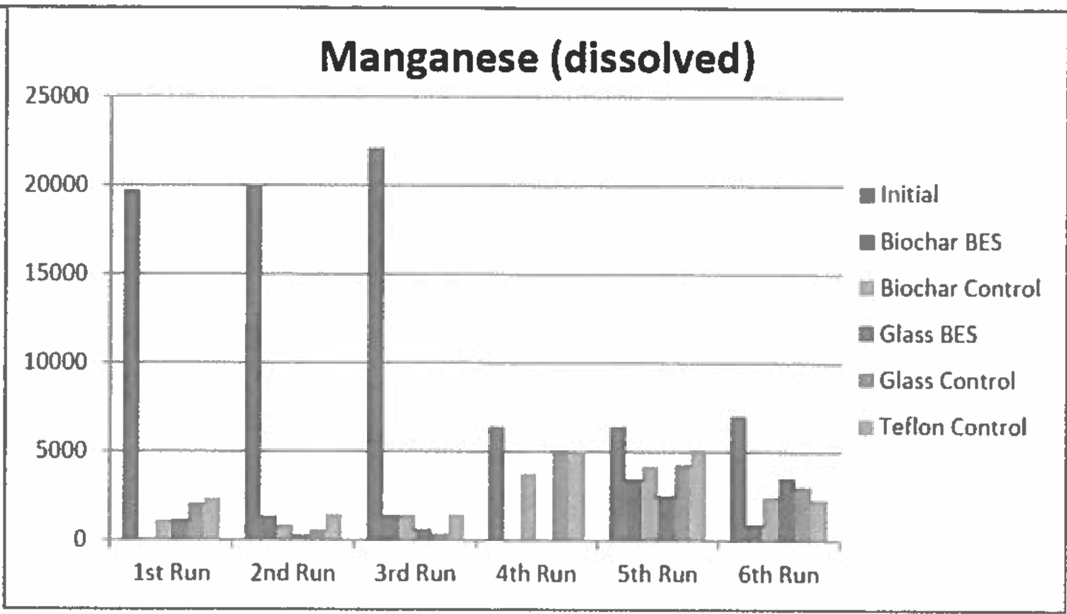

Figure 7 Concentrations of Manganese in ug/l. The differentiation of the initial values between the first 3 runs and the last 3 runs is due to 2 different samplings in the site of interest.

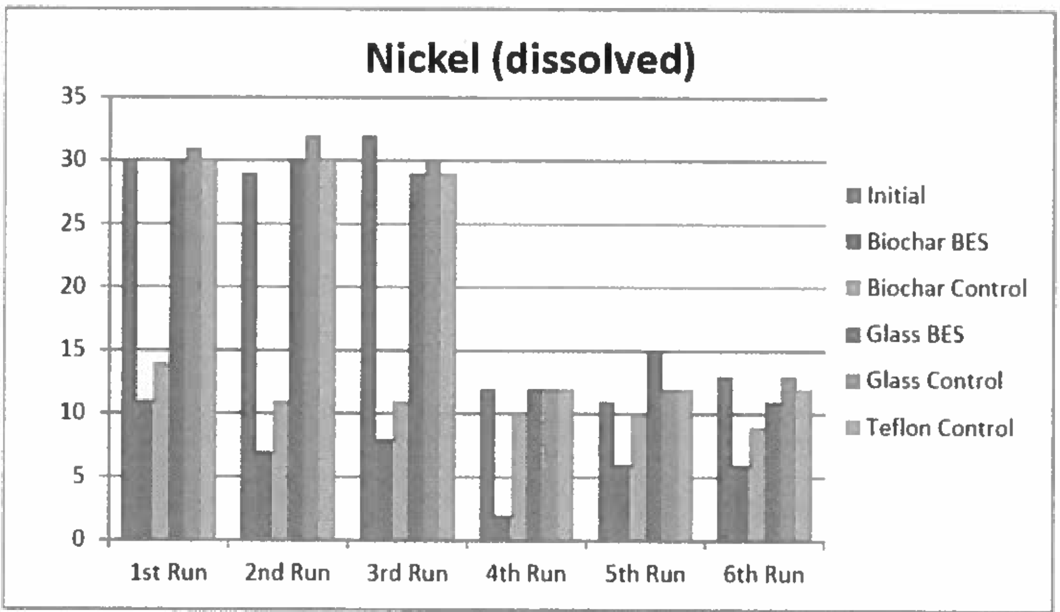

Figure 8 Concentrations of Nickel in ug/l. The differentiation of the initial values between the first 3 runs and the last 3 runs is due to 2 different samplings in the site of interest.

