



Biological Treatment of Hexavalent Chromium
Treatability Study Report for the
Former XXXX Engineering Facility

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1.0 Introduction

A former XXXX was found to be impacted by hexavalent chromium [Cr(VI)]. The site Cr(VI) plume has groundwater concentrations as high as 1,150,000 µg/L in the hot spot(s). Treatability studies were conducted at Shaw Environmental & Infrastructure, Inc.'s Technology Development Laboratory (TDL) in Knoxville, Tennessee, to evaluate treatment technologies to decrease the concentration of Cr(VI) in the groundwater. Chemical treatment was evaluated for high Cr(VI) concentrations from the hot spot(s), while biological treatment was tested on relatively low Cr(VI) concentration soil and groundwater due to potential toxic effects to the microbes from elevated Cr(VI) levels. This report presents the results and discussion of the biological treatment. The chemical treatment results were presented in a separate treatability report; "Chemical Treatment of Hexavalent Chromium Treatability Study Report for the Former XXXX Engineering Facility," dated October 12, 2006.

2.0 Technology Description

2.1 Geochemistry of Chromium

The oxidation states of chromium in the environment range from 0 to +6, with the most prevalent forms being Cr(III) and Cr(VI). Under natural conditions, Cr(III) is the most thermodynamically stable oxidation state. Cr(VI) is the most oxidized, mobile, reactive, and toxic chromium state. Cr(VI) is generally soluble whereas Cr(III) is insoluble over a wider range of conditions. The transformations between Cr(III) and Cr(VI) is a function of pH and oxidation-reduction potential (Eh) of the environment.

Cr(III) exists over a wide range of Eh-pH. Under acidic conditions (pH < 4.6), Cr(III) predominates as dissolved ions (Cr³⁺). When the pH is between 4.6 and 13, Cr(III) exists as an amorphous precipitate, Cr(OH)₃. If the conditions are extremely alkaline (pH > 13), soluble Cr(OH)₄⁻ is formed.

Chromium(VI) exists under strongly oxidizing conditions. With pH < 6.5, Cr(VI) predominates as HCrO₄⁻ (bichromate). With pH > 6.5, CrO₄²⁻ (chromate) is the dominant species of Cr(VI). Both HCrO₄⁻ and CrO₄²⁻ are soluble and toxic species.

A potential in situ treatment methodology is to chemically lower the Eh so that the insoluble Cr(OH)₃ is formed, thereby decreasing the aqueous Cr levels below the cleanup goals. This will involve adjusting the pH to above 5 or so, and decreasing the oxidation potential of the groundwater to less than approximately 0.4 volts. The pH must also be adjusted so that other metals don't exceed

groundwater standards after successfully treating the Cr(VI).

2.2 Biological Reduction of Cr(VI)

Cr(VI) undergoes biologically mediated reduction to Cr(III) under anaerobic conditions. The enhanced anaerobic conditions are typically induced through the addition of a biodegradable substrate, such as molasses and lactate. The indigenous organisms utilize the substrate as a carbon source for biomass generation and as an electron donor for energy production. Cr(VI) is a relatively oxidized compound and therefore can act as an electron acceptor. The biological process to treat Cr(VI) may be a one or two-step process. In the one-step process, the microbes directly interact with Cr(VI). The electrons are directly transferred from electron donor to Cr(VI) results in the oxidation of the electron donor to benign products and the reduction of Cr(VI) to Cr(III). In the two-step process, the anaerobic conditions brought about by the addition of biodegradable substrate result in the reduction of other materials, e.g., iron and sulfur, which then reduce the Cr(VI) to Cr(III).

Numerous organic substrates have been evaluated for establishment of reducing conditions, including molasses, acetate, lactate, emulsified vegetable oil (EVO), and a mixture of carbon source and zero valent iron (ZVI). The substrates tested in this study are molasses, sodium acetate, EVO, and EHC-MTM. The EVO substrate is known as emulsified oil substrate (EOSTM), which is an environmentally safe blend of food grade soybean oil, lactate, nutrients and surfactants, manufactured by EOS Remediation, Inc. Upon injection to subsurface, EOS releases vegetable oil globules which then adhere to soil particles and serve as a long term carbon source. EHC-MTM (Adventus) is a mixture of fibrous carbon material, micro-scale ZVI, and sulfur particles. The organic component of EHC-M is hydrophilic and it supports the growth of bacteria in groundwater environment. The ZVI and sulfur promote chemical reduction which cause additional drop in the redox potential of groundwater.

3.0 Treatability Study Objectives

The specific objectives of the treatability study are as follows:

- Biologically reduce Cr(VI) to Cr(III) so that the total Cr in water phase is less than 50 µg/L;
- Evaluate molasses, acetate, EOS, and EHC-M, as carbon substrates to determine the most effective substrate for achieving biotransformation of Cr(VI) to Cr(III);
- Evaluate the affect of carbon source addition on groundwater pH; and
- Determine whether biological Cr(VI) reduction results in the mobilization of arsenic species.

4.0 Experimental Design and Procedures

The following section describes the experimental approach, which entailed site soil preparation and reagent treatment effectiveness tests on soil and groundwater mixtures.

4.1 Treatability Study Sample Collection and Homogenization

Several batches of soil and groundwater samples were received separately at the TDL between June 2nd, 2006 and August 1st, 2006. The samples were shipped on ice and stored at the TDL at 4°C until they were used in treatability testing. Samples were identified as follows:

<u>Field ID</u>	<u>Type</u>	<u>Amount</u>	<u>Date received</u>	<u>TDL Lab #</u>
MW-1B	GW	5X 1 LITER	6/2/06	10326
MW-7C	GW	5X 1 LITER	6/2/06	10327
W-7F-WRE-28-30	SOIL	8 OZ JAR	6/14/06	10364
W-7F-WRE-30-32	SOIL	8 OZ JAR	6/14/06	10365
W-7F-WRE-32-34	SOIL	8 OZ JAR	6/14/06	10366
W-7F-WRE-50-52	SOIL	8 OZ JAR	6/14/06	10367
W-7F-WRE-52-54	SOIL	8 OZ JAR	6/14/06	10368
W-7F-WRE-54-56	SOIL	8 OZ JAR	6/14/06	10369
W-7F-WRE-56-57.5	SOIL	8 OZ JAR	6/14/06	10370
W-7F-WRE-58-60	SOIL	8 OZ JAR	6/14/06	10371
W-7F-WRE-60-61	SOIL	8 OZ JAR	6/14/06	10372
DP-CORE1-47.5-49	SOIL	8 OZ JAR	6/14/06	10373
DP-CORE1-49-50.5	SOIL	8 OZ JAR	6/14/06	10374
DP-CORE1-50.5-51.5	SOIL	8 OZ JAR	6/14/06	10375
DP-CORE1-51.5-55.5	SOIL	8 OZ JAR	6/14/06	10376
MW-7A	GW	8X 1 LITER	8/1/06	10519

The DP-CORE1 soil samples and groundwater MW-1B were noted as having low concentrations of Cr(VI), and were therefore homogenized for biological treatment test. The soil samples were emptied into a chilled stainless steel mixing bowl and mixed manually to apparent homogeneity at 4°C. The five liters of site groundwater collected from MW-1B were homogenized in a sterile chilled glass container. Samples were stored with zero headspace at 4°C prior to testing. The homogenized site groundwater and site soil was sampled for baseline analysis. Cr(VI) concentration was measured by EPA method 7196A, ferrous iron by the 1,10-phenanthroline HACH spectrophotometric method with AccuVac Ampuls, and metals by EPA method 6010, with water prepared by EPA method

3015, and soil prepared by EPA method 3051. The pH and ORP measurements followed the standard operating procedure (SOP) of TDL.

