ASSESSMENT OF INOCULA TO ENHANCE STARTUP OF ETHANOL-FED AND SOLID-PHASE ORGANIC SULFATE REDUCING BIOREACTORS FOR THE NATIONAL TUNNEL DRAINAGE, CLEAR CREEK/CENTRAL CITY SUPERFUND SITE[1]

Alison Ruhs [2], Linda Figueroa [3], Thomas Wildeman [4], Michael Holmes [5], and David Reisman [6]

Abstract: The U.S. Environmental Protection Agency (EPA) is planning to construct an Anaerobic Passive Treatment System (APTS) to treat acid mine drainage from the National Tunnel in North Clear Creek near the City of Blackhawk, Colorado. North Clear Creek is part of the Clear Creek/Central City Superfund Site, and the National Tunnel is a major contributor of contaminants to this tributary. The EPA would like to determine the feasibility of constructing an APTS at this location.

Two modes of sulfate reducing bioreactor (SRBR) configurations are under consideration. One mode is an ethanol fed SRBR and the other mode is a solid substrate fed SRBR (two different mixtures). Laboratory proof-of-concept studies to test the performance of locally available microbial inoculum and the effects of start-up conditions were conducted.

The rationale for the laboratory experiments was to establish the best start-up inoculum for two different types of bioreactors: solid phase substrate based – wood, corn stover/hay, limestone/quartz and ethanol based – ethanol as the food source, limestone/quartz, reducing additive. Bag tests were conducted with 3 different substrates (two solids phase mixtures and ethanol), and 7 different inoculum. Sulfate and copper removal from the proof-of-concept experiments suggest that domestic sewage sludge provided the best bacterial inoculum for the ethanol-fed SRBR with horse and goat manure tied for second best.

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[1] Poster was presented at the 2007 National Meeting of the American Society of Mining and Reclamation, Gillette, WY, 30 Years of SMCRA and Beyond June 2-7, 2007. R.I. Barnhisel (Ed.) Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

[2] Alison Ruhs, Graduate Student, [3] Linda Figueroa, Associate Professor, [4] Thomas R. Wildeman, Professor Emeritus, respectively, Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO, 80401, [5] Michael Holmes, Remedial Project Manager, US EPA Region 8 (EPR-SR), 999 18th Street, Suite 300 Denver, CO 80202-2466, [6] David Reisman, USEPA, National Risk Management Research Laboratory, ETSC, Cincinnati, OH 45268,

Proceedings America Society of Mining and Reclamation, 2007 pp 706-713
DOI: 10.21000/JASMR07010706

http://dx.doi.org/10.21000/JASMR07010706

Introduction

The goal of this laboratory study was to test the performance of locally available microbial inocula and the effects of start-up conditions for input into the design of ethanol-fed and solid phase substrate sulfate reducing bioreactors (SRBRs). Passive treatment is the recommended alternative for the National Tunnel drainage in North Clear Creek near the City of Black Hawk, Colorado. North Clear Creek is part of the Clear Creek/Central City Superfund Site, and the National Tunnel is a major contributor of metal contamination to this tributary.

Rationale and Approach

The rationale for these experiments and what we expected to find are listed below:

1. We want to establish the best start-up inoculum for two different types of bioreactors:
   - Substrate based – wood, corn stover/hay, limestone/quartz
   - Ethanol based – ethanol as food source, limestone/quartz, reducing additive
2. Bag tests were conducted with 3 different substrates (CSM mix, Golder mix, Ethanol), and 7 different inocula to establish the best inoculum for each type of reactor.
3. Pine was used because it is a soft wood, which should make it more bioavailable. It was also readily available, free, and untreated.
4. Limestone was used as the base media because it would help with neutralization and in a real bioreactor it would help maintain hydraulic conductivity.
5. Ethanol reactors had Fe° as the reducing additive, this would allow us to see which inoculum started the fastest without adding a food supplement such as glucose, soy protein, alfalfa, etc. The second set of tests will look at which additive works better.
6. Ethanol concentration was based on the Leviathan reactor at the Leviathan Mine Aspen Seep, California, which adds between 400-600 milligrams of ethanol per liter. Using the density of ethanol = 0.789 g/mL, and assuming the original concentration of ethanol to be 95%, we ended up with an ethanol concentration = 500mg/L.
7. Inoculum determined based on statewide availability and likelihood of SRB being present.
   - *Horse manure* – it is used frequently as an inoculum in bioreactors, very similar to cow manure.
   - *Anaerobic digester* – specialized microbes, already anaerobic, expect that these microbes could adjust rapidly to an ethanol based diet.
   - *Brewery waste* – (spent grain), thought maybe if there were microbes present they could utilize ethanol, ultimately having a fast start up.
   - *Septic tank* – anaerobic, good consortium of microbes, should be a health population of microbes.
   - *Pond scum* – anaerobic section, healthy population of microbes.
   - *Activated sludge* – although these microbes are aerobic, there should be a healthy population, could prove to be a good start-up inoculum.

- *Goat manure* – known to be able to eat anything, could have a diverse population of anaerobic microbes.

8. It is thought that goat, horse, and cow manure will work equally.

9. The anaerobic digester and septic tank we predict will work well, followed by pond scum, activated sludge, and finally brewery waste.

Measurements

1. Conductivity, pH, and reduction potential (-Eh) were measured at time zero, and once a week after that until conclusive results were established. Bags were also monitored for H_2S and visual indications of metal precipitation. Once the experiments were complete metals analysis was conducted using the ICP-AES, to determine metals removal.

2. Duplicates were conducted as well to insure quality of measurements.

First Set of Bag Tests

Below is the labeling system used to organize the data collected during the bag experiments.

Labeling	**Contents**	**Abbreviation**
1A	Horse Manure, ***CSM***	HM
2A	Anaerobic Digester, ***CSM***	AD
3A	Brewery Waste, ***CSM***	BW
4A	Septic Tank, Ethanol, ***CSM***	ST
5A	Pond Scum, ***CSM***	PS
6A	Activated Sludge, ***CSM***	AS
7A	Goat Manure, ***CSM***	GM
1B	Horse Manure, ***CSM***	HM
2B	Anaerobic Digester, ***CSM***	AD
3B	Brewery Waste, ***CSM***	BW
4B	Septic Tank, Ethanol, ***CSM***	ST
5B	Pond Scum, ***CSM***	PS
6B	Activated Sludge, ***CSM***	AS
7B	Goat Manure, ***CSM***	GM
1C	Horse Manure, ***Golder mix***	HM,G
2C	Anaerobic Digester, ***Golder mix***	AD,G
3C	Brewery Waste, ***Golder mix***	BW,G
4C	Septic Tank, Ethanol, ***Golder mix***	ST,G
5C	Pond Scum, ***Golder mix***	PS,G
6C	Activated Sludge, ***Golder mix***	AS,G
7C	Goat Manure, ***Golder mix***	GM,G
E1A	Horse Manure, ***Ethanol***	HM,E
E2A	Anaerobic Digester, ***Ethanol***	AD,E
E3A	Control (no inoculum), ***Ethanol***	C,E
E4A	Brewery Waste, ***Ethanol***	BW,E
E5A	Septic Tank, ***Ethanol***	ST,E
E6A	Pond Scum, ***Ethanol***	PS,E
E8A	Activated Sludge, ***Ethanol***	AS,E
E9A	Goat Manure, ***Ethanol***	GM,E

Results and Discussion

Within 10 days of starting the bag tests a couple of the inocula appeared to be working just based on simple observations, such as the appearance of black precipitates (presumably metal sulfides) that had formed in the bottom of the bag. The Septic Tank sludge was the only inoculum within 10 days to produce H_2S gas in the ethanol containing bags, while several inocula had produced H_2S and black precipitates in the CSM and Golder substrate containing bags. These findings suggest that the septic tank would be the best inoculum for the ethanol reactors in the field. The selected photos show the black precipitate that developed after 10 days, and a comparison can be made in the color difference between different inocula used.

