NOVEL BIOAUGMENTED SORPTION TREATMENT TECHNOLOGY FOR CVOCS AND 1,4-DIOXANE

FINAL REPORT

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1,4-DX	1,4-dioxane
cis-DCE	cis-1,2-dichloroethene
CVOC	chlorinated volatile organic compound
DO	dissolved oxygen
DoD	Department of Defense, United States
EBCT	empty bed contact time
FID	Flame Ionization Detector
g	gram(s)
GAC	granular activated carbon
GC	Gas Chromatograph
gpm	gallons per minute
H_2O_2	hydrogen peroxide
HRT	hydraulic retention time
ID	internal diameter
L	liter(s)
mg	milligram(s)
mg/L	milligrams per liter
mg/g	milligrams of sorbate/gram of sorbent
min	minutes
mL	milliliter(s)
mL/min	milliliters per minute
m	meter
mm	millimeter
MNA	Monitored Natural Attenuation
NA	not applicable
ng	nanogram(s)
nmol	nanomole(s)
OU	operable unit
PCE	tetrachloroethene
TCE	trichloroethene
US EPA	United States Environmental Protection Agency
µg	microgram(s)
µg/L	micrograms per liter

EXECUTIVE SUMMARY

Background

Chlorinated volatile organic compounds (CVOCs) and 1,4-dioxane are persistent chemicals that oftentimes co-occur in groundwater at DoD sites. Conventional treatment methods such as advanced oxidation are very expensive, while sorption or air stripping and typically ineffective for the complete removal of both CVOCs and 1,4-dioxane due to the hydrophilicity of 1,4-dioxane.

Project Objective

The overarching objective of this project was to demonstrate an ex-situ adsorption/biodegradation treatment train to irreversibly remove CVOCs and 1,4-dioxane from waters at a DoD site using bioaugmented adsorbents. The desired outcome of this project was to ultimately limit the cost associated with existing pump and treat technologies (e.g. advanced oxidation) by minimizing costs associated with energy inputs and/or chemical inputs (e.g. costs associated with UV sources and chemical oxidants), and also be applied to remediation efforts for other chemical mixtures.

Key Findings

- Bioaugmented reactors performed 5X better than abiotic reactors even in the absence of key parameter optimization.
- Nutrients and oxygen supply were identified as crucial parameters for aerobic bioGAC systems.
- Insufficient nutrient input will halt microbial activity and stall 1,4-dioxane removal
- Proper and consistent H_2O_2 dosing is critical to ensuring aerobic conditions while mitigating sudden desorption events.
- Data suggest that a shorter 10 hr HRT yields higher removal of 1,4-dioxane on a percentage and mass basis, implicating the possibility of faster remediation than previously expected.
- DNA extraction and amplification of site groundwater indicated the presence of naturally occurring CB1190-like organisms that can potentially degrade 1,4-dioxane in non-bioaugmented reactors.
- This is the first instance where direct metabolic treatment of comingled 1,4-dioxane and CVOCs was applied in hybrid field-scale bioreactors.

Implications for Practitioners

- The hybrid biological/physicochemical process ultimately provides sustainable real time sorbent bioregeneration
    - Low capital and operational costs attributed to sorbent investment (1.5% w/w total)
    - Longer operational lifetime for sorbent beds
        - No changeout anticipated for bioaugmented process
    - Amount of RCRA waste will be minimized with combined biodegradation
        - Ensures little to no accumulation of toxic byproducts
- Biodegradation of 1,4-dioxane and some CVOCs minimizes leaching concerns associated with potential secondary release (landfilling)

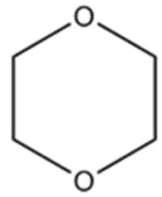
1.0 INTRODUCTION

1.1 Project Objective

This demonstration/validation supports NDCEE's mission to reduce the DoD's liabilities by developing sustainable, cost-effective technologies for expedited site cleanup and closure by ex situ remediation of 1,4-dioxane and CVOCs in groundwater. Specifically, this project demonstrated the simultaneous removal of CVOCs and 1,4-dioxane from water by using abiotic and bioaugmented adsorbents. This approach will ultimately limit the cost associated with existing pump and treat technologies by minimizing costs associated with energy inputs and/or chemical inputs (e.g. costs associated with UV sources and chemical oxidants), and also be applied to remediation efforts of other comingled chemicals. Further, stakeholders will achieve faster groundwater and site remediation using this approach.

1.2 Background

1,4-Dioxane is a cyclic ether which has been historically used as a stabilizer for 1,1,1-trichloroethane (Doherty, 2000; Figure 1), and this has caused it to commonly co-occur with a variety of chlorinated volatile organic compounds (CVOCs) in contaminated groundwater through historical use and handling (Adamson et al., 2015; Adamson et al., 2014; Anderson et al., 2012; Mohr et al., 2010). Due to its high aqueous miscibility and low Henry's law constant (4.88×10-6 atm·m3/mol), 1,4-dioxane is highly mobile and persistent in the environment and challenging to separate from aqueous solutions (Mohr et al., 2010). Chemical oxidation has been investigated for the removal of 1,4-dioxane (Chitra et al., 2012; Vescovi et al., 2010; Zhao et al., 2014; Zhong et al., 2015), but requires large chemical and energy inputs with the potential for generating harmful transformation products (DiGuiseppi et al., 2016; Li et al., 2018).



1,4-dioxane

Figure 1: Chemical structure of 1,4-dioxane.

1,4-Dioxane biodegradation mediated by various microorganisms has been well documented (Zhang et al., 2017). While biodegradation of 1,4-dioxane has been demonstrated as an effective treatment mechanism with pure cultures (Mahendra and Alvarez-Cohen, 2005; Mahendra and Alvarez-Cohen, 2006; Mahendra et al., 2007; Zhao et al., 2018), mixed cultures (Polasko et al., 2018), and indigenous microorganisms (Gedalanga et al., 2016), one of its primary drawbacks is the sensitivity of microbial activity to environmental conditions, including inhibitory comingled CVOCs (Mahendra et al., 2013; Zhang et al., 2016; Figure 2).

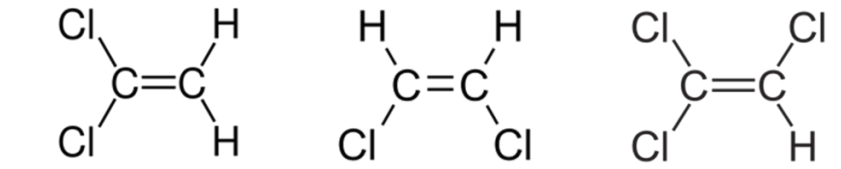


Figure 2: Examples of common CVOCs comingled with 1,4-dioxane.

Until recently, adsorption was thought to be an ineffective means of removing 1,4-dioxane from water due to its high aqueous solubility and low octanol-water partitioning coefficient (McGuire and Suffet, 1978; McGuire et al., 1978; Mohr et al., 2010). Some adsorbents have been studied for 1,4-dioxane removal capabilities, such as activated carbon (Norit 1240 GAC; adsorption capacity of ~38 mg/g) (Myers et al., 2018), a synthetic polymeric adsorbent (Ambersorb 560; adsorption capacity of ~40 mg/g at aqueous equilibrium concentration of 80 mg/L) (Woodard et al., 2014), and a titanium silicalite zeolite (TS-1; maximum adsorption capacity of 85.17 mg/g) (Chen et al., 2019). However, few adsorbents have been reported that provide sufficient capacity for 1,4-dioxane or have considered their removal capabilities in the presence of CVOCs. GAC is often a first choice when considering adsorption for contaminant removal, however it is not always effective and has the potential for desorption under transient influent conditions.

Recent work suggests that adsorbents bioaugmented with 1,4-dioxane metabolizing or co-metabolizing bacteria can be more effective at removing 1,4-DX alone or while in mixtures with CVOCs from water than abiotic adsorbents (Myers et al., 2018; Johnson et al., 2018; Figures 3 and 4). The bacteria are able to mineralize 1,4-DX while the CVOCs are strongly adsorbed by the adsorbent. This has the added benefit of making the CVOCs non-bioavailable to the bacteria, which can help mitigate inhibitory effects.

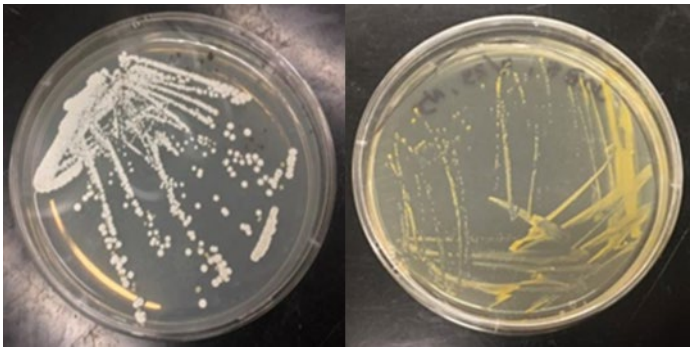


Figure 3: Colonies of *Pseudonocardia dioxanivorans* CB1190 (left) and *Mycobacterium austroafricanum* JOB5 (right)

While others have attempted to use abiotic adsorbents to remediate 1,4-DX or CVOCs separately, to our knowledge, no other efforts have used adsorbents to treat these chemicals while in mixtures. Additionally, the efficacy of bioaugmented adsorbents for the simultaneous treatment of both 1,4-DX and CVOCs is not yet reported. This technology has been demonstrated to be effective for other contaminant mixtures such as the analogous mixtures of MtBE/tBA/BTEX. While other projects exist that seek to treat 1,4-DX and CVOC mixtures,

most are only feasible under specific site conditions, require significant chemical addition (e.g. co-metabolic treatment), or significant cost. This technology can be installed in existing pump and treat infrastructure and requires minimal nutrient and oxygen amendment for success.