4.2 Biological Reduction Effectiveness Tests

Biological treatability testing with substrates was performed on slurries of soil and groundwater from the relatively low Cr(VI) concentration area. Specifically, the following reagent tests were performed:

- 1) Molasses
- 2) EOS – emulsified oil substrate
- 3) Sodium acetate
- 4) EHC-M – organic material with microscale ZVI
- 5) Control – no reagent

The amount of substrate added was calculated to meet the stoichiometric demand of Cr(VI), nitrate, and sulfate reduction, with a safety factor of 5 to account for 20 percent microbial efficiency. The pH buffer and mineral nutrients were also added to the test bottles amended with substrates. Each reagent test was sampled at four times: day 1, day 14, day 56, and day 84. All test conditions were run in triplicate. Each test sample was prepared by mixing 25 g of composite soil and 100 mL of composite groundwater in 160-mL serum bottle, with amendment of appropriate amount of chemical reagents, as detailed in Table 4-1. These bottles were then sealed with Teflon faced septa and aluminum crimp caps. At each sample point, a set of triplicate samples for each test condition was sacrificed for analysis. Water samples were collected and analyzed for total chromium and arsenic, ferrous iron (Fe⁺²), Cr(VI), pH and oxidation/reduction potential (ORP). The soil samples from initial and final sampling points were collected and analyzed for total chromium, iron, arsenic and Cr(VI).

Table 4-1. Biological Treatment Effectiveness Test Summary

Reagent	Test ID	Amount of Reagent Added ^a (mg)	Nutrient and pH Buffer Added	Sample Points (days)			
				T-1 Day 1	T-2 Day 14	T-3 Day 56	T-4 Day 84
Molasses	M	145.5	Yes	XXX	XXX	XXX	XXX
EOS	N	30.1	Yes	XXX	XXX	XXX	XXX
Sodium acetate	O	34.1	Yes	XXX	XXX	XXX	XXX
EHC-M	P	78.7	Yes	XXX	XXX	XXX	XXX
Control	R	None	No	XXX	XXX	XXX	XXX

Notes: ^a. Reagents were added to 25 g soil and 100 mL groundwater mixture;

5.0 Testing Results and Discussion

A complete dataset of the results is provided in attachment 1. To visually compare the performance

of different treatment options tested, Figure 5-1 through Figure 5-9 present the trend of parameters, including Cr(VI) in water, total Cr in water, total Cr in soil (dry weight based), pH, ORP, ferrous iron, nitrate, sulfate, and total arsenic in water. Each treatment option is discussed in detail in the following section.

5.1 Unamended control

The parameters monitored in the unamended control samples did not change significantly during the test. The pH remained neutral between 7.4 and 7.8, without a monotonic trend over time. Sulfate concentration after 12 weeks didn't change from the initial ~30 mg/L, whereas nitrate concentration remained unchanged at ~15 mg/L until after 12 weeks when it completely disappeared, indicating that the redox condition in these microcosms was nitrate-reducing but not yet sulfate reducing. This is consistent with the ORP trend in the control samples, which decreased slightly from the initial 233 mV to 161 mV after 12 weeks of anaerobic incubation. The aqueous phase Cr(VI) dropped by ~12% (36.8 mg/L to 32.5 mg/L) after 12 weeks, which may be attributed to chemical and/or biological reduction activity. However, the total Cr in aqueous phase did not change much from the initial 33 mg/L to 34 mg/L after 12 weeks. Overall the results indicated that the intrinsic biological reduction of Cr(VI) is not significant, if it exists at the XXXX aquifer.

5.2 Molasses Treatment

Molasses was applied at a nominal concentration of 1450 mg/L. Microbial nutrients (ammonia-nitrogen and orthophosphate) and pH buffer were added to the treated samples to get the optimal condition for enhanced biological activity. Analytical results showed that molasses treatment achieved 38.6% reduction of aqueous Cr(VI) in two (2) weeks, and almost complete removal of Cr(VI) from the water phase (99.9%) in twelve (12) weeks. In consistence, the total Cr in the soil phase increased from the initial 40.2 mg/kg (dry weight) to 125.5 mg/kg in twelve (12) weeks, due to transformation of aqueous Cr(VI) to Cr(III) precipitate in the form of chromium hydroxide.

The molasses treatment caused the ORP to drop from the initial 336 mV to the final -69 mV, ferrous concentration in water phase increased dramatically after 8 weeks of incubation (0.12 mg/L to 9.6 mg/L). Indeed molasses was the only treatment that resulted in negative ORP and significant increase of ferrous iron among all 4 treatments. The pH of the molasses amended microcosms were the lowest of all tests. The acidifying effect of molasses treatment appeared on day 1, with a pH 6.33 ± 0.36 , and the pH kept decreasing to 6.04 ± 0.15 after 12 weeks. Nitrate and sulfate concentrations were consistent with the overall trend of ORP change and Cr(VI) reduction, with nitrate completely consumed by nitrate-reducing bacteria in two weeks, and sulfate gradually decreasing until

completely disappeared in twelve weeks. Fermentation of molasses produced acetic acid, propionic acid, and butyric acid, probably with lactic and formic acids as the intermediates as they only appeared in week 2 and week 8 samples.

Ironically, although molasses treatment reduced the total Cr in water by 98.9% with a final level of 0.33 mg/L in twelve weeks, it was not able to achieve the treatment goal of 0.05 mg/L (50 µg/L) total Cr in water within the treatment time frame, even with the strongest reducing condition. This is probably due to the persistence of Cr(OH)₃ at relatively low pH associated with molasses treatment, which is not likely the case in the field condition if the aquifer material provide adequate buffering capacity.

5.3 EOS treatment

Emulsified oil substrates (EOS 598B42) was applied at the dose of 300.8 mg/L, together with nutrients and pH buffer, to the microcosms. EOS achieved complete removal of Cr(VI) and total Cr from water phase and reached the drinking water standards for total Cr in eight weeks. Reduction of Cr(VI) to Cr(III) and the following precipitation of Cr(OH)₃ to solid phase caused dramatic increase of total Cr in soil from the beginning 36.4 mg/kg (dry weight) to the final 137.8 mg/kg after twelve weeks in EOS treated samples.