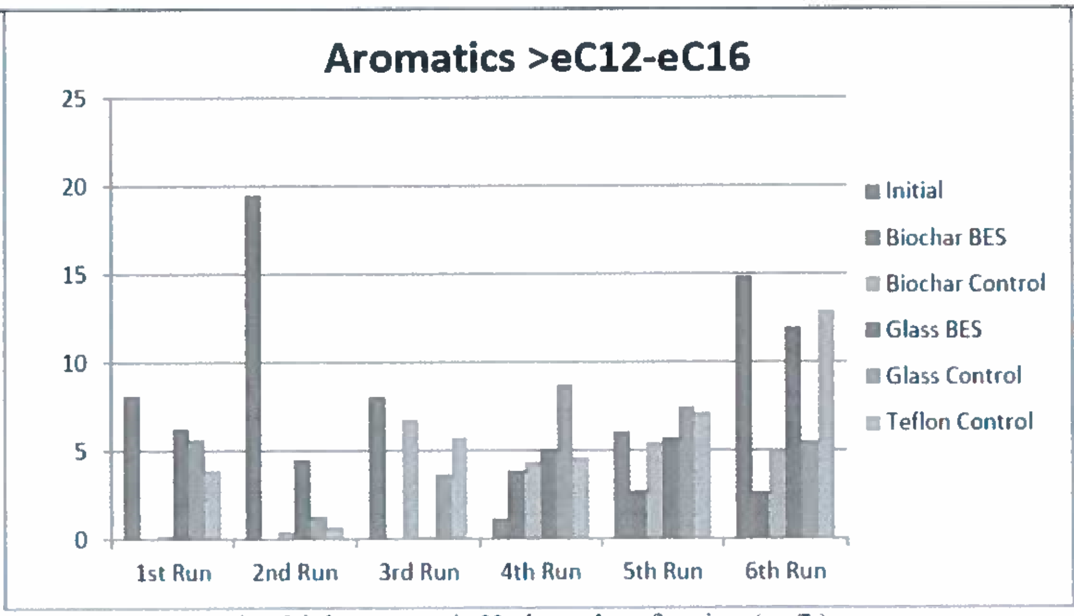

Figure 9 Representative Light Aromatic Hydrocarbon fraction (ug/L)

All Results in mg/L	Biochar BES	Biochar Control	Glass BES	Glass Control	Teflon control	% Reduction Biochar BES
Ali nC8->eC10	0.21	0.35	4.80	13.65	18.33	98.84
Ali nC10->nC12	0.68	1.82	65.94	33.90	21.17	96.80
Ali nC12->nC16	0.24	0.44	15.05	8.50	40.14	99.40
Ali nC16->nC21	0.03	0.17	0.40	0.32	4.08	99.24
Ali nC21->nC35	1.27	1.88	2.03	1.77	9.65	86.84
Ali nC35->nC44	0.17	0.43	0.42	0.30	2.61	93.34
Aro eC8->eC10	0.66	1.68	12.49	11.68	22.50	97.05
Aro eC10->eC12	5.74	19.20	860.69	779.69	1255.10	99.54
Aro eC12->eC16	0.67	1.52	70.88	54.01	131.55	99.49
Aro eC16->eC21	0.03	0.36	8.30	5.61	22.21	99.86
Aro eC21->eC35	0.25	0.34	0.46	0.38	8.60	97.08
Aro eC35->eC44	4.82	5.40	4.80	4.63	10.52	54.17
total aliphatics	2.60	5.10	88.63	58.43	95.98	**97.29**
total aromatics	12.18	28.51	957.62	856.00	1450.48	**99.16**
total ali/aro	14.78	33.60	1046.25	914.42	1546.45	**99.04**

Table 1 Worst Case Scenario using highly contaminated gasworks contaminated groundwater measured using TPHCWG method for GCGC-FID. In this case the Biochar BES is compared with the 'Teflon control' (no sorption / BES) to workout % reduction.