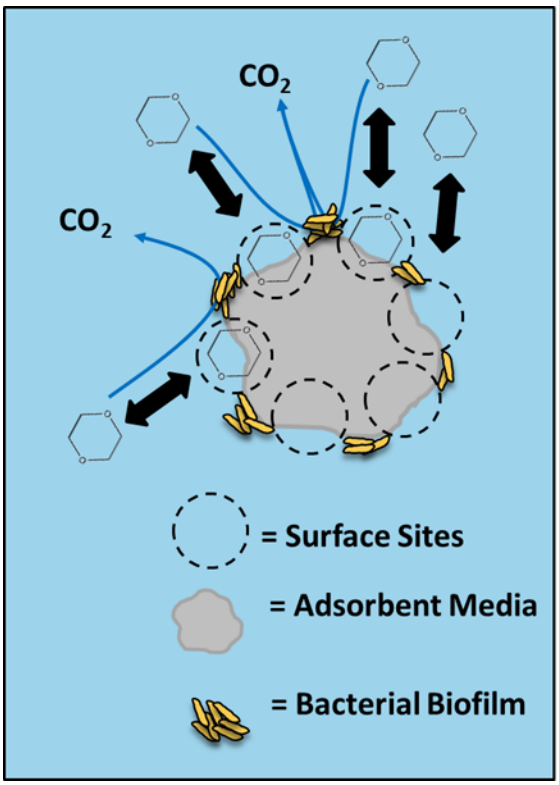


Figure 4: Animation depicting biofilm attachment to filter media and subsequent 1,4-dioxane degradation.

2.0 METHODS AND APPROACH

Bioaugmented adsorbents using two different adsorbents and two different bacterial strains were assessed under site-specific conditions (e.g. temperature, water characteristics) in laboratory scale column flow-through reactors. More information regarding experimental details are listed below in subsequent sections.

2.1 Chemicals

1,4-Dioxane (anhydrous, 99.8%) was purchased from Sigma-Aldrich. Trichloroethene (TCE; 99.5%, ACS Grade), cis-1,2-dichloroethene (cDCE; 97%; ACROS Organics), and 1,1-dichloroethene (1,1-DCE; >95%; Ultra Scientific) were purchased from Fisher Scientific and used to prepare saturated stocks with deionized (DI) water. Granular activated carbon (GAC; Norit 1240™) was purchased from Fisher Scientific. Silica sand (20-30 mesh, ASTM C-778) was purchased from Aqua Solutions, Inc.

2.2 Culture Conditions

Propane-oxidizing bacterial cells of JOB5 were grown in pre-sterilized, sealed bottles containing a ratio of 1 part nitrate mineral salts (NMS) medium (Whittenbury et al., 1970) to 4 parts

headspace (to ensure sufficient oxygen mass transfer) with 25 % (v/v) filter-sterilized propane added (to ensure stoichiometric excess) into the headspace using sterile syringes. All bottles were incubated at 30˚C with 150 rpm agitation. To maintain aerobic conditions, all cell cultures were aerated with filter-sterilized air when the oxygen content fell below 10% as measured using an oxygen and carbon dioxide analyzer (Quantek Instrument, Grafton, MA) followed by adding 25% (v/v) propane into the headspace.

Pseudonocardia dioxanivorans CB1190 was grown as a pure culture in either ammonium minerals salts (AMS) medium or UCLA modified media as described in Parales et al. (1994) and Polasko et al. (2018), respectively. CB1190 was grown in 2 L baffle flasks with a 1:4 ratio of liquid to headspace to ensure sufficient oxygen mass transfer. Culture flasks were shaken in 30°C incubators with a rotational rate of 150 rpm. The cultures were fed 100 mg/L of 1,4-dioxane and allowed to degrade the cyclic ether to below detection on a gas chromatograph fitted with a flame ionization detector (GC-FID). This cycle was repeated a total of three times before the culture was used for experiments to ensure sufficient biomass and microbial activity.

2.3 Bio-GAC Treatability Studies

GAC was prepared as abiotic or bioaugmented with 1,4-DX-metabolizing bacteria (*Pseudonocardia dioxanivorans* CB1190) or co-metabolizing bacteria (*Mycobacterium austroafricanum* JOB5) that have been proven to biodegrade 1,4-DX and some CVOCs and then packed into reactors/columns. The columns were placed in an environmental chamber and held at temperatures typical of NASNI OU-11. Reactors were conducted in upflow mode to minimize fouling. Influent waters will consisted of synthetic waters prepared to match site specific water quality parameters. Nutrients and oxygen releasing compounds were supplied to the reactor influent ensure bacterial growth and biodegradation activity. 1,4-DX and CVOC concentrations were monitored using gas chromatography. Biomass and functional gene analysis were monitored using qPCR. These laboratory column feasibility studies using GAC will be used to optimize the treatment process and operation requirements (e.g. nutrient addition, oxygen requirements, reactor size, etc.) for site-specific contaminant mixtures and water quality characteristics.

Columns were constructed according to the diagram and photograph shown in Figures 5 and 6. The columns consisted of stainless-steel tubes (3.4798 cm D. X 30.48 cm L.) with luer sampling ports installed along the length at 2.54, 5.08, 10.16, 15.24, 20.32, and 25.40 cm. The stainless-steel tubes were sealed with stainless steel endcaps that ended in hose barbs via tri-clamp connections using PTFE gaskets. Silicone vacuum grease was used to ensure water-tight connections. One-way stopcocks were attached to all sampling ports on the columns. Prior to all experiments, the apparatus was recirculated with either hydrochloric acid solution (5% v/v as described above) or denatured ethanol (70% v/v) to sterilize the interior. The apparatus was then flushed with DI water to remove the acid or ethanol residues. A PTFE membrane filter was placed at the column inlet to prevent biological contamination of the column. PTFE tubing was used to prevent absorption of the CoCs. All fittings were either polycarbonate or polypropylene. The pump tubing consisted of PTFE tubing compatible with a peristaltic pump head to prevent absorption of the CoCs. Sampling valves were located before the pump tubing, before the inlet filter, and after the column. When appropriate, samples were taken from ports along the length of the column.

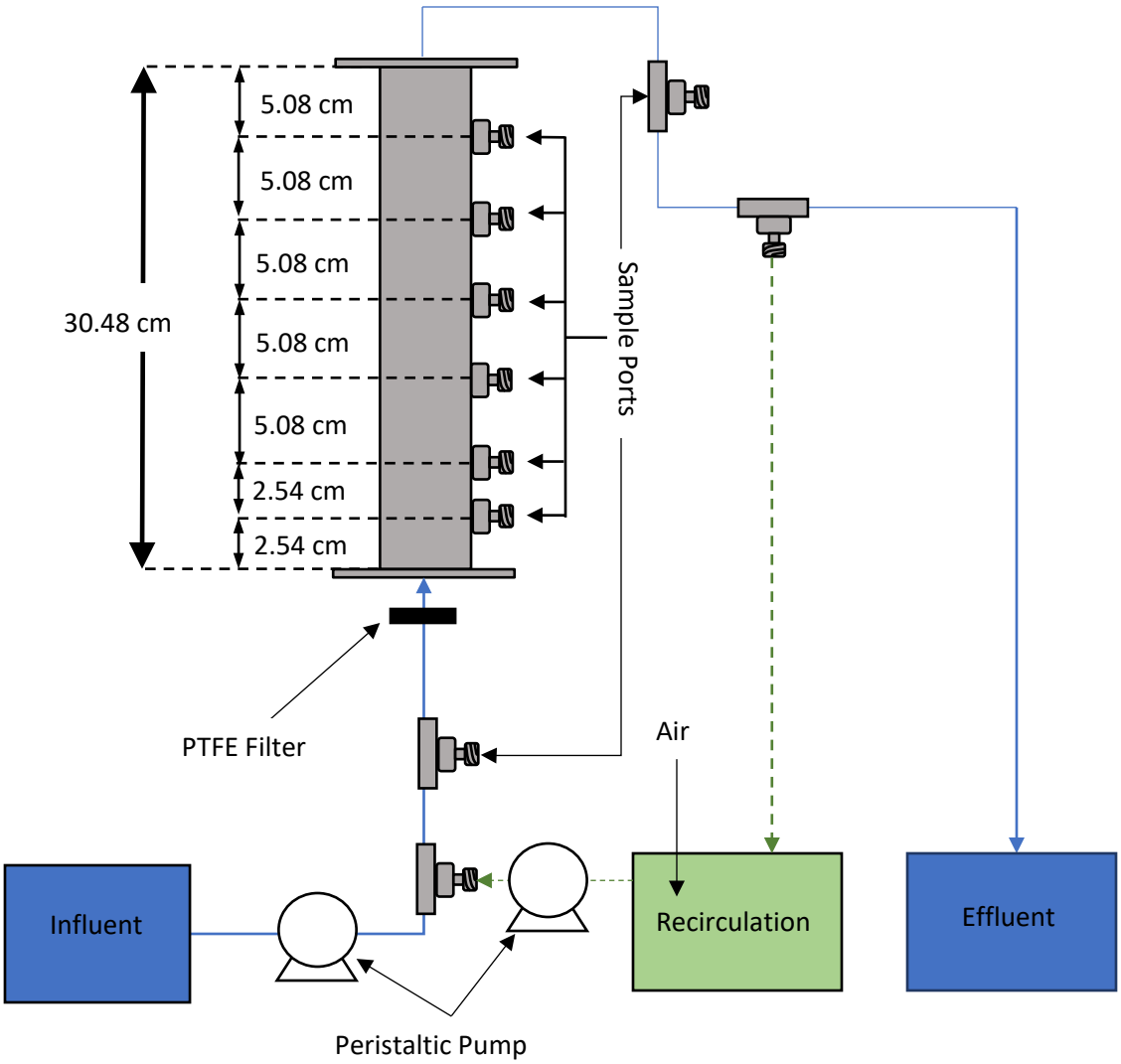


Figure 5: Column Schematic. The columns were made of stainless steel (30.48 cm H x 3.4798 cm D). The endcaps consisted of stainless-steel tri-clamp connections with hose barbs for tubing connection.