The reduction of Cr(VI) in EOS treated samples was associated with a drop of ORP (307 mV to 187 mV), ferrous production, nitrate reduction, and sulfate reduction, but to a less extent than the molasses treatment. EOS treatment also lowered the pH of the microcosms to below 7 compared to the unamended control, but the pH of the EOS treated samples fluctuated around 7 within the treatment time frame. Surprisingly, no metabolic acids were detected in EOS treated samples. It is possible that the fermentation of EOS is a slow, rate-limiting step due to the slow biodegradation of oil, such that any metabolic acids produced are rapidly degraded to methane and carbon dioxide during the Cr(VI) reduction process, or other microbial anaerobic processes, and therefore can't be detected.

5.4 Acetate treatment

Acetate treatment used sodium acetate with a nominal concentration of 340.9 mg/L. Mineral nutrients and pH buffer were spiked into the acetate treated samples. Cr(VI) removal in acetate treatment was not as fast as in the other treatments, as there were still 62% left after eight weeks of incubation, and no further reduction was observed after twelve weeks of treatment in two of the triplicates. Total Cr in soil in twelve-week acetate treated samples showed moderate increase

compared to the other treatments. One of the triplicate samples with twelve weeks of incubation (O4-T) has 0.03 mg/L Cr(VI) (99.9% removal) and 0.13 mg/L total Cr (99.6% removal) in water phase, and its total Cr concentration almost double that of the other triplicates. The variation in O4, O4-D, and O4-T caused large error bars in Figures 5-1, 5-2, and 5-3, which is probably due to the heterogeneity in soil sample.

Acetate treatment lowered the pH and ORP, and increased ferrous concentration slightly over time. Complete nitrate reduction was observed in two weeks in acetate treated samples, but sulfate remained unchanged through eight weeks and decreased by 40% in twelve weeks. Acetic acid was only detected in the initial acetate treated samples, no other metabolic acids was detected throughout the treatment time frame, except for 1.16 mg/L propionic acid and 25.61 mg/L acetic acid in one of the twelve week incubated samples which had highest Cr(VI) removal (O4-T). It appears that the acetate added to the treatments was rapidly consumed, and resulted in carbon limited treatments and only a slight reduction in Cr(VI). Therefore, acetate is not a recommended substrate for field application of biological Cr(VI) reduction.

5.4 EHC-M treatment

EHC-M, a mixture of micro-scale ZVI, organic carbon source and some sulfur grains, was added to the microcosms to get a concentration of 787 mg/L EHC-M in the samples. Mineral nutrients and pH buffer were added to these samples. Complete removal of Cr(VI) and total Cr from water phase was achieved in eight weeks with EHC-M treatment. Total Cr in soil phase increased from the beginning 38.8 mg/kg (dry weight) to the final 184.5 mg/kg due to precipitation of Cr(III) species.

The reduction of Cr(VI) in EHC-M treatment was associated with a steady decrease of ORP and slightly lower pH relative to the unamended controls. Nitrate disappeared in two weeks. Sulfate concentration increased by three times in twelve weeks, due to the oxidation of sulfur which is one of the components of EHC-M. Interestingly, ferrous production in EHC-M treated samples was not significant compared to the other treatments although there was ZVI in EHC-M, this indicated that the chemical reduction through ZVI is not the predominant process of Cr(VI) reduction, as shown in the chemical treatment study (addressed in detail in the Chemical Treatment Treatability Study report, October 12, 2006). No organic acids were detected in EHC-M treated samples, indicating slow biodegradation of the carbon source in EHC-M.

5.5 Cr(VI) in soil

Being a soluble species, Cr(VI) is expected to exist primarily in water phase. If there is non-

detectable Cr(VI) in water, there should be virtually no Cr(VI) in soil phase. However, this is not the case in this study, as shown in Table 5-1 and 5-4. In the initial samples (one day), Cr(VI) ranged from below 0.4 mg/kg to 1.13 mg/kg; however, in the final samples, Cr(VI) was detected between 3.5 to 9.4 mg/kg (dry weight) in all treated samples, with less than 0.1 mg/L of Cr(VI) in the water phase in most of them. The Cr⁺⁶ in the final sample soil phase was approximately five (5) percent of the Cr⁺⁶ removed from the aqueous phase by treatment. A possible explanation of this phenomenon is that Cr(VI) was bonded to the biomass and encapsulated on the surface of soil particles by a biofilm. This bio-film could be destroyed during the soil digestion analytical process and the bound Cr(VI) could be released and detected. This hypothesis is supported by the negative correlation between aqueous Cr(VI) and solid phase Cr(VI) (see Table 5-4), since strong microbial activity will result in more reduction of Cr(VI) in water phase but also more binding of Cr(VI) to the biomass in solid phase.

5.6 Arsenic mobilization

The results of all the treatments indicated that arsenic removal from the water phase is associated with Cr(VI) removal from water phase, namely there is no detectable arsenic in molasses treatment, EOS treatment, and EHC-M treatment, within eight weeks of treatment time (Figure 5-9). However, metal analysis of solid phase didn't show corresponding increase of arsenic in soil due to detection limitation and potential matrix interferences in the initial (one day) samples. But overall arsenic analysis showed that reduction of Cr(VI) didn't cause mobilization of arsenic.

6.0 Conclusions and Recommendations

Overall, this treatability study indicated that EOS and EHC-M are both effective amendments at stimulating the microbial reduction of Cr(VI) in groundwater at the XXXX site with concentrations up to 34 mg/L. Complete removal of Cr(VI) and total Cr from water phase was observed in eight weeks in these two treatments, with amendment dose at 5X of the stoichiometric demand. No adverse metal mobilization effect was observed in these two treatments. Molasses was effective in removing Cr(VI) from water but it failed to meet the treatment goal of total Cr in water. Acetate treatment resulted in a decrease of Cr(VI) and total Cr in water phase by 40% which is far from enough to meet the treatment goal, likely due to the short longevity of acetate.

Based on the treatment results, EOS and EHC-M are recommended to be applied at the XXXX site to induce in-situ biological reduction of Cr(VI). A dose level at 5X of the stoichiometric demand is appropriate for optimum microbial activity. The specific choice between these two should depend on the site conditions. EOS is in liquid form, which is suitable for injection if the treatment zone is deep

in the aquifer. EHC-M is solid form, which is a better choice for in-situ mixing in shallow aquifer or application through trenches.

Attachment 1.