Ethanol bags: E6 contains pond scum
E5 contains septic tank sludge

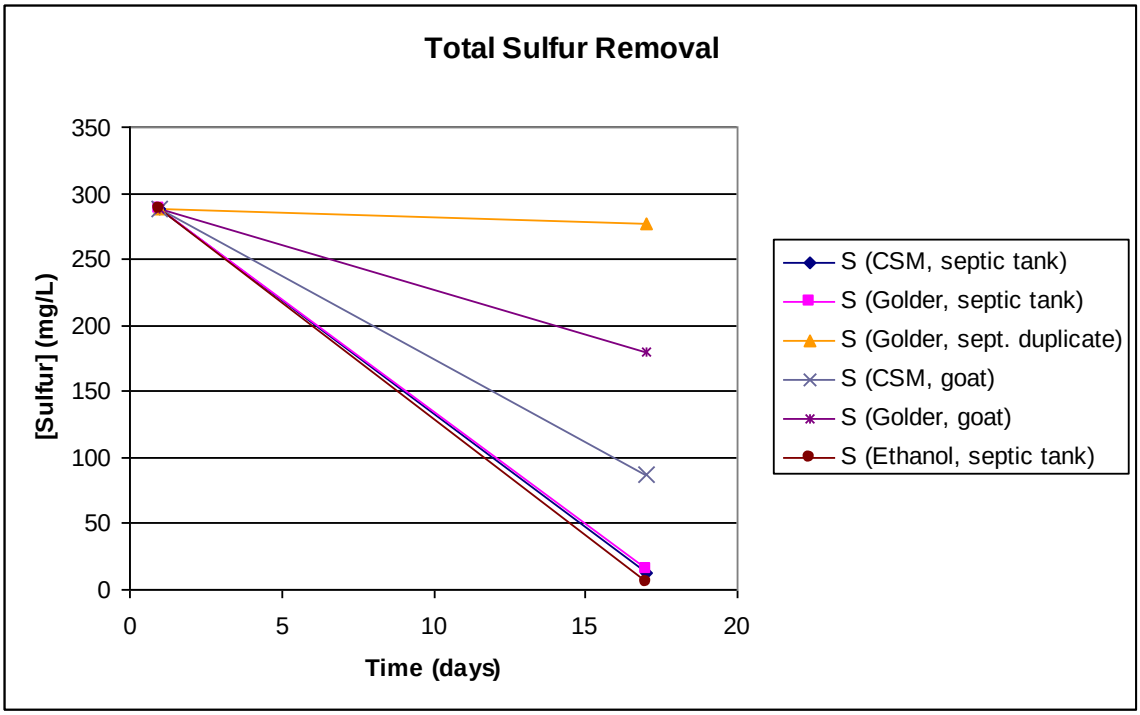
Ethanol bags: (left) Activated Sludge
(Right) Goat Manure