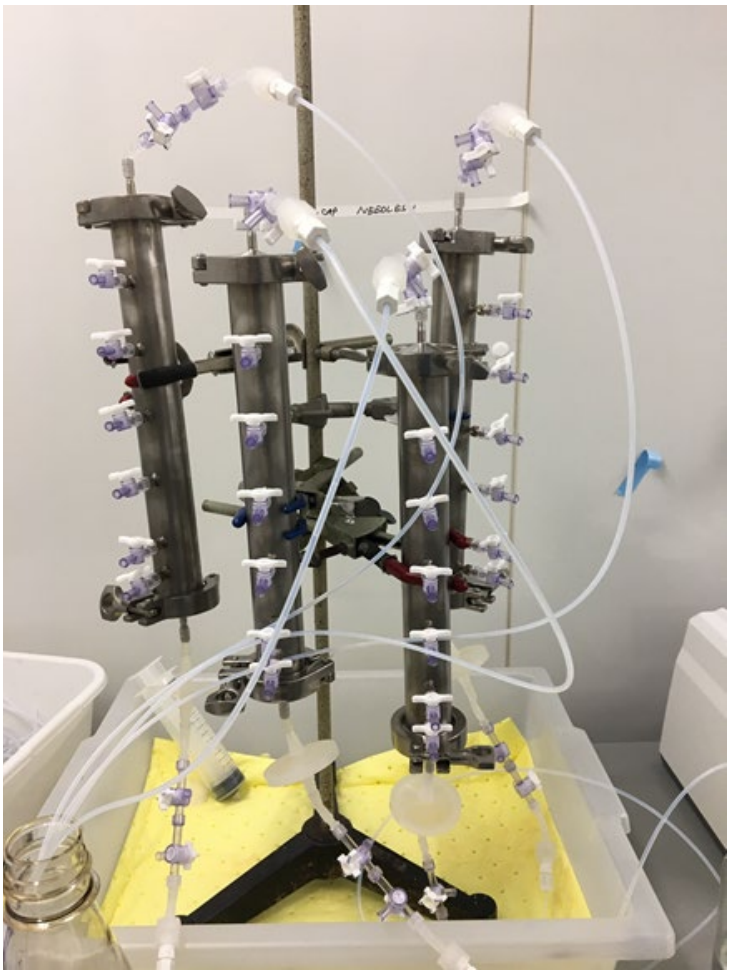


Figure 6: Column apparatus photographs. Stainless-steel columns with sampling ports used in different bio-GAC column experiments.

Solid media (GAC, sand, or mixtures of the two) were inoculated with pure CB1190 culture in two ways. For CVOC-free and bed composition experiments, GAC, sand, and GAC/sand were inoculated in beakers using different volumes of culture (15, 185, and 200 mL) that were chosen to be proportional to the volumes of solids in each container (5%, 95%, 100%). This was done to help keep bioaugmentation relatively similar between the layered- and mixed-bed columns. The beakers were covered with flame-sterilized aluminum foil and allowed to incubate in the previously mentioned shaking incubators with less agitation to prevent the GAC from splashing up out of solution onto the container walls (130 rpm). The inoculated solid media was incubated thusly for a total of three cycles of 1,4-dioxane addition as previously mentioned. After 1,4-dioxane fell below the detection limit of the GC-FID on the last cycle, the solid media were washed with 1,4-dioxane-free AMS or UCLA modified medium and wet packed into acid-sterilized glass chromatographic or ethanol-sterilized stainless-steel columns for the CVOC-free and bed composition experiments, respectively. After packing, 1,4-dioxane-free AMS or UCLA modified medium was recirculated through the columns for 48 h in upflow mode (1 mL/min) to allow any 1,4-dioxane that was adsorbed during the inoculation step to desorb and be biodegraded by the biofilm grown on the solid media.

For the hydraulic retention time (HRT) and synthetic groundwater experiments, the solid media was inoculated by recirculation of pure CB1190 culture through the columns for an extended period of time. The media was first mixed and packed into the columns before being autoclave sterilized (121°C, 45 min sterilization time). After sterilization, pure CB1190 culture (200 mL) was placed in serum bottles that served as reservoirs for recirculation at a flow rate of 1 mL/min. Air was sparged into the bottles using 6 in. long stainless-steel needles with 0.02 μm filters that were connected to a dry air cylinder. Recirculation was maintained for 7-10 d before the culture was replaced with UCLA modified medium and a filter was placed at the column inlet to prevent contamination. The 1,4-dioxane-free UCLA modified medium was recirculated through the columns for 48 h to ensure that 1,4-dioxane adsorbed during the inoculation phase was desorbed and degraded. Air was sparged into the medium to ensure aerobic conditions.

Influent solution was prepared to reflect groundwater characteristics for an impacted site located in Southern California. To prevent differential partitioning over time as influent was removed from the influent reservoir, collapsible 1 L tedlar gas sampling bags were selected as the influent containers. For the CVOC-free, bed composition, and HRT experiments, influent solution containing all components that are not volatile at room temperature (including 1,4-dioxane, inorganic ions, etc.) was aerated for 30 min to ensure enough dissolved oxygen (DO) for aerobic biodegradation to occur. After aeration, the solution was added into the tedlar bags and the CVOCs were then injected into the bags. The solutions were allowed to equilibrate for 24 h prior to use in the experiments. For the synthetic groundwater experiment, instead of aerating the influent solution, liquid hydrogen peroxide was added to serve as an oxygen source (1.96 x 10-1 mM or 6.6 mg/L hydrogen peroxide) in combination with the 3 mg/L DO that accumulated in the medium during preparation.

To evaluate the impact of hydraulic retention time on the bio-GAC column systems, 3 bio-GAC packed columns were run in parallel with different target retention times including 10, 20, and 30 h, which correspond to EBCTs of 25.6, 51.3, and 76.9 h, respectively. The columns were operated in upflow mode with influent concentrations consisting of 3325 μg/L 1,4-dioxane, 1900 μg/L TCE, 3400 μg/L cDCE, and 3125 μg/L 1,1-DCE. A flow rate of 0.125 mL/min was targeted to achieve an HRT of ~15 h (EBCT of 38 h).

A final experiment was conducted using a mixture of bacterial growth medium and synthetic groundwater to mimic expected conditions for a planned pilot-scale study. The synthetic groundwater recipe is presented in Table 1. The influent matrix initially consisted of 90% synthetic groundwater and 10% UCLA modified medium by volume. The initially targeted hydraulic retention time was 20 h and the targeted contaminant levels in the influent were kept the same as in the bed composition and HRT experiments.

Table 1: Synthetic groundwater composition

Compound	Concentration g/L
Sodium bicarbonate	0.645
Sodium chloride	0.800
Magnesium sulfate	0.025
Potassium nitrate	0.250

2.4 Ex-situ Field Reactor Operation and Monitoring

Ex-situ pilot-scale reactors were constructed at NASNI OU-11 to validate laboratory results in a field setting. Gravity fed reactors were constructed by installing sampling ports at 1/6, 1/3, 1/2, & 2/3 of the length of the reactor, starting from the bottom. An additional influent sampling port was installed at bottom of reactor. To preserve reactor rigidity and integrity, holes were installed in a staggered orientation (Figure 7). Prior to filling and operation, quality control measures were conducted to test the integrity of the system. After installing sampling ports, the reactors were leak tested and elevated 2-feet above the influent tote to ensure sufficient head (Figure 7).

Figure 7: Images of reactor construction and quality control testing.

Following quality control testing, the drum reactors were packed with solid media. Filter bed material was homogenized in small batches, where 0.21 kg GAC was mixed with 18.6 kg sand (roughly 1.5% w/w GAC loading). To establish fixed CB1190 growth in bioaugmented reactors, growing cultures (at least 5 gallons) of CB1190 (10^6 CB1190 16S rRNA copies / mL) were added to the GAC and sand mixture in 5 gallon buckets, and contents were homogenized prior to addition to bioaugmented reactors (Figure 8). Non-bioaugmented reactors were prepared in a similar fashion, but without the addition of CB1190 culture. This process was repeated 15 times until the reactors were 2/3 full, leaving headspace in 1/3 of the reactor. A peristaltic pump rated between 50-500 mL/min was attached to the influent port of the reactor.

Figure 8: Internal view of reactors before and after filling with the GAC/sand mixture.

Bioaugmented reactors were recirculated prior to operation in order to ensure homogenous and robust CB1190 establishment. This was performed by first connecting the bottom of a recirculation tote to a port at bottom of reactor and then connecting the top of reactor to top of influent tote using silicon tubing. A concentrated liquid nutrient solution (5 gallons), 100 mg/L 1,4-dioxane, added 0.03% hydrogen peroxide were added to the influent tote. This volume was brought up to 50 gallons with tap water. The bioaugmented tanks were recirculated at 450 mL/min using Viton tubing and 1,4-dioxane, DO, and pH were monitored every other day during recirculation. Key parameters were monitored and adjusted if necessary. For example, if 1,4-dioxane fell below 1 mg/L an additional 25 mL of pure 1,4-dioxane was added in two separate pulses (50 mL total), or if 1,4-dioxane degradation appeared to stall, an additional 5 gal of concentrated liquid nutrient solution was added. Oxygen maintenance during recirculation was also critical, and if dissolved oxygen fell below 3 mg/L, an additional 250 mL of 30% hydrogen peroxide was added to the recirculation tank.

Following completion of CB1190 establishment, the recirculation tote was emptied to commence flow through operation. Groundwater from OU11 was collected and used to fill a 275 gallon tote, and this was subsequently covered with a tarp to prevent algal growth. Hydrogen peroxide (0.03%), 5 gallons of liquid nutrient solution, and 1L of diammonium phosphate was added to the tote containing OU11 groundwater. Groundwater was pumped into the influent port at 180 mL/min (5 hr – 7.5 hr HRT) or 45 mL/min (20 hr – 30 hr HRT). Samples were periodically withdrawn from the bottom inlet port and top effluent port daily and analyzed for 1,4-dioxane and CVOCs. Select samples were sent to a third party certified analytical laboratory to support the findings of the primary laboratory (UCLA). Biomass samples (liquid and solid) were collected at specified times to determine biomass abundance on the fixed bed by total nucleic acid extraction and measurement by qPCR (UCLA).

2.5 Analytical Methods

1,4-Dioxane (1-1000 mg/L) was measured by Hewlet Packard 6890 GC-FID containing a Restek® Stabilwax-DB capillary column (30 m x 0.53 mm x 1 µm) as previously described (Zhang et al., 2016). 1,4-Dioxane (5-1000 µg/L) were measured by a Hewlett Packard 6890 gas chromatograph fitted with a mass spectrometer (GC-MS) containing a Restek® Rxi-624Sil MS

GC column (30 m x 0.25 mm id x 1.4 μm) using a previously described method (Johnson et al., 2019). Prior to analysis, 1,4-dioxane samples were extracted into dichloromethane using a previously described frozen microextraction method (Li et al., 2011).

CVOCs were measured using a GC-MS by static headspace analysis. Briefly, 1.6 mL of sample was injected into butyl stopper-sealed, atmospheric pressure-equilibrated amber serum vials with a total sealed volume of 8 mL. The vials were then stored stopper side down in a 60°C water bath and allowed to equilibrate for at least 30 min. Volumes of 250 μL were then taken from the headspace of the vials and then manually injected into a GC-MS splitless injection port. The injection port and mass spectrometer temperatures were held at 120°C and 280°C, respectively. The carrier gas was helium with a flow rate of 3.0 mL/min. The oven program consisted of a starting temperature of 60°C for 1 min, followed by a thermal ramp of 100°C/min for 0.2 min, and then held at 180°C for 1.8 min.