Tables of Analytical Data

Table 5-1. Analytical results with one day incubation

Sample ID	Treatment	pH	ORP (mV)	Fe ²⁺ (mg/L)	Cr(VI) in water (mg/L)	Cr(VI) in soil (mg/kg, dry)	Inorganic anions (mg/L)					Organic acid (mg/L)				Metals in water (mg/L)			Metals in soil (mg/kg, dry)		
							Chloride	Nitrate	Phosphate	Sulfate	Acetic	Propionic	Lactic	Formic	Butyric	As	Cr	Fe	As	Cr	Fe
M1	Molasses, 1450 mg/L	5.91	358.9	0.12	37.2	0.4 U	396.83	13.71	274.36	27.14	3 U	3 U	3 U	3 U	3 U	0.072	29.7	0.44	3.02 J	42.54	13975.01
M1-D		6.55	320.9	0.12	34.8	0.4 U	402.46	14.06	274.21	32.28	3 U	3 U	3 U	3 U	3 U	0.074	31.7	0.075 U	2.23 J	43.85	13357.53
M1-T		6.53	329	0.12	38.8	0.4 U	395.43	13.29	272.91	27.5	3 U	3 U	3 U	3 U	3 U	0.074	31.4	0.075 U	2.47 J	34.20	11560.34
N1	EOS, 300.8 mg/L	6.67	312.5	0.02 UJ	34.8	0.4 U	382.05	12.95	131.27	18.34	3 U	3 U	3 U	3 U	3 U	0.069	32	0.075 U	3.49 J	42.84	13662.95
N1-D		6.74	311.2	0.02 UJ	34.4	0.4 U	392.23	13.26	140.04	26.35	3 U	3 U	3 U	3 U	3 U	0.068	32	0.075 U	2.82 J	38.03	13968.03
N1-T		6.7	297.9	0.02 UJ	34	1.73	389.83	13.35	164.83	26.3	3 U	3 U	3 U	3 U	3 U	0.067	31.5	0.075 U	2.34 J	28.31	10059.61
O1	Sodium acetate, 340.9 mg/L	6.82	292.2	0.04 UJ	33.6	0.49	391.87	13.65	190.61	26.43	169.14	3 U	3 U	3 U	3 U	0.068	32.3	0.075 U	3.61 J	39.70	14069.49
O1-D		6.76	287.2	0.04 UJ	32.8	1.13	385.86	13.6	219.97	26.59	160.33	3 U	3 U	3 U	3 U	0.065	31.8	0.075 U	2.85 J	37.03	13360.72
O1-T		6.79	283	0.02 UJ	37.2	0.4 U	390.32	13.38	149.88	26.05	163.05	3 U	3 U	3 U	3 U	0.068	32.4	0.075 U	3.57 J	39.71	14984.84
P1	EHC-M, 787 mg/L	6.78	276.4	0.02 UJ	35.2	0.4 U	410.74	13.97	210.32	26.27	3 U	3 U	3 U	3 U	3 U	0.068	32.9	0.075 U	2.76 J	37.47	14348.94
P1-D		6.86	262.2	0.02 UJ	36.4	0.4 U	404.83	13.79	155.91	26.33	3 U	3 U	3 U	3 U	3 U	0.069	32.6	0.123	3.04 J	30.42	14294.36
P1-T		6.89	253.9	0.02 UJ	35.6	0.4 U	407.3	13.85	149.89	27.24	3 U	3 U	3 U	3 U	3 U	0.066	32.3	0.075 U	2.42 J	48.51	14109.23
R1	Control	7.49	236.5	0.02 UJ	37.6	0.4 U	15.46	13.97	3 U	30.33	3 U	3 U	3 U	3 U	3 U	0.055	33.2	0.286	2.84 J	34.23	12892.66
R1-D		7.53	236.2	0.02 UJ	36	0.4 U	16.91	15.02	3 U	28.33	3 U	3 U	3 U	3 U	3 U	0.054	33.4	0.315	3.59 J	46.76	15914.79
R1-T		7.47	226.3	0.02 UJ	36.8	0.4 U	15.16	14.4	3 U	29.53	3 U	3 U	3 U	3 U	3 U	0.054	32.6	0.156	2.89 J	35.76	12967.00

Table 5-2. Analytical results with two weeks incubation

Sample ID	Treatment	pH	ORP (mV)	Fe ²⁺ (mg/L)	Cr(VI) in water (mg/L)	Inorganic anions (mg/L)					Organic acid (mg/L)				Metals in water (mg/L)		
						Chloride	Nitrate	Phosphate	Sulfate	Acetic	Propionic	Lactic	Formic	Butyric	As	Cr	Fe
M2	Molasses, 1450 mg/L	6.2	329	0.12	21.75	426.37	3 U	196.21	26.2	19.54	3 U	5.49	17.75	3 U	0.072	19.3	4.45
M2-D		6.21	314.5	0.12	21.75	425.56	3 U	194.77	28.95	24.16	3 U	4.2	22.94	3 U	0.071	19.2	2.67
M2-T		6.27	298.8	0.12	24.25	438.04	3 U	188.71	25.02	22.04	3 U	4.85	21.6	3 U	0.078	19.2	6.92
N2	EOS, 300.8 mg/L	6.59	304.4	0.02 UJ	31	425.78	3 U	126.17	25.82	3 U	3 U	3 U	3 U	3 U	0.074	29	57.4
N2-D		6.63	302.1	0.02 UJ	32.75	413.92	3 U	108.9	23.34	3 U	3 U	3 U	3 U	3 U	0.042	28.8	219
N2-T		6.74	296.8	0.02 UJ	20.5	430.23	3 U	110.36	27.24	3 U	3 U	3 U	3 U	3 U	0.06	17.8	5.02
O2	Sodium acetate, 340.9 mg/L	6.91	286.2	0.04 UJ	29.75	445	3 U	111.16	23.07	3 U	3 U	3 U	3 U	3 U	0.072	24.5	3.1
O2-D		6.93	272.1	0.04 UJ	25.25	425.38	3 U	99.26	24.21	3 U	3 U	3 U	3 U	3 U	0.076	23.4	6.59
O2-T		6.89	267.9	0.02 UJ	28.5	446.1	3 U	124.85	26.71	3 U	3 U	3 U	3 U	3 U	0.073	23.9	38.3
P2	EHC-M, 787 mg/L	6.65	309.8	0.02 UJ	31.25	433.58	3 U	132.42	45.72	3 U	3 U	3 U	3 U	3 U	0.079	26.2	3.29
P2-D		6.69	307	0.02 UJ	28.75	423.79	3 U	126.3	44.89	3 U	3 U	3 U	3 U	3 U	0.072	25.3	35.7
P2-T		6.66	305	0.02 UJ	31.25	449.35	3 U	124.18	39.29	3 U	3 U	2.54 UJ	3 U	3 U	0.076	24.7	4.85
R2	Control	7.43	273.7	0.02 UJ	40.75	17.29	15.34	3 U	24.61	3 U	3 U	3 U	3 U	3 U	0.066	33	3.07
R2-D		7.45	275.8	0.02 UJ	35.25	18.08	15.32	3 U	27.39	3 U	3 U	3 U	3 U	3 U	0.057	34.3	18.5
R2-T		7.43	272.9	0.02 UJ	38.75	18.98	15.37	3 U	25.93	3 U	3 U	3 U	3 U	3 U	0.055	32.6	14.5

Notes: U – Laboratory reporting limits;

UJ – Values under laboratory reporting limits.

J – Estimated value due to potential matrix interference.