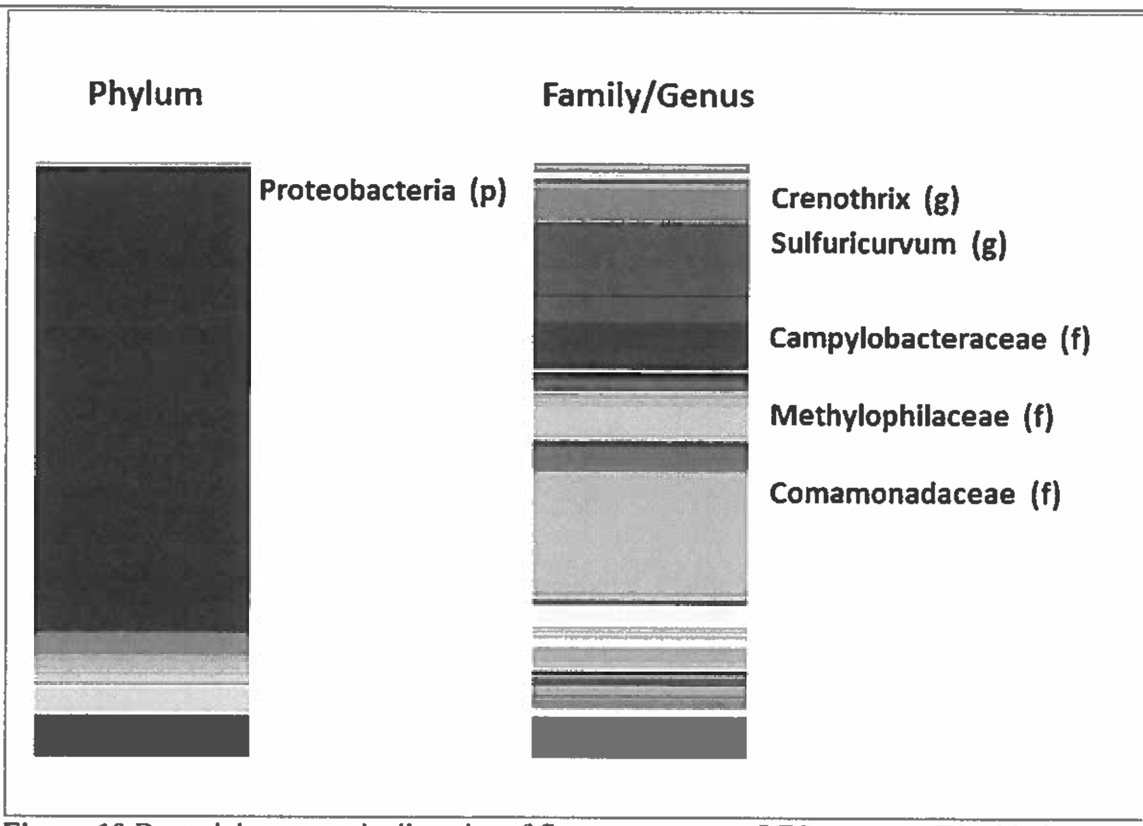

Figure 10 Bacterial taxonomic diversity of first water run on BES systems (T0). Phylum and lower lever resolution obtained represented (genus/family).

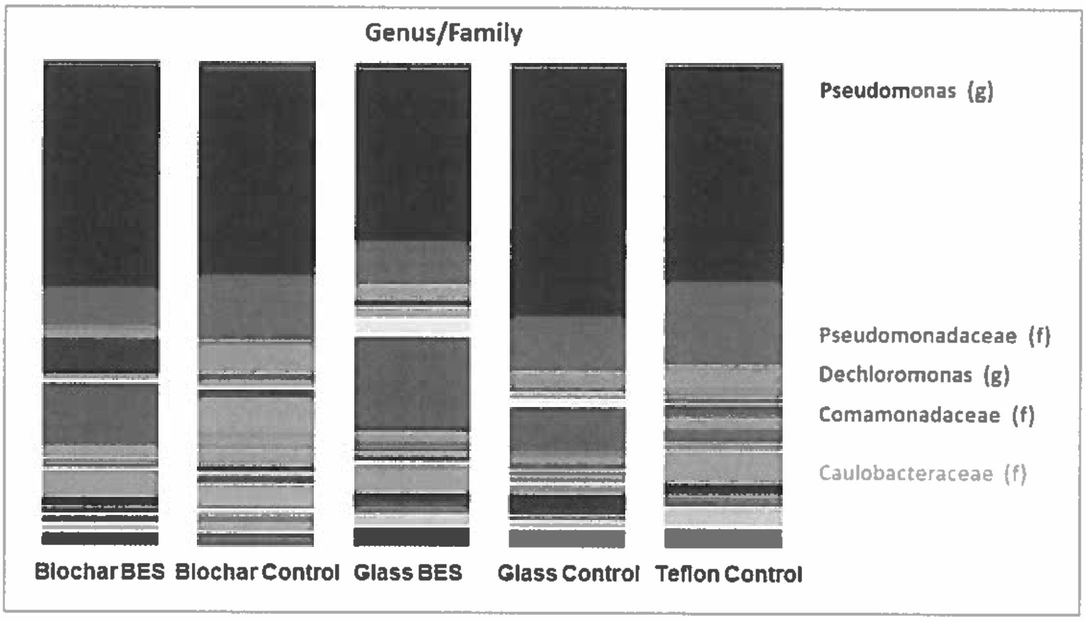

Figure 11 Bacterial taxonomic diversity of a worst case BES run with BTEX and PAH-contaminated groundwater. Lower level resolution represented (genus/family).