2.6 Biomass Quantification

DNA abundance was used as a surrogate measure for biomass. Total nucleic acids were extracted from liquid or solid samples using a phenol/chloroform/isoamyl extraction method as described by Gedalanga et al. (2014). DNA abundance was measured using quantitative polymerase chain reaction (qPCR) targeting 16S, *dxmBI,* and aldH (Gedalanga et al., 2014).

3.0 RESULTS

The following section describes results collected over the course of the laboratory and field component of this project. Data in this section cover lab treatability results using columns operated in the presence and absence of CB1190, where both 1,4-dioxane and CVOC removal are monitored across three different HRTs. These data are then accompanied by 1,4-dioxane and CVOC removal results from both field scale reactor runs, as well as results from 16S and functional gene analysis.

3.1 Synthetic Groundwater Column Experiments with CVOCs

A mixed-bed CB1190-bioaugmented column experiment was constructed to provide a preliminary evaluation on the effects scaled hydraulic retention times on 1,4-dioxane removal performance. These data may be found in Figure 9. Of the 3 targeted retention times, 10 h had the lowest peak and steady state effluent concentrations (1790 and ~1000 μg/L, respectively). Peak effluent concentrations increased with increasing HRT (3826 and 2892 μg/L for 30 and 20 h HRTs, respectively). Additionally, the time to reach the peak effluent decreased with increasing HRT (10.7, 8.0, and 5.5 BVs for 10, 20, and 30 h HRTs, respectively).

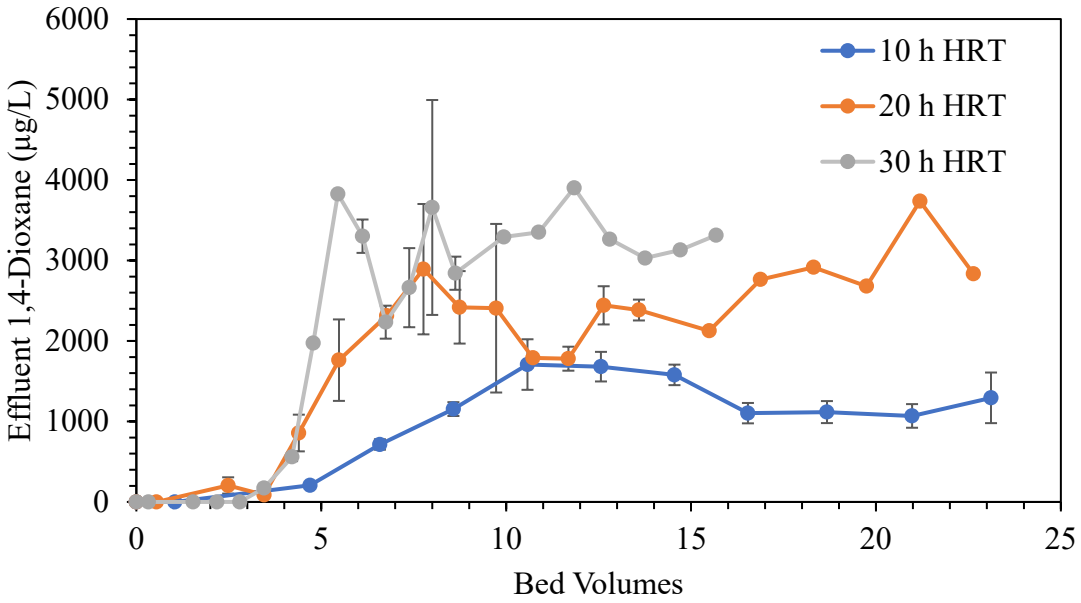


Figure 9: Effects of HRT on both peak 1,4-dioxane breakthrough and steady-state effluent 1,4-dioxane levels.

Small-scale treatability testing offers a cost-effective means to provide critical information with respect to factors affecting performance for eventual process scale up, and following optimization of nutrient and oxygen delivery, a column study similar to that which was previously described was constructed. This study evaluated long term (~100-200 pore volumes) 1,4-dioxane and CVOC removal performance in synthetic groundwater at 10 hr, 20 hr, and 30 hr HRTs. 1,4-dioxane removal data from this study can be visualized in Figures 10-12. Similar to data observed in Figure 9, columns operated at the lowest HRT (10 hrs) showed the greatest sustained removal performance, most notably between 50 and 125 pore volumes (Figure 10). In contrast, cumulative 1,4-dioxane removal for reactors operated at 20 hr and 30 hr HRTs saw diminished performance at 60 pore volumes and 15 pore volumes, respectively (Figures 11 and 12).

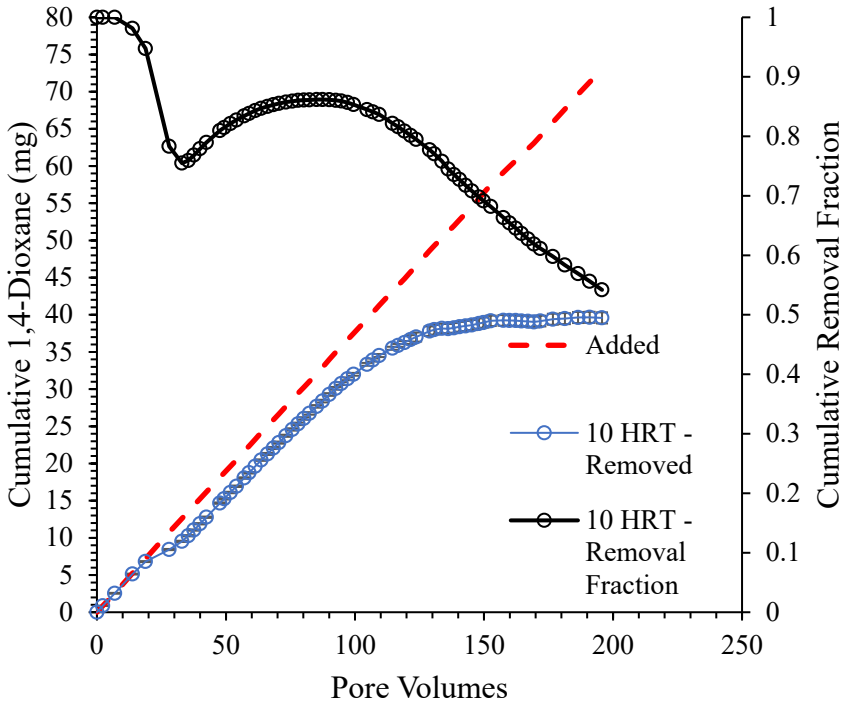


Figure 10: 1,4-dioxane removal in lab scale reactors at a 10 hr HRT

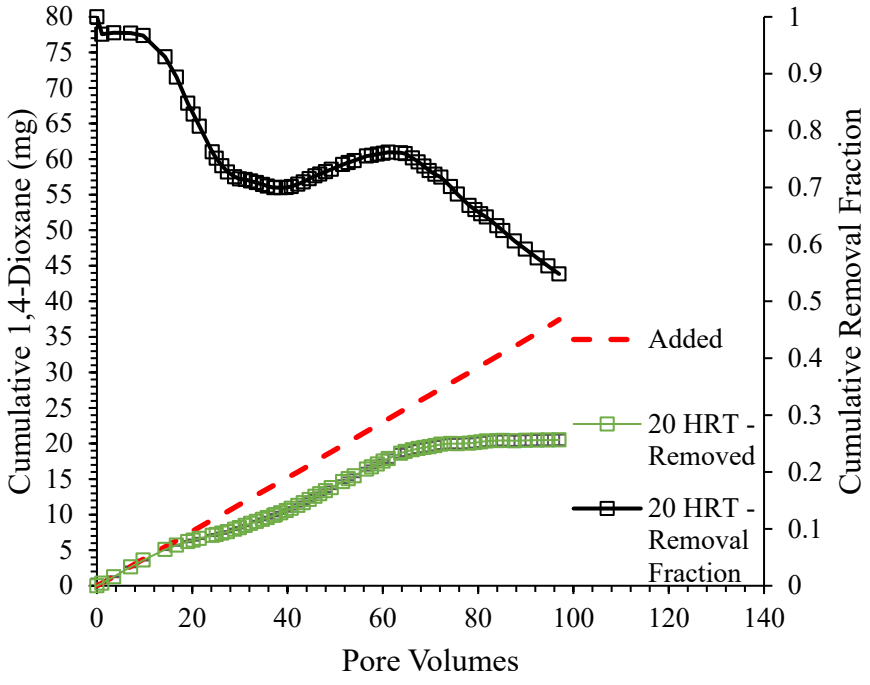


Figure 11: 1,4-dioxane removal in lab scale reactors at 20 hr HRT

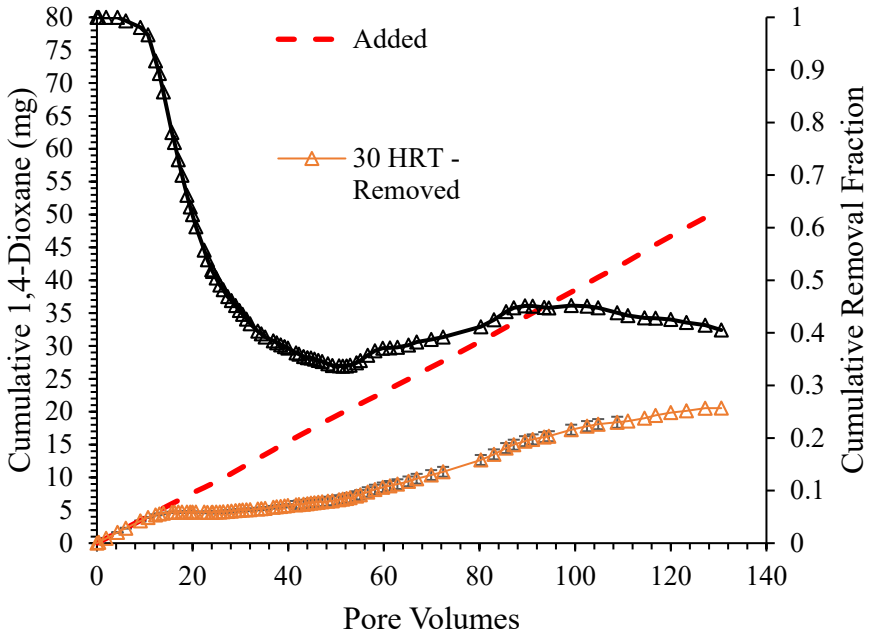


Figure 12: 1,4-dioxane removal in lab scale reactors at 30 hr HRT

While HRT variability appeared to have a considerable effect on 1,4-dioxane degradation, CVOC removal performance remained relatively constant over time across all HRTs tested (Figures 13-15). However, it should be noted that due to the extended operation of the column operated at HRT = 10 hrs, a considerably larger cumulative CVOC mass was removed in comparison to that of reactors operated with 20 and 30 hr HRTs (Figures 13-15).