Table 5-3. Analytical results with eight weeks incubation

Sample ID	Treatment	pH	ORP (mV)	Fe ²⁺ (mg/L)	Cr(VI) in water (mg/L)	Inorganic anions (mg/L)				Organic acid (mg/L)				Metals in water (mg/L)			
						Chloride	Nitrate	Phosphate	Sulfate	Acetic	Propionic	Lactic	Formic	Butyric	As	Cr	Fe
M3	Molasses, 1450 mg/L	6.07	-3.2	11	0.094	413.4	3 U	59.64	2 U	617.26	26.5	1.44 J	43.36	3 U	0.026 U	0.49	0.743
M3-D		6.09	-30.1	10	0.021	418.2	3 U	55.55	2 U	725.33	30.18	1.55 J	23.06	3 U	0.026 U	0.51	0.993
M3-T		6.13	-29.9	7.8	0.019	390.57	3 U	71.92	4.88	761.65	26.6	2.75 J	3 U	3 U	0.026 U	0.539	0.3 U
N3	EOS, 300.8 mg/L	7.11	175.9	0.13	0.041	411.94	3 U	59.8	6.65	3 U	3 U	3 U	3 U	3 U	0.026 U	0.05 U	0.3 U
N3-D		7.26	168.7	0.15	0.043	408.23	3 U	51.41	5.82	3 U	3 U	3 U	3 U	3 U	0.026 U	0.05 U	0.3 U
N3-T		7.35	191.6	0.12	0.04	417.83	3 U	54.43	5.74	3 U	3 U	3 U	3 U	3 U	0.026 U	0.05 U	0.494
O3	Sodium acetate, 340.9 mg/L	7.22	203.8	0.18	18	414.88	3 U	80.65	26.7	3 U	3 U	3 U	3 U	3 U	0.05	20.7	0.3 U
O3-D		7.2	209	0.18	20.8	410.04	3 U	126.7	26.07	3 U	3 U	3 U	3 U	3 U	0.05	22.3	0.3 U
O3-T		7.25	212.3	0.14	20.8	413.99	3 U	130.66	26.79	3 U	3 U	3 U	3 U	3 U	0.048	23.4	0.3 U
P3	EHC-M, 787 mg/L	7.38	202	0.14	0.032	427.54	3 U	45.55	100.82	3 U	3 U	3.67	4.57	3 U	0.026 U	0.05 U	0.3 U
P3-D		7.15	198.9	0.17	0.052	421.03	3 U	67.95	104.34	3 U	3 U	3 U	3 U	3 U	0.026 U	0.05 U	0.3 U
P3-T		7.12	199.8	0.13	0.048	413.07	3 U	64.73	92.6	3 U	3 U	3 U	3 U	3 U	0.026 U	0.05 U	0.3 U
R3	Control	7.74	168.9	0.06 UJ	29.5	16.4	14.99	3 U	28.3	3 U	3 U	3 U	3 U	3 U	0.026 U	33.5	0.3 U
R3-D		7.8	169.7	0.06 UJ	29.3	15.95	14.47	3 U	27.99	3 U	3 U	3 U	3 U	3 U	0.026 U	33.6	0.3 U
R3-T		7.68	168.2	0.08 UJ	29.2	15.78	14.11	3 U	28.08	3 U	3 U	3 U	3 U	3 U	0.026 U	33.8	0.3 U

Table 5-4. Analytical results with twelve weeks incubation

Sample ID	Treatment	pH	ORP (mV)	Fe ²⁺ (mg/L)	Cr(VI) in water (mg/L)	Cr(VI) in soil (mg/kg, dry)	Inorganic anions (mg/L)				Organic acid (mg/L)				Metals in water (mg/L)			Metals in soil (mg/kg, dry)			
							Chloride	Nitrate	Phosphate	Sulfate	Acetic	Propionic	Lactic	Formic	Butyric	As	Cr	Fe	As	Cr	Fe
M4	Molasses, 1450 mg/L	5.89	-75.2	10.5	0.03	7.37	420.76	3 U	24.75	2 U	596.38	58.86	3 U	3 U	14.07	0.033 U	0.316	0.316	1.16 U	118.65	12070.10
M4-D		6.04	-55.2	4.95	0.03	9.42	414.94	3 U	29.72	2 U	554.76	81.8	3 U	3 U	10.8	0.033 U	0.367	0.674	1.16 U	129.23	12590.07
M4-T		6.18	-77.9	10.9	0.05	7.99	400.91	3 U	11.15	2 U	556.3	28.5	3 U	3 U	44.98	0.033 U	0.297	0.382	1.16 U	128.74	11279.50
N4	EOS, 300.8 mg/L	6.88	203	1.54	0.06	8.26	401.61	3 U	54.02	2 U	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	115.43	13768.57
N4-D		6.91	204	1.32	0.05	7.20	416.16	3 U	47.41	2.97	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	124.07	13193.77
N4-T		6.84	153.7	1.32	0.04	7.13	315.85	3 U	38.39	2.98	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	174.01	13541.93
O4	Sodium acetate, 340.9 mg/L	6.96	201.2	1.32	20.90	3.58	407.72	3 U	79.16	25.36	3 U	3 U	3 U	3 U	3 U	0.058	21.9	0.3 U	1.16 U	56.37	10860.10
O4-D		7.05	208.7	0.1 U	22.40	3.47	420.22	3 U	87.18	39.27	3 U	3 U	3 U	3 U	3 U	0.065	23.2	0.3 U	1.16 U	50.21	11297.46
O4-T		7.18	117	0.08 UJ	0.03	6.96	405.6	3 U	54.06	15.6	25.61	1.16 J	3 U	3 U	3 U	0.033 U	0.13	0.3 U	1.16 U	113.25	12190.00
P4	EHC-M, 787 mg/L	6.88	103.2	0.06 UJ	0.03	6.63	413.42	3 U	44.75	113.82	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	157.75	13647.42
P4-D		6.81	119.1	0.11	0.01	7.60	421.97	3 U	51.29	114.86	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	164.68	14044.64
P4-T		6.8	97.1	0.08 UJ	0.04	4.14	424.66	3 U	50.94	121.58	3 U	3 U	3 U	3 U	3 U	0.033 U	0.075 U	0.3 U	1.16 U	230.93	20487.97
R4	Control	7.42	155	0.02 UJ	32.80	4.00	17.38	15.36	3 U	33.39	3 U	3 U	3 U	3 U	3 U	0.037	34.4	0.3 U	1.16 U	35.38	12091.90
R4-D		7.55	160	0.02 UJ	32.70	0.93	16.42	15.52	3 U	27.95	3 U	3 U	3 U	3 U	3 U	0.037	33.8	0.3 U	1.16 U	32.84	12175.36
R4-T		7.57	167.4	0.02 UJ	31.90	0.90	16.1	15.45	3 U	50.9	3 U	3 U	3 U	3 U	3 U	0.041	34.4	0.3 U	1.16 U	21.42	9374.63

Notes: U – Laboratory reporting limits;

UJ – Values under laboratory reporting limits.

J – Estimated value due to potential matrix interference.

