Treatment of 1,4-Dioxane and cVOCs Using an Isobutane-Fed Fluidized Bed Bioreactor: Bench Studies

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Project Team

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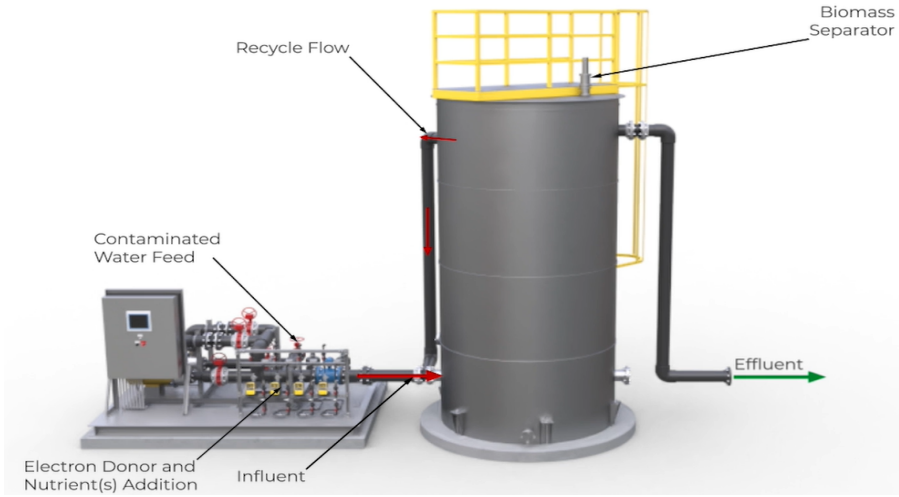
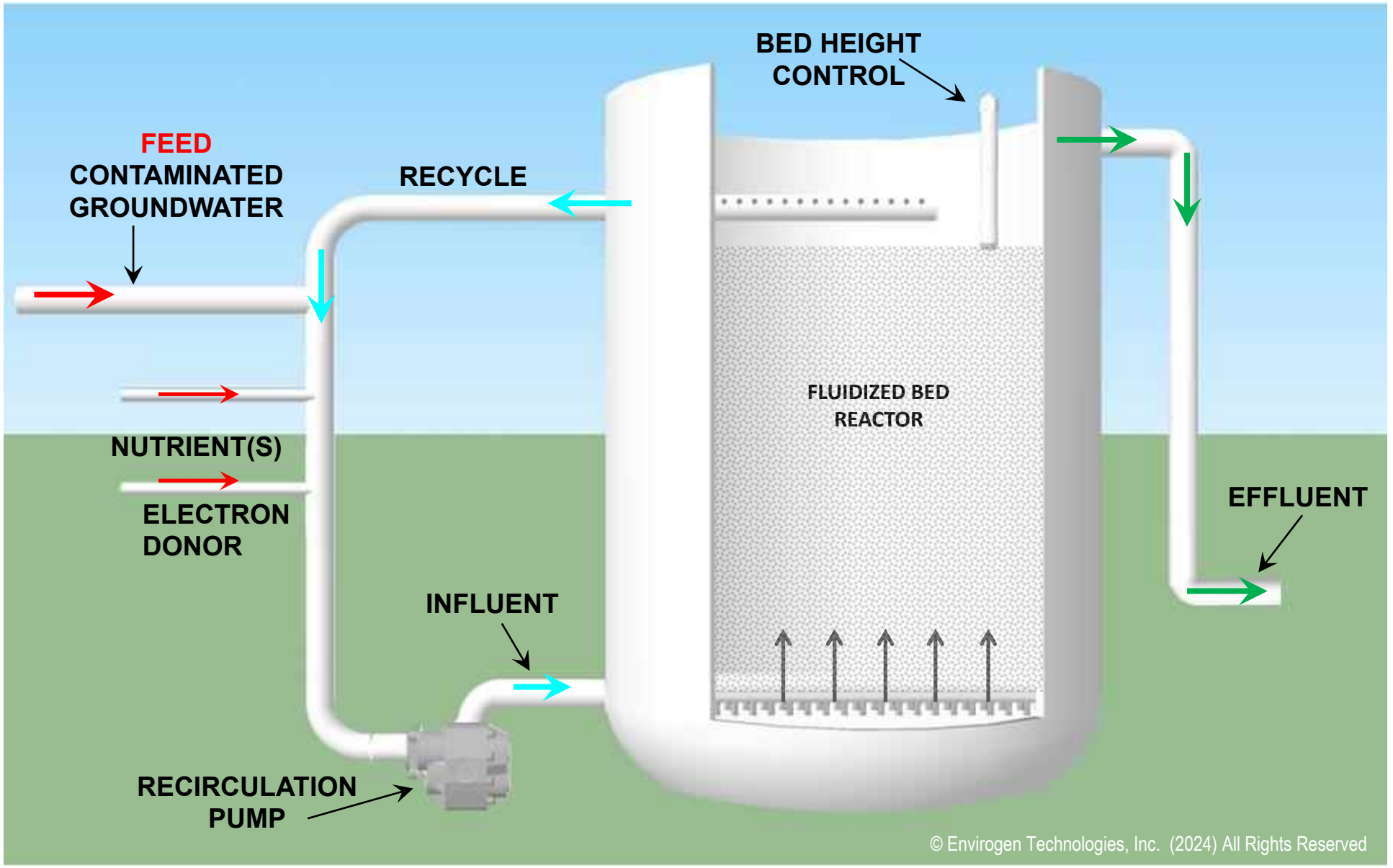
ESTCP PROJECT # ER22-7543