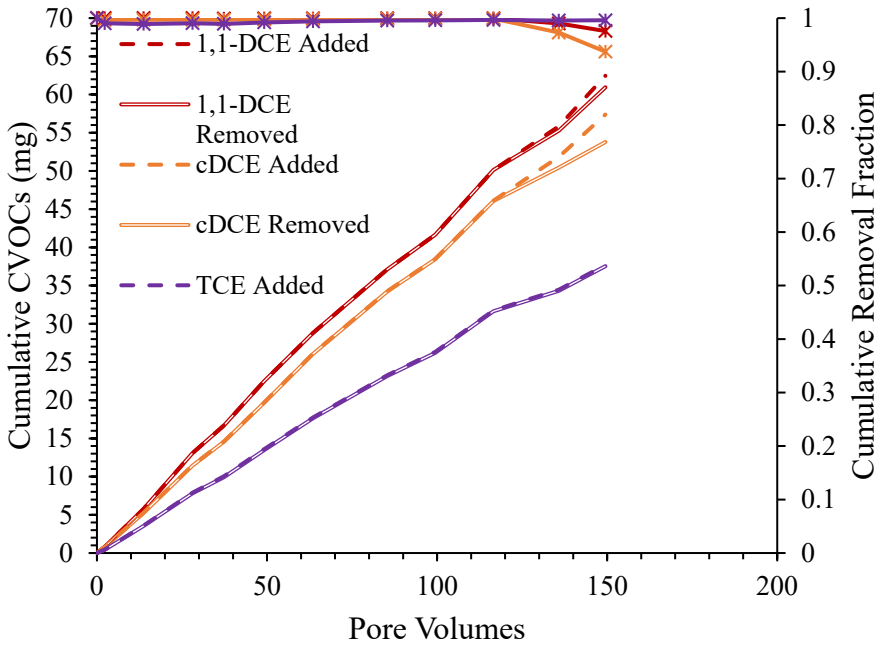


Figure 13: CVOC removal in lab scale reactors at a 10 hr HRT

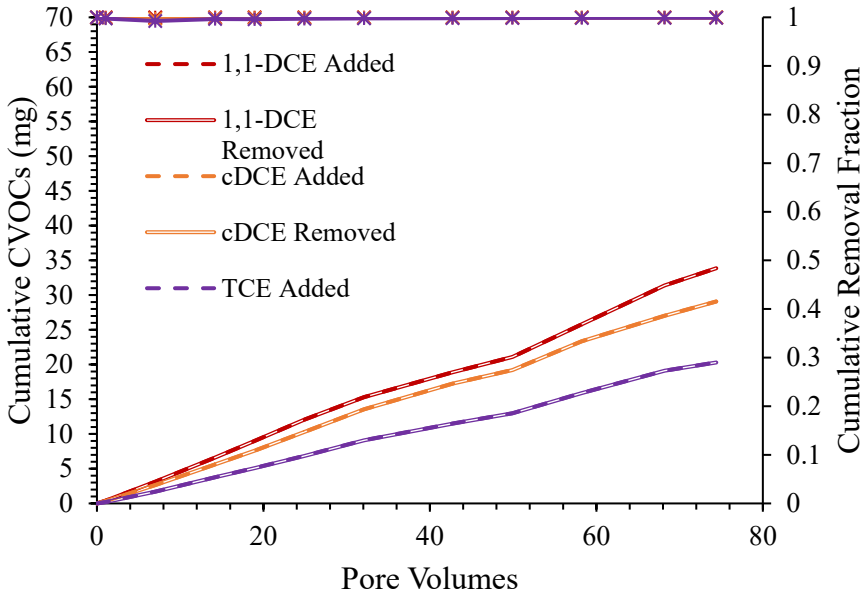


Figure 14: CVOC removal in lab scale reactors at 20 hr HRT

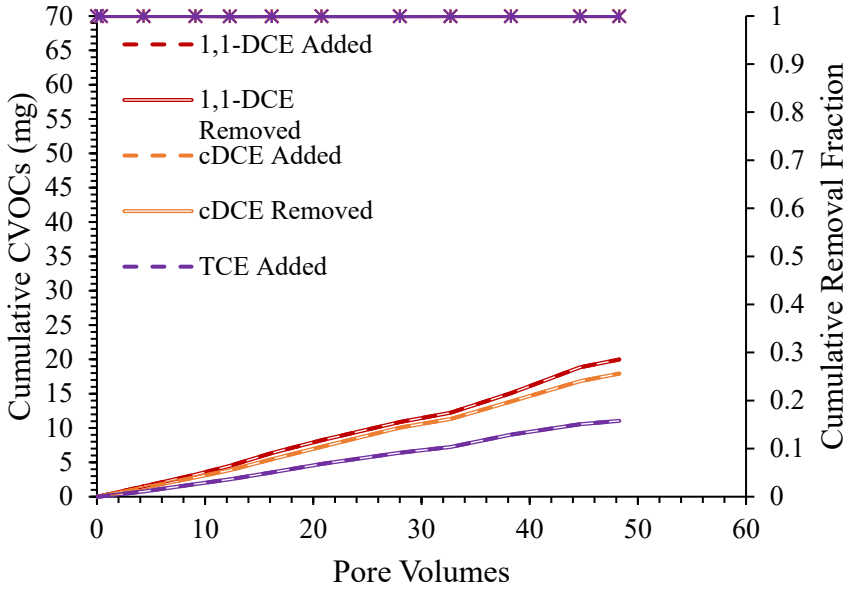


Figure 15: CVOCs removal in lab scale reactors at 30 hr HRT

3.2 Reactor Operation with Site-Derived Groundwater

Two separate reactor studies were constructed at NASNI OU11 to assess the effects of HRT on 1,4-dioxane and CVOC removal using impacted site groundwater. Field scale reactors were operated for up to 90 days, and 1,4-dioxane and CVOC removal data from these studies may be found in Figures 16, 17, 19 and 20 below.

The initial reactor study constructed at OU11 was operated at a 20 hr HRT. The initial dip in fractional removal observed between 0 and 5 days of operation is attributed to a ripening period where CVOCs preferentially sorb to the GAC surface causing residual 1,4-dioxane desorption from the initial recirculation period (Figure 16). The bioaugmented reactor operated at a 20 hr HRT achieved a removal fraction of roughly 80% before performance gradually declined around 14 days of operation, where corresponding data were observed for cumulative 1,4-dioxane removal.

Biomass (16S) analysis of the bioaugmented reactor operated at a 20 hr HRT revealed that total 16S bacterial populations exceed the CB1190 fraction by roughly two orders of magnitude at the top of the reactor, indicating reactor colonization by consortia native to OU11 groundwater (Figure 17). Based off of these data, it was assumed that CB1190 was eventually out competed by groundwater consortia for available nutrients and oxygen, providing a reasonable explanation for decline in reactor performance after 14 days.

Data from this initial reactor study further suggest that two bioaugmented reactors would yield similar levels of 1,4-dioxane removal as three non-bioaugmented reactors, contributing to a 1/3 reduction in GAC costs. Both reactors contained 3.4 kg (7.5 lbs) of GAC.