Attachment 2

Figures

Figure 5-1. Aqueous Cr(VI) concentration change over time

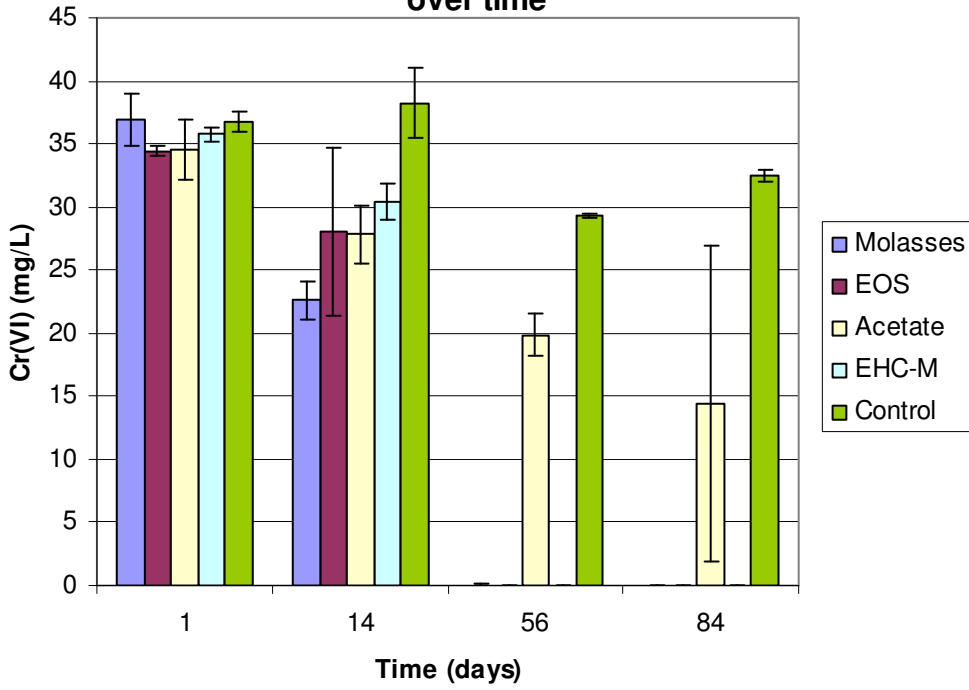


Figure 5-2. Aqueous total Cr change over time

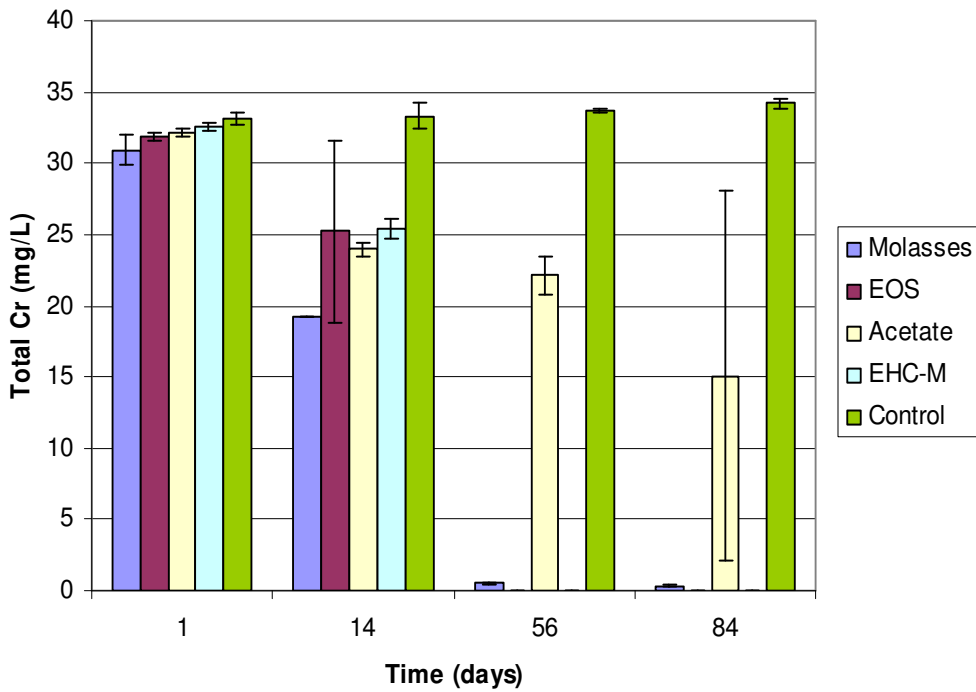


Figure 5-3. Total Cr in soil (dry weight) over time

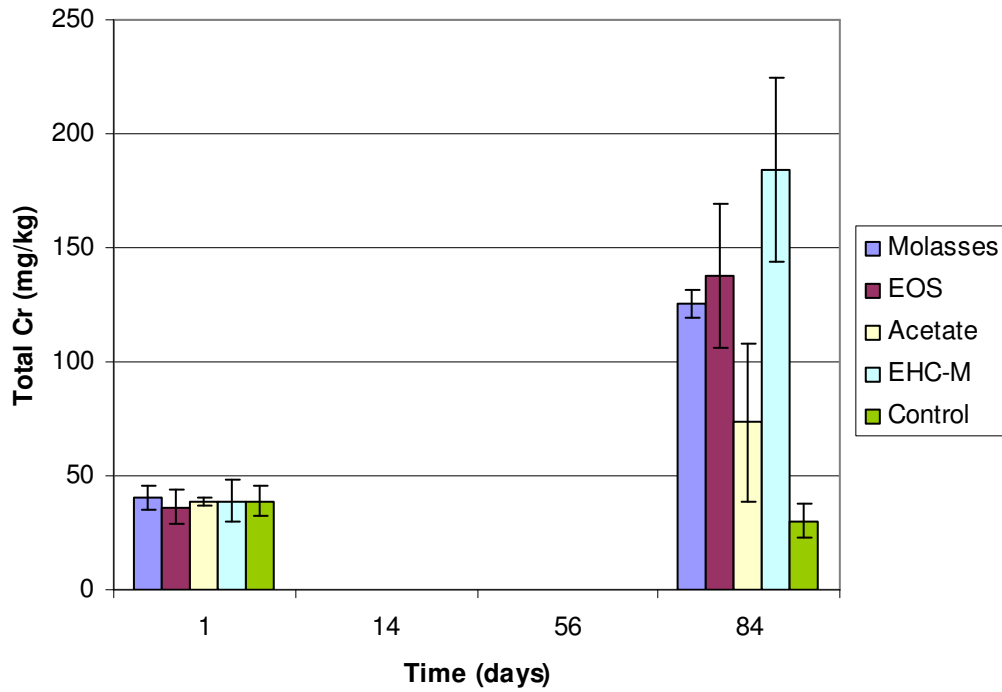