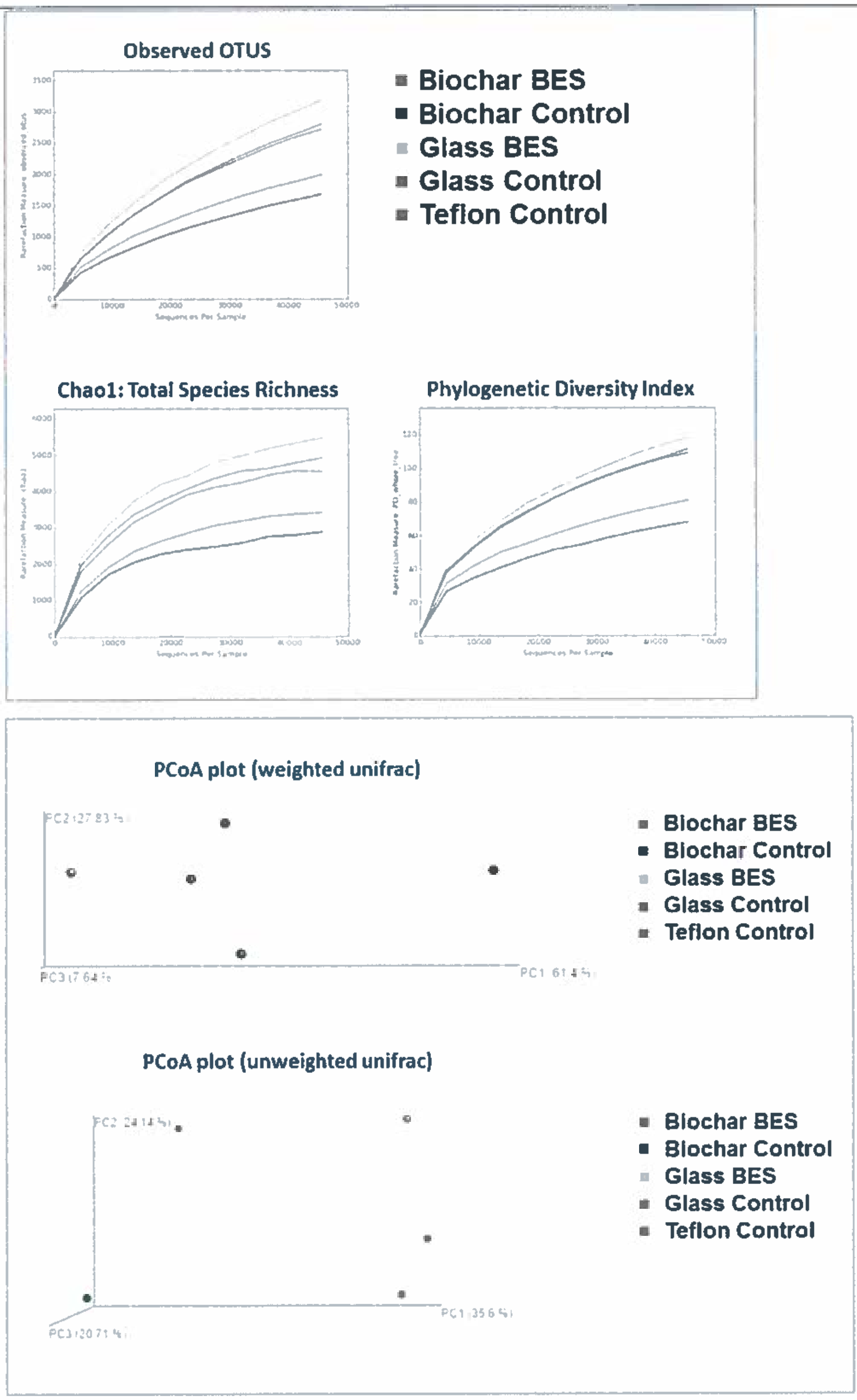

Figure 13 Beta diversity PCA plots comparing cells of previous BES run with organic-contaminated groundwater from an old gasworks site. Weighted Unifrac Index (quantitative abundance of microorganisms) and Unweighted Unifrac Index (qualitative abundance of microorganisms) PCA plots represented.