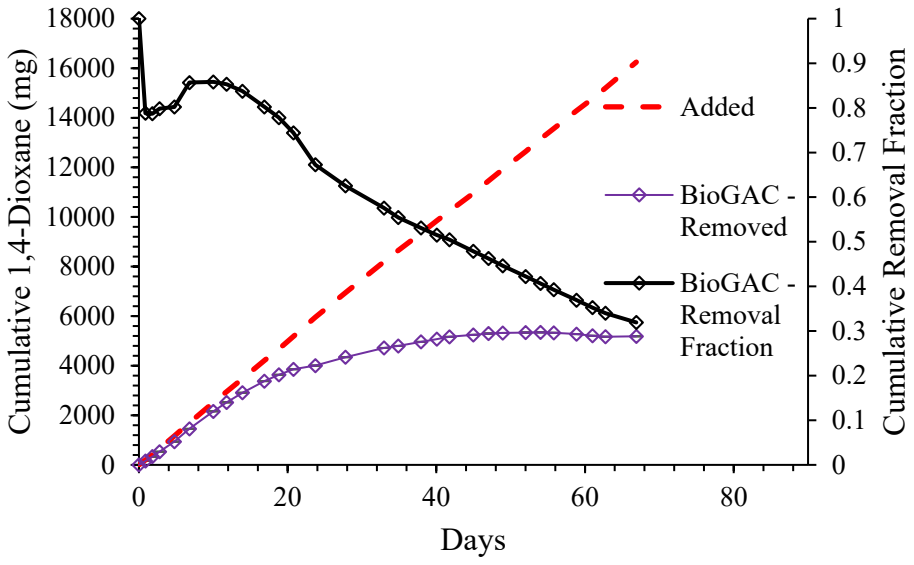


Figure 16: BioGAC reactor trial #1 – 1,4-dioxane removal at HRT of 20 hrs

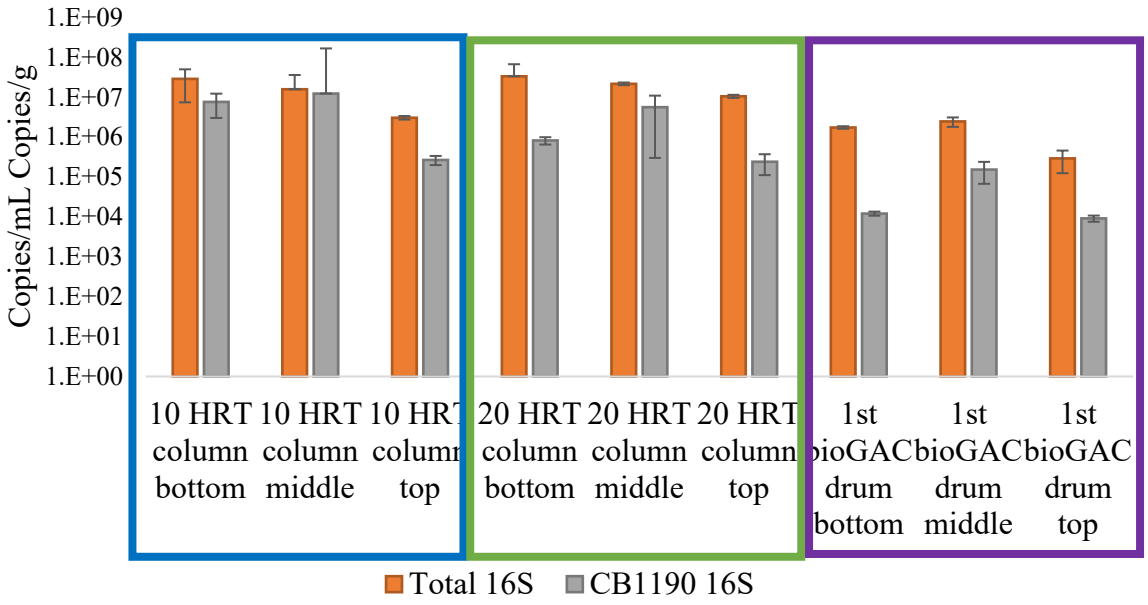


Figure 17: Attached biomass in CB1190-GAC lab scale and field scale reactors

CVOC removal performance remained consistent throughout reactor operation at a 20 hr HRT over the course of 20 days of analysis. Complete removal of all CVOCs present in groundwater was observed, where nearly 5 grams cumulative CVOCs were removed after 20 days of operation (Figure 18).

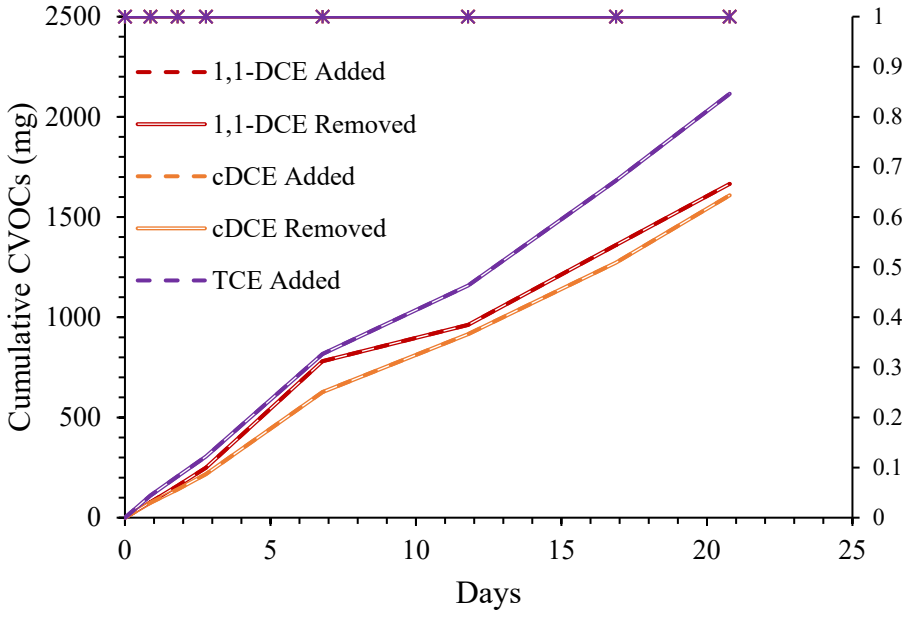


Figure 18: CVOC removal in BioGAC reactor trial 1.

Data from the laboratory scale column reactors suggested that a shorter 10 hr HRT yields higher removal of 1,4-dioxane on a percentage and mass basis, implicating the possibility of faster remediation than previously expected (Figure 19). Consistent 1,4-dioxane removal over a greater

timeframe was observed in reactors operated at 10 hr HRTs in comparison to those operated at 20 and 30 hr HRTs, indicating the possibility for extended reactor operation without any sharp decline in performance. Naturally, these observations led to the construction of field scale reactors operated at a 10 hr HRT.

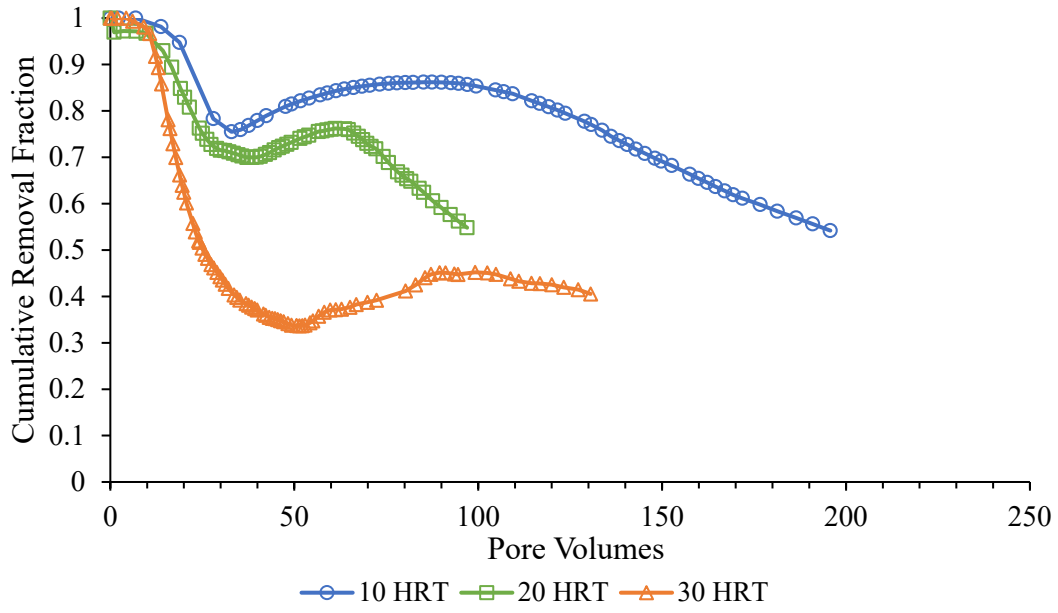


Figure 19: Dioxane removal fraction – lab scale reactor performance at varying HRTs

Results from the bioaugmented reactor operated at a 10 hr HRT are shown in Figure 20. Total reactor operational duration was 90 days, and similar to the laboratory scale results observed in Figure 18, consistent 1,4-dioxane removal performance was observed over an extended period of operation. Roughly 90-100% 1,4-dioxane contained in NASNI OU-11 groundwater was removed over 60 days of operation, following the reactor start up period (Figure 20). To the best of our knowledge, this is the first instance of both high and consistent metabolism of 1,4-dioxane in a bioreactor system. Further studies to optimize 1,4-dioxane feeding cycles during initial recirculation can help minimize this ripening stage, increase fractional removal, improve reactor operation, and decrease GAC costs.

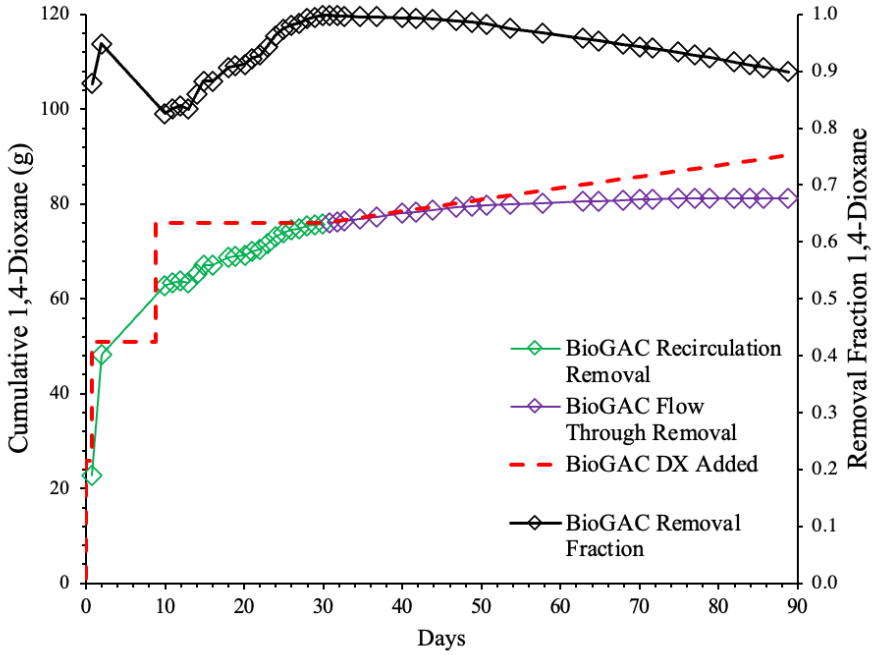


Figure 20: BioGAC reactor trial #2 – dioxane removal at HRT of 10 hrs

CVOC data for the 10 hr HRT BioGAC reactor are listed in Figure 21. These data indicate nearly complete disappearance of TCE, with roughly 55% and 70% of 1,1-DCE and cis-DCE removed, respectively.

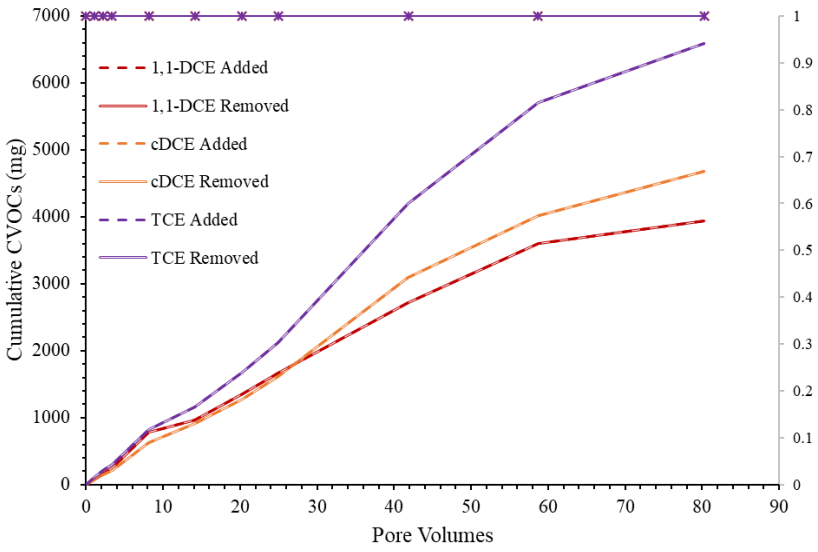


Figure 21: CVOC removal in BioGAC reactor at HRT of 10 hrs

3.3 Microbial Assessment of Native 1,4-Dioxane Degrading Capacity at OU11

Functional and 16S rRNA genes derived from NASNI OU11 groundwater (reactor influent feed) were quantified to better understand 1,4-dioxane removal results observed in non-bioaugmented

reactors operated at a 20 hr HRT. The 20 hr HRT reactor pair was operated at a rate of 120 gal/wk, and roughly 50% more 1,4-dioxane was removed in comparison to the non-bioaugmented control (Figure 22). Theoretical 1,4-dioxane removal in laboratory-scale reactors were estimated to remove 2 g of 1,4-dioxane while the field scale, non-bioaugmented reactor removed a total of 3.5 g 1,4-dioxane, indicating the potential for 1,4-dioxane degradation in the presence of native groundwater consortia.