Technical Objectives

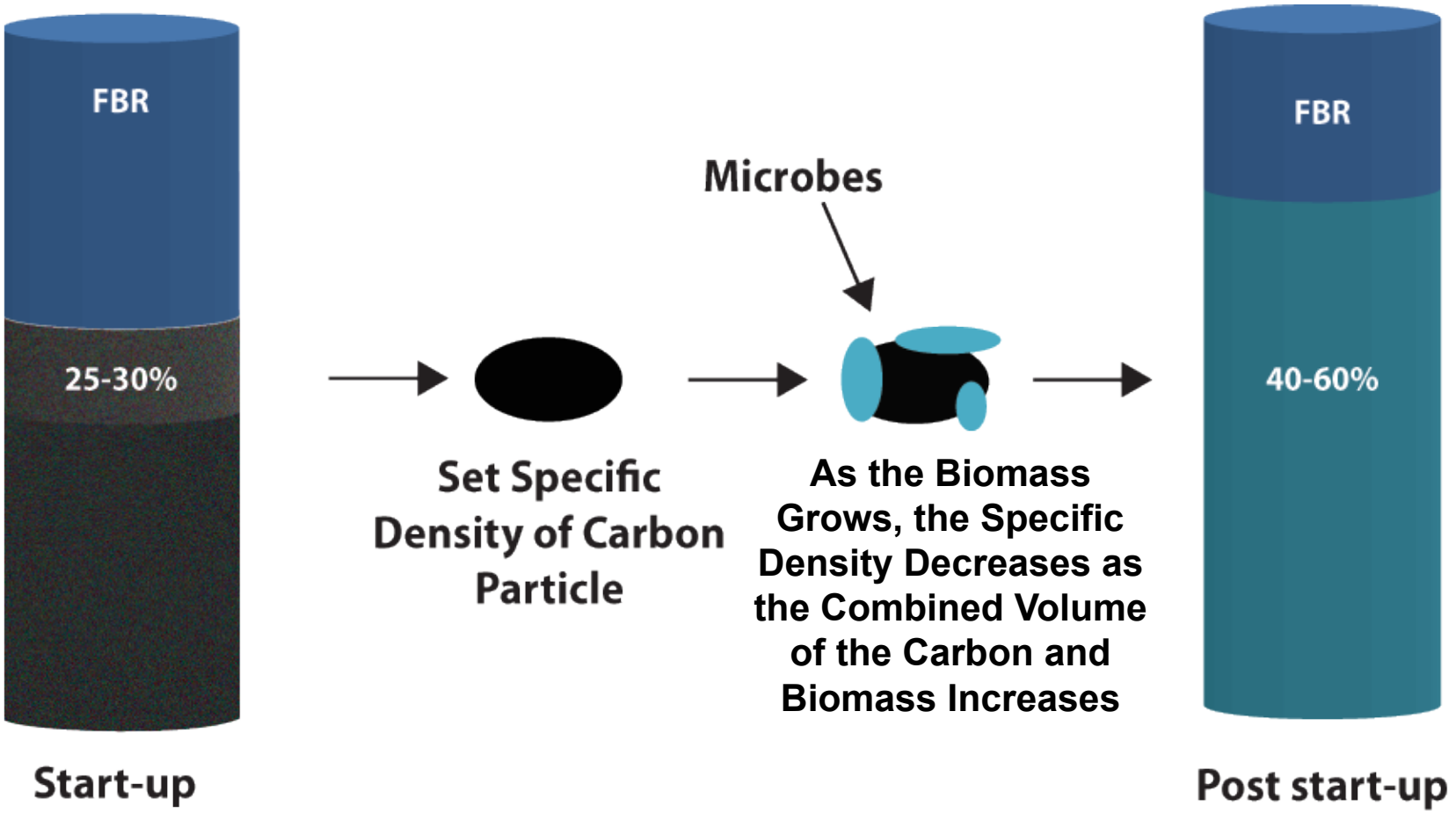
- To demonstrate and validate the performance of an isobutane-fed biological fluidized bed reactor (FBR) for the treatment of 1,4-Dioxane and cVOCs in groundwater.
  - Seed with a known isobutane degrading organism with co-metabolism capabilities of 1,4-D and cVOCs.
  - Treatment of 1,4-D to > 90+% and cVOCs to relevant MCLs
  - 60-70 minute Hydraulic Residence Time (HRT)
  - Demonstrate system performance, robustness and reliability
- Develop costing and design for a pilot-scale and full-scale FBR for simultaneous treatment of 1,4-D and cVOCs.
  - System size
  - Chemical usage
  - Operations and maintenance requirements