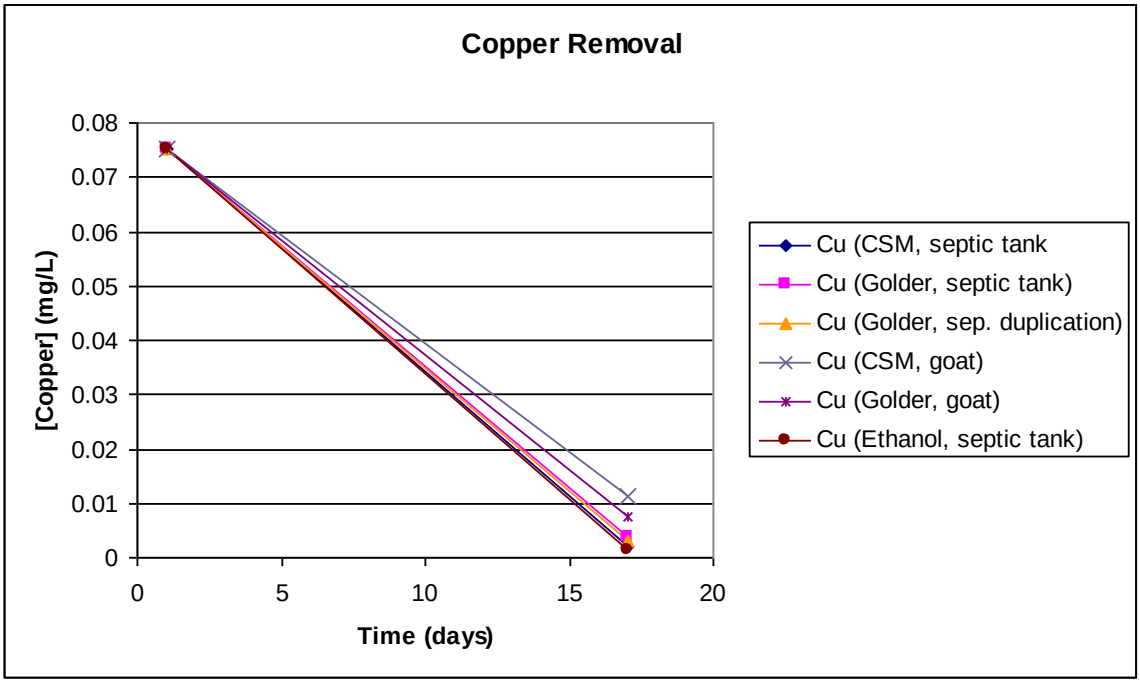
Table 1 below shows the results from the first set of bag tests:

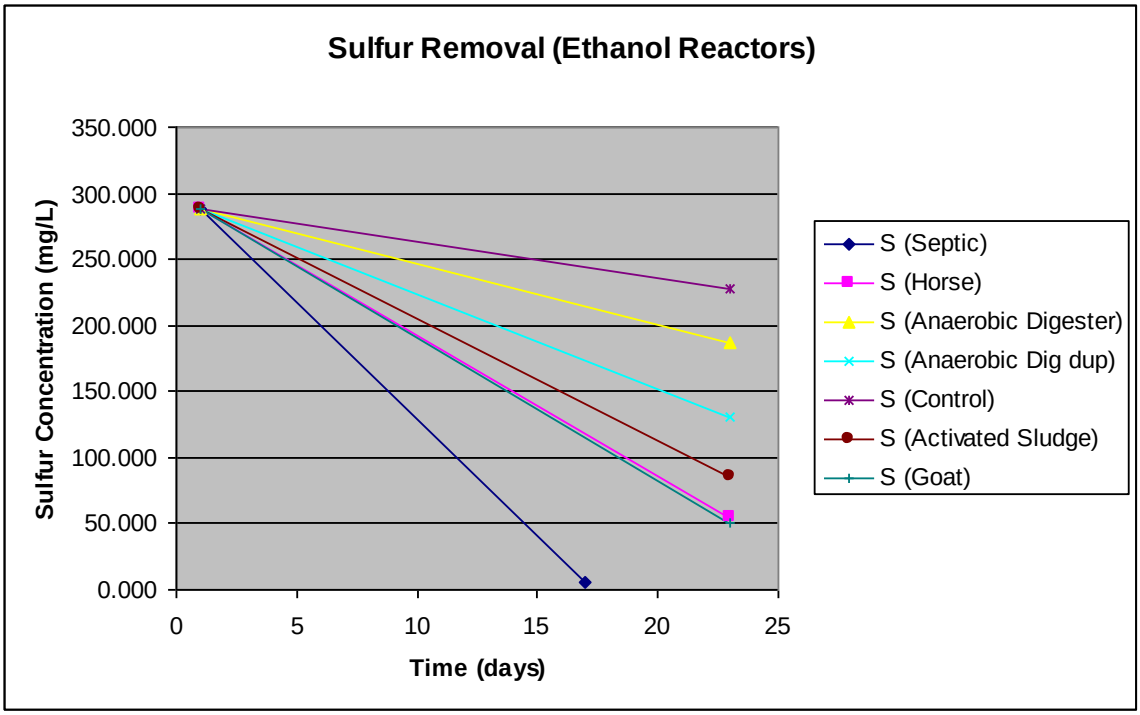
TABLE 1

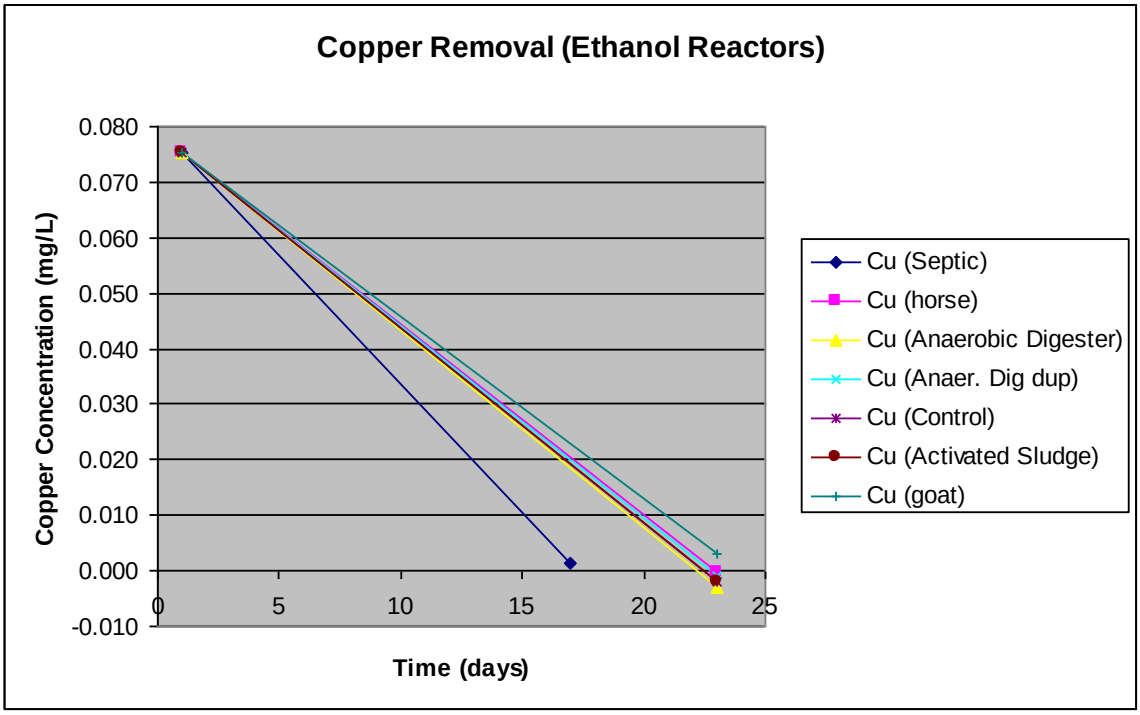
Bag	Time: day 0 pH	Eh *actual*	Conductivity	Smell?	Time: day 3 pH	Eh *actual*	Conductivity	Smell?	Time: day 10 pH	Eh *reading*	Conductivity	Smell?
E1A	6.95	162	981		6.59	620	2190		7.2	480	2220	
E2A	6.53	40	1655		7.24	-390	1480		7.1	440	1430	
E2A *dup.*	6.39	20	1730		7.37	250	1529		7.8	340	1510	
E3A (control)		160				160			6.5	600	1370	
E4A	6.07	110	1450		6.23	80	2120		6.4	30	4330	
E5A	6.54	-401	1790		7	-100	2080		7.8	-290	1570	H2S
E6A	6.76	47	1425		7.02	215	1330		7.5	130	1130	
E8A	6.56	60	1379		6.98	182	1340		7.5	10	1350	
E9A	8.48	38	4640		7.65	-100	5360		8.1	-170	5300	
1A	5.9	470	1960		5.96	130	2090		7.3	-20	2180	
2A	6.09	275	1310		6.23	-70	1780	slight H_2S	7	-120	1720	
2A *dup.*	6.16	220	1850		6.22	-90	1820		7.1	-90	1780	
3A	5.34	318	1710		5.02	900	2150		6	-21	2840	
4A	5.78	200	1740		5.96	460	1920		6	-105	1700	H2S
5A	5.82	250	1680		6.14	360	1760		6.8	-20	1770	
6A	5.81	247	1619		5.91	440	1710		6.9	0	1750	
7A	7.39	227	3200		6.39	120	3620		7.1	-120	3570	H2S
1B	6.02	362	1970		5.85	235	2080		6.7	-20	1840	
2B	6.03	210	1750		6.18	110	1750	slight H_2S	6.9	-60	1720	H2S
3B	5.31	480	1620		4.92	205	2070		5.6	40	2600	
4B	5.79	222	1510		5.91	60	1850		7.1	-100	1730	H2S
5B	5.76	260	1657		6.11	105	1750		7.1	-15	1790	
6B	5.84	265	1585		5.98	610	1660		7	-15	1720	
7B	7.43	263	3260		6.5	170	3720		7.2	-110	3730	H2S
7B *dup.*	7.05	273	2840		6.22	225	3060		7.1	-60	2970	
1C corn + hay	6.92	295	2120		5.5	240	2150		6.5	-30	2450	
1C hay only	6.23	274	1970		5.72	195	2370		6.9	-60	2400	
2C	6.11	285	1930		6.22	90	1870		7.2	-50	1930	
3C	5.48	258	1810		5.04	150	2320		6.5	-50	2020	H2S
4C	5.82	265	1970		5.87	31	1870		7.3	-130	2440	H2S
4C *dup.*	5.71	225	1740		5.69	125	2360		7.3	-25	1970	
5C	5.82	216	1850		5.81	50	2000		7.1	-35	2000	
6C	5.84	214	1765		5.77	134	1900		7.2	-45	1960	
7C	7.09	202	2960		6.13	45	3680		7.1	-110	1750	H2S