DNA extraction and amplification of 16S and functional genes related to 1,4-dioxane degrading populations (e.g. dxmB and aldH) from NASNI OU11 groundwater indicated the presence of CB1190-like organisms that may potentially degrade 1,4-dioxane (Figure 23). Both 1,4-dioxane removal data in the non-bioaugmented controls and the relatively high occurrence of biomarkers associated with microbial 1,4-dioxane degradation support the potential enrichment of 1,4-dioxane degrading microbial communities over decades of 1,4-dioxane exposure at OU11, and this was not made apparent until optimal/controlled bioreactor conditions were applied in this study.

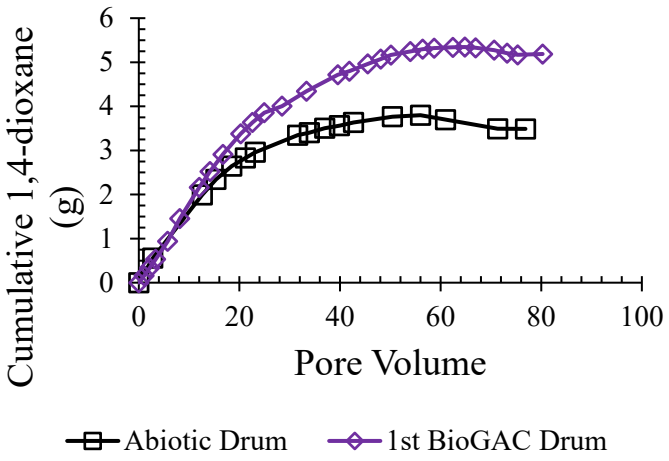


Figure 22: Comparison of BioGAC and non-bioaugmented reactor performance (HRT = 20)

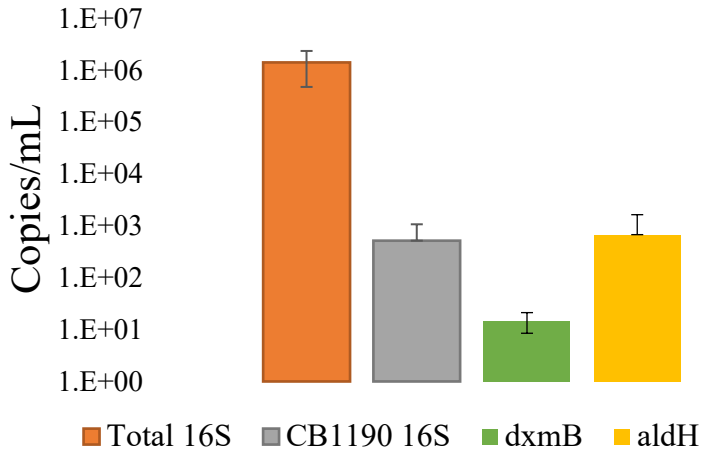


Figure 23: NASNI OU11-10 groundwater biomarkers

4.0 COST ANALYSIS

This study applied cost data from a small-scale point of entry (POE) system in McAdenville, North Carolina where groundwater 1,4-dioxane concentrations ranged from 12.5 to 16 µg/L. The average flow rate used in this calculation was 200 gallons per day. Upfront capital costs were kept the same for both treatment systems to reflect long term returns on operational costs. Steady state removal conditions were assumed for the bioreactor system operating at HRT = 10. Assumptions for the bioaugmented reactors can be found in Table 2 below. It was assumed that GAC replacement costs were factored into annual operational costs ($11,120) for the existing POE system; annual disposal costs for the existing GAC treatment system (500 lbs) were added to estimated operational costs classified as RCRA waste disposal (~$120/wet ton). Process costs were scaled over 30 years of treatment life cycle at 50 potential sites.

Table 2: Cost breakdown of potential capital and operational costs for the bioaugmented treatment system. For the purpose of this analysis, this effort used similar capital costs described in a 2015 North Carolina Department of Environmental Quality report.

Capital vs. Operational Costs	Item	Unit Cost	Cost per Gallon GW	Cost per Year	Rationale
Capital	GAC (1.5% w/w; @ ρ ~ 0.48 g/mL)	$45/ft^3	N/A	N/A	Filter media changeout will disrupt degrading microbial population
Capital	Coarse Sand (97% w/w)	$9.91/ft^3	N/A	N/A	Filter media changeout will disrupt degrading microbial population
Operational	Monoammonium Phosphate	$3.20/lb	$0.00213	$155.60	Nutrient solution constituent
Operational	Potassium Phosphate Monobasic	$2.70/lb	$0.00225	$164.36	Nutrient solution constituent
Operational	Hydrogen Peroxide (12% Stock)	$30/gal	$0.00025	$18.26	Oxygen release compound

A cost analysis tool provided by the Navy Environmental Sustainability Development to Integration program was used to better understand total savings throughout the lifecycle of new technology implementation versus existing GAC pump and treat systems. Previously listed considerations were used for inputs for this analysis. Cost data from these calculations can be found in Tables 3-6 below.

Table 3: Per unit cost totals

Process	Yearly $K	One Time $K
Existing	11.2	22.0
New	0.3	22.0

Table 4: Per unit cost totals (unit present value)

Process	Unit Present Value $K
Existing	**250.7**
New	**28.9**

Table 5: Extended RDTE CER and extended present value

Extended RDTE Cost Effectiveness Ratio	Extended Present Value of Savings $K
16.11	9990.3
RDTE CER ≥ 1 indicates cost savings surpass RDTE cost, but greater indicates increased effectiveness.	Total cost savings over the process life for immediate integration is adjusted into present value and multiplied by the number of units to reflect total DoD-wide savings.

Table 6: Savings to investment ratio (per unit)

Unit: Savings-to-Investment Ratio
10.08
SIR is the ratio of operating savings (adjusted to present value) to the increase in capital cost.

5.0 CONCLUSIONS AND RECOMMENDATIONS

For well over a century, humankind has made great strides in harnessing biological systems for treating large volumes of carbon and nutrient impacted waters, ultimately curtailing several public health and environmental concerns in developed countries where water and wastewater treatment infrastructure are readily available. However, as anthropogenic environmental impacts become increasingly complex, novel treatment strategies will require development to better adapt to some of the more recalcitrant chemicals found in water on a global scale.

Up until this last decade, treatment of groundwater containing 1,4-dioxane alone was considered improbable, and the co-occurrence of 1,4-dioxane and CVOCs in groundwater is a relatively new phenomenon with which practitioners and researchers alike have only recently become acquainted. Present strategies to address the mitigation of these chemicals often involves energy

and/or cost intensive processes due to1,4-dioxane and CVOC chemical characteristics – efforts conducted under this project sought to significantly lower these costs, as well as enable more seamless technology integration into existing pump and treat systems. This study further documented the first instance where direct metabolic treatment of comingled 1,4-dioxane and CVOCs was applied in hybrid biological/physicochemical, field-scale bioreactors.

Several valuable lessons were garnered from both laboratory optimization studies and the field demonstration, and these are listed in Table 7 below. One of the most impactful findings from this study was that increasing bioreactor processivity alone (HRT = 10 hrs vs 20 and 30 hrs) enhanced 1,4-dioxane degradation capacity to nearly 90-100% during steady state operation. Poor 1,4-dioxane and CVOC removal was observed at higher HRTs, and this is likely attributed to overgrowth and nutrient competition from microbial consortia native to site groundwater. Therefore, lowering the HRT improved both CB1190 1,4-dioxane degradation kinetics and created unfavorable growth conditions for competing microorganisms.

Table 7: Guidance for constructing and operating BioGAC reactors for combined 1,4-dioxane and CVOC treatment.

Considerations	*Solutions*	*Reasoning*
For ideal reactor operation, the sand + GAC bed must be uniformly mixed	Mix sand, GAC, & bacterial culture in small batches in 5 gal buckets and add to reactor	To avoid short circuiting and dead zones within the reactor. Discrete bed layers are not recommended.
Maintaining a steady DO ~5 mg/L ensures presence of oxygen in our reactor for steady microbial utilization and 1,4-dioxane removal	Provide dissolved oxygen via dilute additions of hydrogen peroxide (0.03% for recirculation and 0.0045% for flow through)	Other oxygen delivery methods such as air sparging will volatilize CVOCs and may fluidize the bed. ORCs elevate pH.
Faster 1,4-dioxane removal will result in faster oxygen utilization that can lead to anoxic conditions	Increase dosing of hydrogen peroxide to provide more dissolved oxygen (DO)	Maintaining a steady DO ~5 mg/L will ensure presence of oxygen in our reactor for steady microbial utilization and 1,4-dioxane removal
Inadequate nutrient dosing limits the rate and extent of 1,4-dioxane mass removal	Increase dosing of liquid nutrient and add in diammonium phosphate	More bioavailable nutrients will increase bacterial population and metabolic activities, yielding more 1,4-dioxane destruction
Growth of indigenous microbes and algae decrease pH during recirculation	Use a leaner, more selective liquid nutrient	A more selective media would allow our bacterial culture to thrive without significant competition from indigenous microbes
Supply of gaseous primary substrate to induce co-metabolism of 1,4-dioxane can sorb to GAC	Pulse input of primary substrate	Sorption will be limited. Reduce competition and improve 1,4-dioxane degradation kinetics
Cold temperatures (<14°C, 57° F) slows 1,4-dioxane degradation	House bioGAC reactors inside building or shed to provide warmer, more consistent temperatures	Warmer temperatures are preferred by our bacterial culture. More consistent temperatures allow for a stable environment for the bacterial culture to degrade 1,4-dioxane

Sunlight causes algae bloom during recirculation which will compete with our bacterial culture	Cover influent tote with tarp to prevent algal growth	Absence of light will minimize algal growth
Microbial population and activity is affected by groundwater biogeochemistry	Preliminary feasibility studies should be conducted with site specific groundwater prior to reactor setup	Microbes for bioremediation may be affected by groundwater characteristics and native bacteria
1,4-dioxane may initially leach from the GAC surface when switching from recirculation to flow through	Further optimization of 1,4-dioxane feeding cycles during recirculation may depress GAC's "ripening period" to yield better reactor performance	Sorption – desorption equilibrium will shift when changing flow regime
Bioaugmented culture may possibly outcompeted by the indigenous microbiome during recirculation	Increasing the volume or density of culture to be bioaugmented will improve reactor performance	Biodegradation rates will depend on the relative abundance of bioaugmented culture with respect to total microbial population

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APPENDIX A: Contact Information

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APPENDIX B: Supplementary Information

Bioaugmented lab scale reactors removed 500% more 1,4-dioxane than abiotic controls. Abiotic laboratory controls used sterile reactors with sterile influent filtered through a 0.2 um nylon filter.