Figure 5-4. ORP change over time

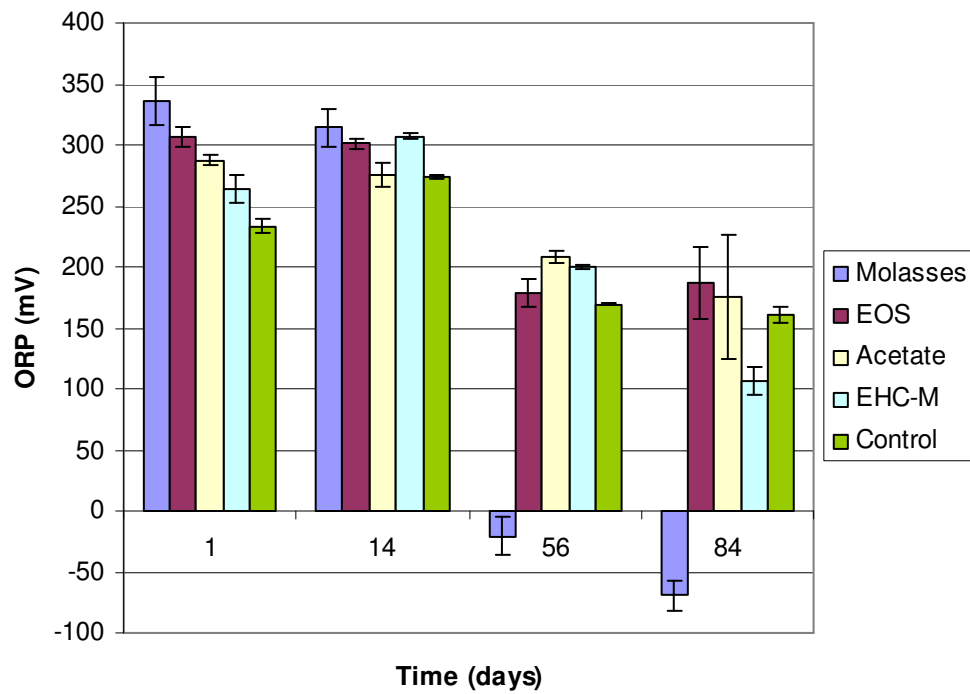


Figure 5-5. pH change over time

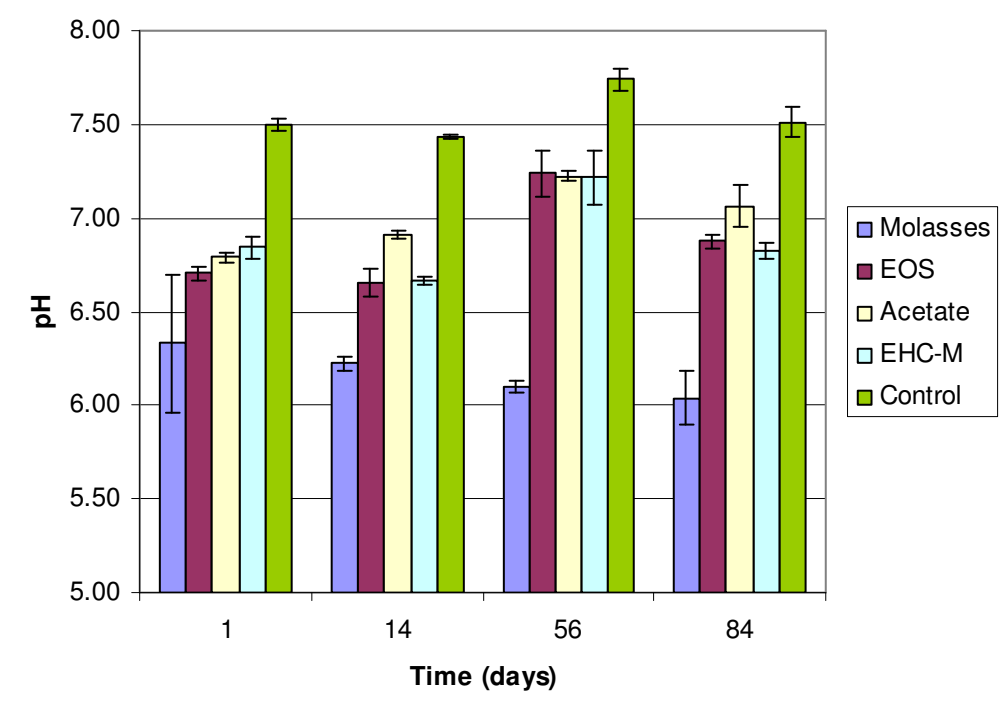


Figure 5-6. Aqueous ferrous concentration change over time

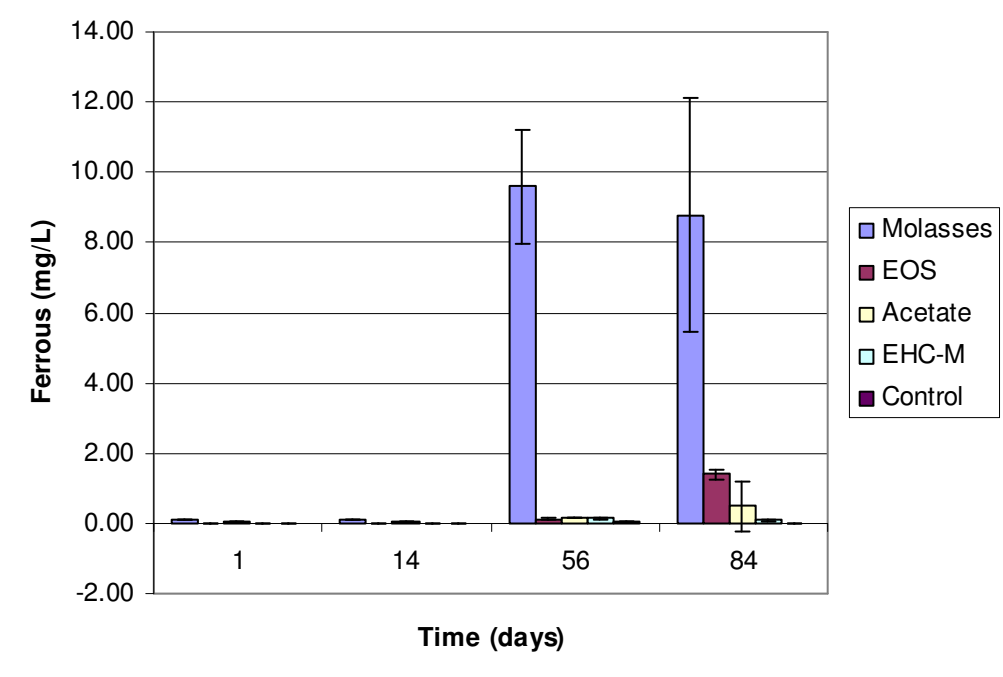