ICP analysis was conducted on the initial mine drainage water and the two best performing inoculum (septic tank sludge and goat manure) were selected for ICP analysis after day 17. Another ICP analysis was conducted after 23 days for four of the best inoculum in the ethanol containing bags (based on ppt. and H_2S gas detection), plus a blank. The results for total sulfur removal and Cu removal can be seen in the 4 figures that follow. It appears that the septic tank sludge in the ethanol bags out performed the other inoculum with respect to sulfur removal and Cu removal.


Total Sulfur Removal
[Sulfur] (mg/L)
350
300
250
200
150
100
50
0
0
5
10
15
20
Time (days)
S (CSM, septic tank)
S (Golder, septic tank)
S (Golder, sept. duplicate)
S (CSM, goat)
S (Golder, goat)
S (Ethanol, septic tank)


Copper Removal
[Copper] (mg/L)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
5
10
15
20
Time (days)
Cu (CSM, septic tank
Cu (Golder, septic tank)
Cu (Golder, sep. duplication)
Cu (CSM, goat)
Cu (Golder, goat)
Cu (Ethanol, septic tank)





Second Set of Bag Tests:

Based on the first set of bag tests, it was decided to test the goat manure and septic tank with two different concentrations of ethanol and reducing additives such as glucose or alfalfa in place of the elemental iron to try and optimize performance. Below is the labeling system used to organize the data for the second set of bag tests.