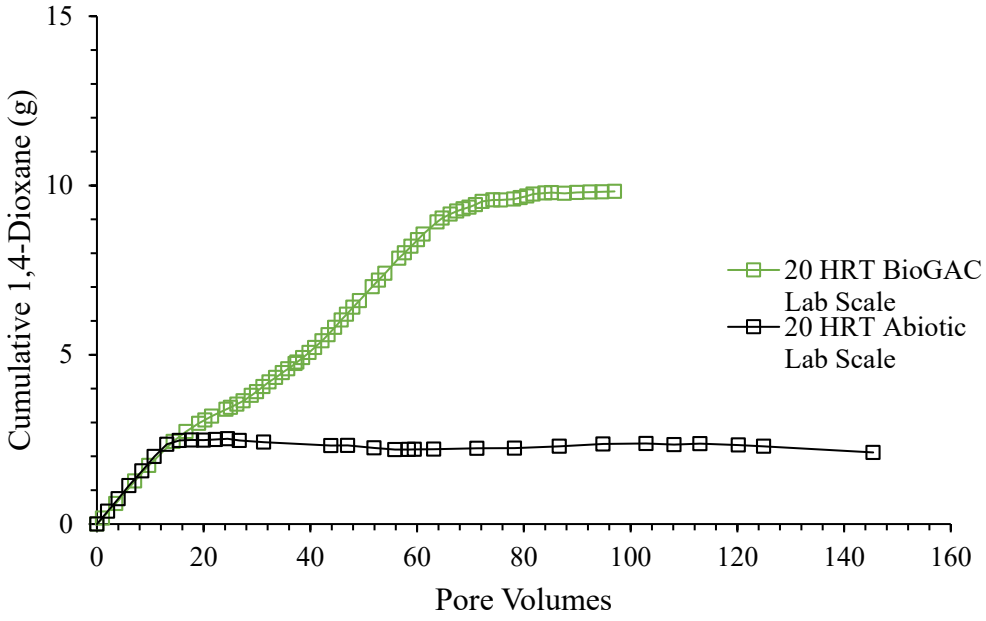


Figure B1: Comparison of BioGAC and abiotic lab scale reactor performance at a 20 hr HRT

Instructions for Reactor Preparation and Startup

Constructing Gravity Fed Reactor

1. Drill holes to install sampling ports (1/6, 1/3, 1/2, & 2/3 from bottom of reactor). Drill hole to install influent sampling port at bottom of reactor. To preserve reactor rigidity and integrity, holes should not be drilled directly on top of the other.
2. Install sampling ports
3. Leak test reactor
4. Elevate reactor 2 feet above influent tote to ensure sufficient head
5. Obtain peristaltic pump rated between 50 mL/min to 500 mL/min

Packing Reactor (2/3 solid media + 1/3 headspace)

To ensure homogenous mixture of sand, GAC, and bacterial culture, mixing will be done in small batches with at least 5 gal of bacterial culture at 10^6 CB1190 16S rRNA copies / mL

1. Weigh out GAC: 0.21 kg, 0.46 lbs (~400 mL)
2. Weigh out Sand: 18.6 kg, 41 lbs
3. Add GAC + sand into 5 gal bucket and pour in bacterial culture
4. Mix thoroughly and pour into reactor
5. Repeat 15 times

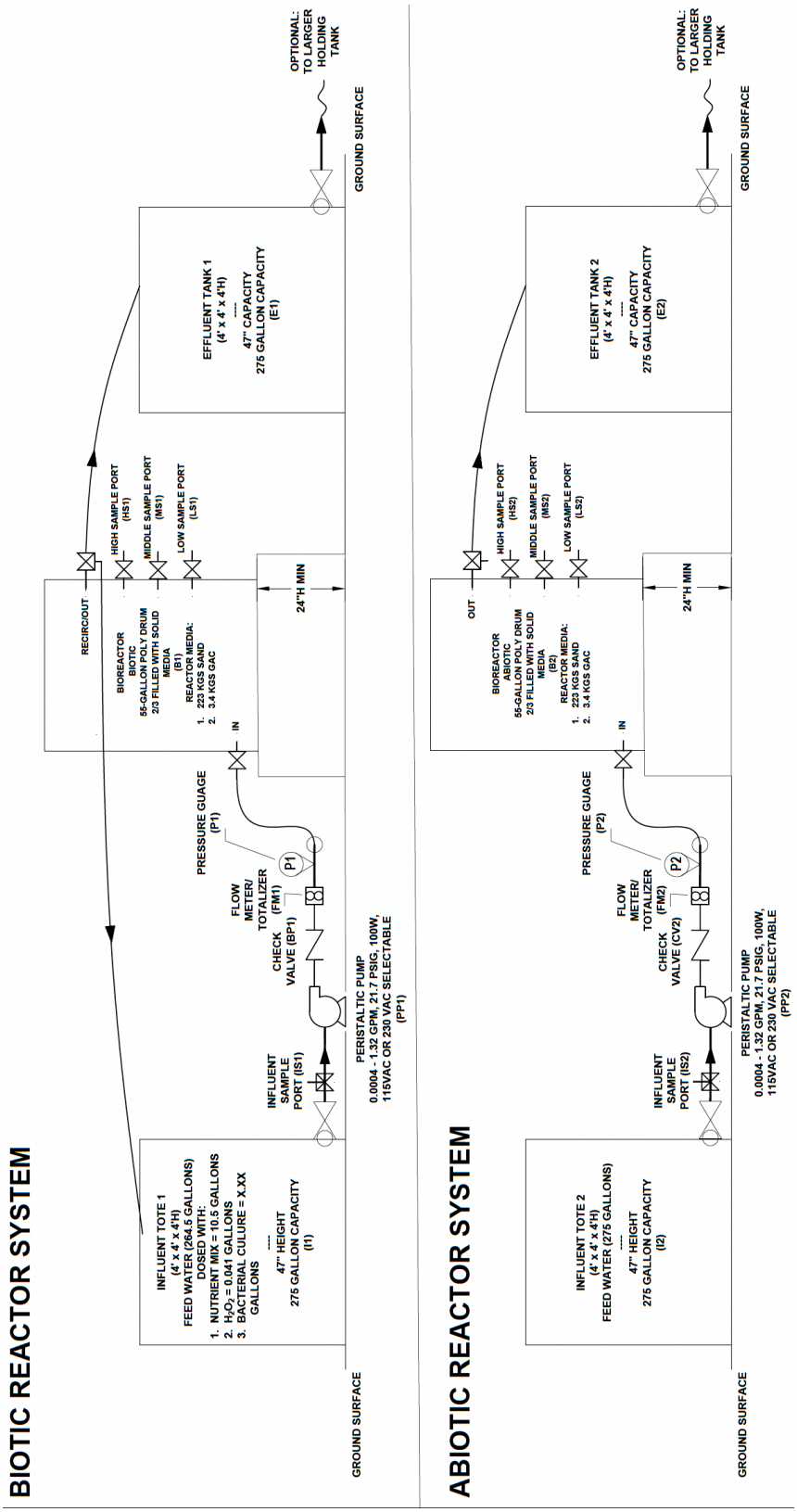
Recirculation Mode of Operation (Bioaugmented Reactor Only)

1. Connect bottom of recirculation tote to port at bottom of reactor using silicone tubing
2. Connect top of reactor to top of influent tote using silicon tubing
3. Add in 5 gal of concentrated liquid nutrient
4. Add in 25 mL of pure 1,4-dioxane or 250 mL of 10% 1,4-dioxane (100 mg/L final concentration)
5. Add in 250 mL of 30% hydrogen peroxide (0.03% final concentration)
6. Fill tote to 50 gal mark with tap or hydrant water
7. Operate recirculation at 450 mL/min, 0.12 gpm using Viton pump tubing
8. Take liquid samples every other day from any sampling port. Measure for 1,4-dioxane. Measure for dissolved oxygen. Measure for pH.
    a. If 1,4-dioxane is below 1 mg/L
    → add in another 25 mL of pure 1,4-dioxane, repeat 2 times
    b. If 1,4-dioxane concentrations stall
    → add in another 5 gal of concentrated liquid nutrient
    c. If dissolved oxygen is below 3 mg/L
    → add in another 250 mL of 30% hydrogen peroxide
9. Empty out recirculation tote in preparation for switching to flow through

Reactor: Flow Through Mode of Operation

1. Fill 275 gal tote with groundwater

2. Cover tote with tarp to prevent algal bloom
3. Immediately before starting recirculation, add the following to the influent tote:
    a. 150 mL of 30% hydrogen peroxide per 275 gal of formation water (or 75 mL per 137 gal)
    b. 5 gal of concentrated liquid nutrient per 275 gal of formation water (or 2.5 gal per 137 gal)
    c. 1 L of 222 g/L diammonium phosphate (DAP) per 275 gal of formation water (or 0.5 L per 137 gal)
4. Operate reactor at 180 mL/min (5 hr – 7.5 hr HRT) or 45 mL/min (20 hr – 30 hr HRT). At 180 mL/min, each tote will last 4 days. At 45 mL/min, each tote will last 16 days (2 weeks)
5. Sample from bottom inlet port and top effluent port daily and analyze for 1,4-dioxane



Process flow diagram prepared by NOREAS.inc