Figure 5-7. Nitrate concentration change over time

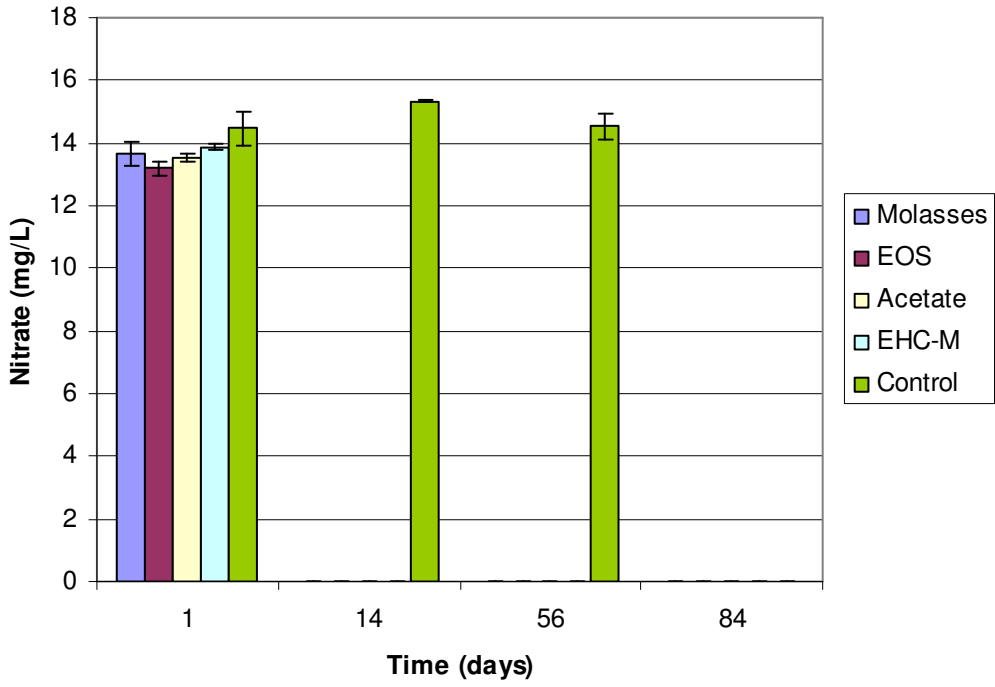
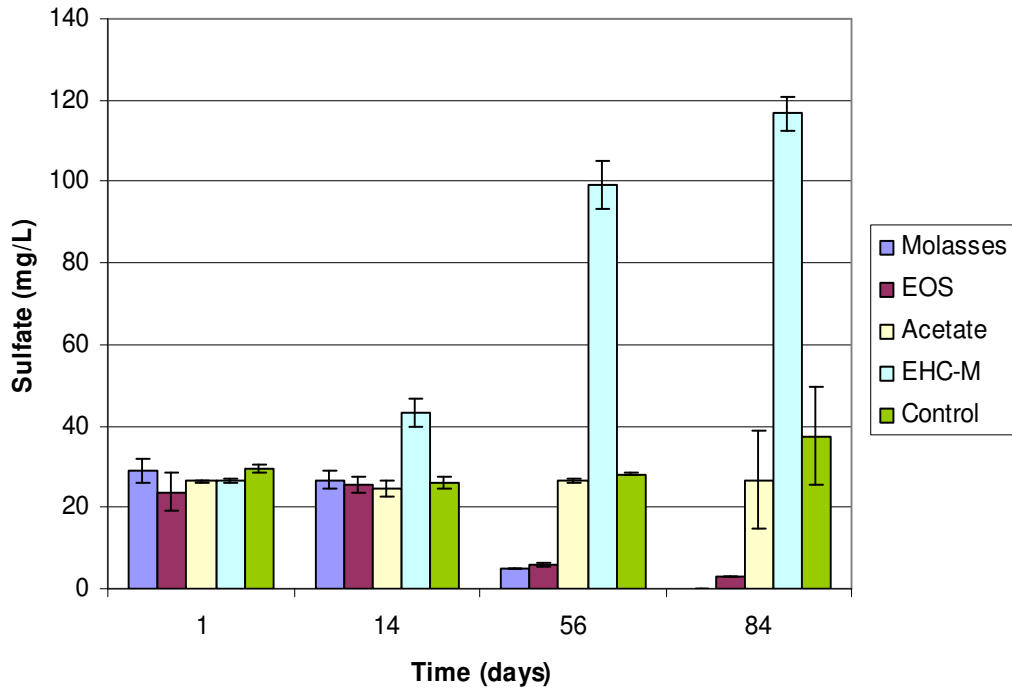
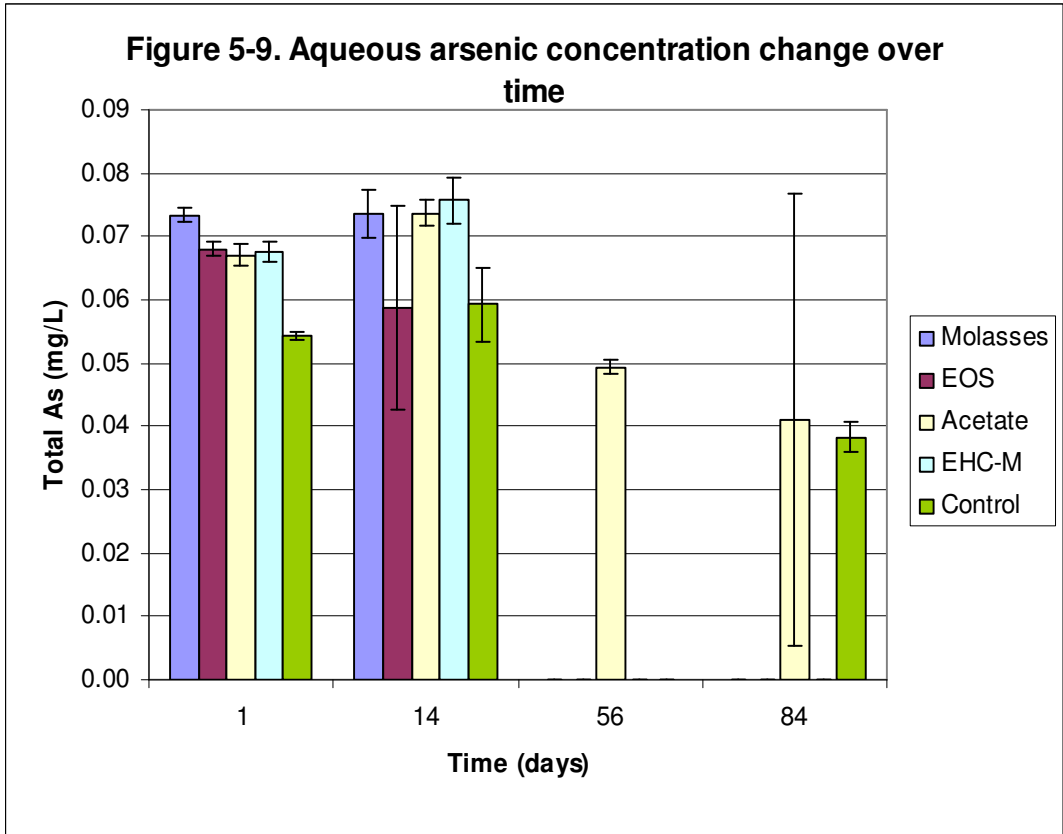


Figure 5-8. Sulfate concentration change over time





Notes: Error bars show one standard deviation calculated from triplicate samples