Labeling	Contents	Additive
SE1n	Septic Tank, ***Ethanol*** (0..42 mL/L)	none
SE1a	Septic Tank, ***Ethanol*** (0.42 mL/L)	alfalfa
SE1g	Septic Tank, ***Ethanol*** (0.42 mL/L)	glucose
SE2n	Septic Tank, ***Ethanol*** (0.67 mL/L)	none
SE2a	Septic Tank, ***Ethanol*** (0.67 mL/L)	alfalfa
SE2g	Septic Tank, ***Ethanol*** (0.67 mL/L)	glucose
GE1n	Goat Manure, ***Ethanol*** (0.42 mL/L)	none
GE1a	Goat Manure, ***Ethanol*** (0.42 mL/L)	alfalfa
GE1g	Goat Manure, ***Ethanol*** (0.42 mL/L)	glucose
GE2n	Goat Manure, ***Ethanol*** (0.67 mL/L)	none
GE2a	Goat Manure, ***Ethanol*** (0.67 mL/L)	alfalfa
GE2g	Goat Manure, ***Ethanol*** (0.67 mL/L)	glucose

Below in Table 2 are the results from the second set of bag tests.

TABLE 2

Bag	Time: day 0				Time: day 7				Time: day 21				
	pH	Eh *actual* (mV)	Conductivity (µS/cm)	Smell?	pH	Eh *actual* (mV)	Conductivity (µS/cm)	Smell?	pH		Eh *actual* (mV)	Conductivity (µS/cm)	Smell?
SE1n	7.5	368	1960		7.3	-39	2140	slight H2S	7.59	-250	-89	2520	slight H2S
SE1a	6.9	284	6090		6.3	-59	9470	slight H2S	6.2	-260	-99	9500	
SE1g	7.4	333	1310		4.7	61	6010		5.14	-200	-39	9450	
SE2n	7.4	333	1770		7.1	-39	1730	slight H2S	7.35	-205	-44	1780	slight H2S
SE2a	7	288	5170		5.7	11	9290		6.08	-240	-79	10500	
SE2g	7.2	318	1210		4.8	21	5310		5.21	-285	-124	8510	
GE1n	8	293	3220		7.2	-39	3610		7.4	-240	-79	3500	slight H2S
GE1a	7.1	283	7800		5.3	31	13940		5.27	-220	-59	12900	
GE1g	8	268	2470		4.8	-89	5450		4.89	-310	-149	8730	
GE1g dup	8.1	278	2380		4.7	-139	5090		4.84	-290	-129	8510	
GE2n	8.2	278	3880		7.3	-134	4630	slight H_2S	7.46	-280	-119	4490	
GE2a	7.2	278	7990		5.5	-39	13890		5.56	-200	-39	14300	
GE2g	8	268	2530		5	-119	5400		5.07	-330	-169	8770	

Using glucose as a reducing additive seemed like a good idea, because it could also provide some of the initial nutrition for the bacteria, but this simply caused a production in organic acids, such as butyrate (determined by the vomit like odor produced), which caused the pH to drop. Alfalfa didn't appear to help the process during the short time the experiment was run, but perhaps it could be a good source of nitrogen for the bacteria in the long term.

Conclusions

Although the septic tank sludge proved to be an excellent inoculum, and readily available, it was decided that the human pathogen risks associated with it was much greater than animal manure. Horse manure was ultimately used in the reactors in the field reactors because it worked reasonably well in the bag tests, is readily available and has been shown to work well in other passive treatment studies.

Acknowledgements

We are grateful to Ms. Judith Bolis of URS Corporation for her assistance is setting up the bag tests and to Mr. James Gusek of Golder Associates for his advice on reactive mixtures and collaboration on the ensuing field study.