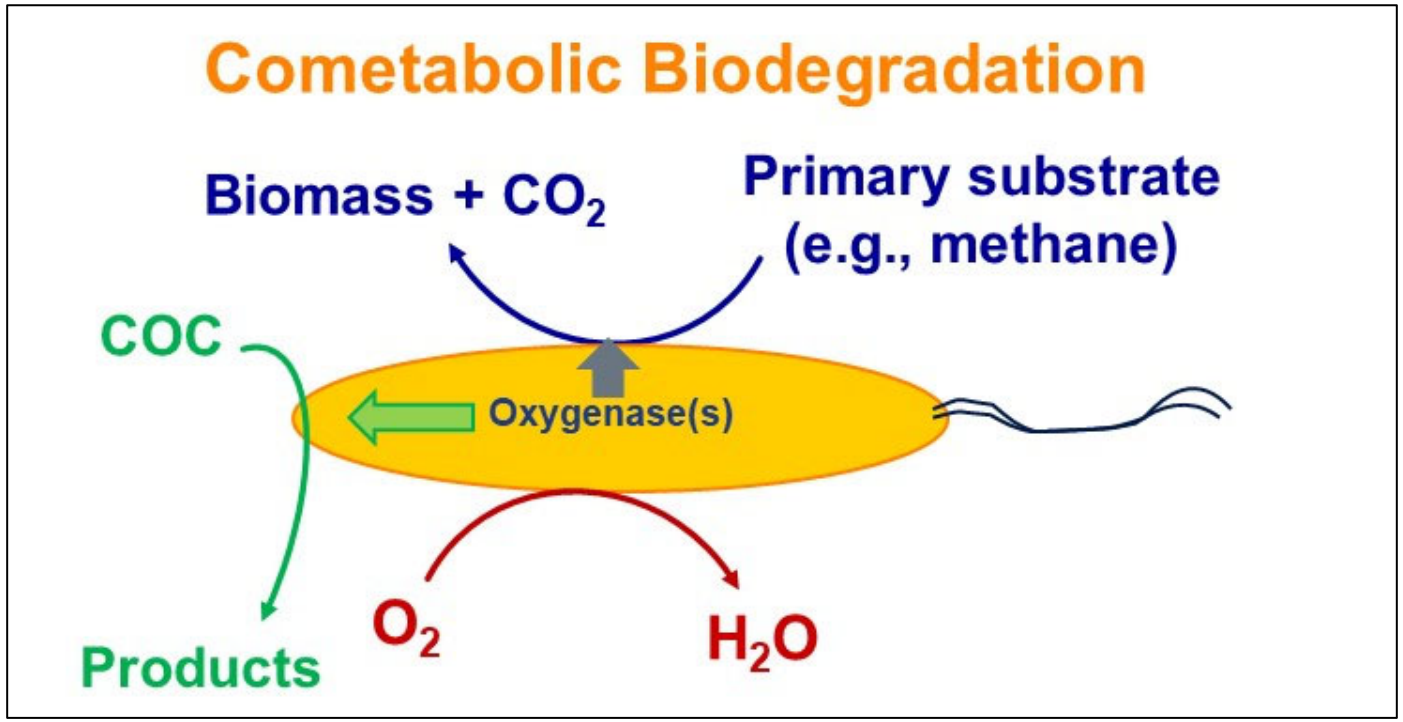
How the FBR Works



Fluidization of Media

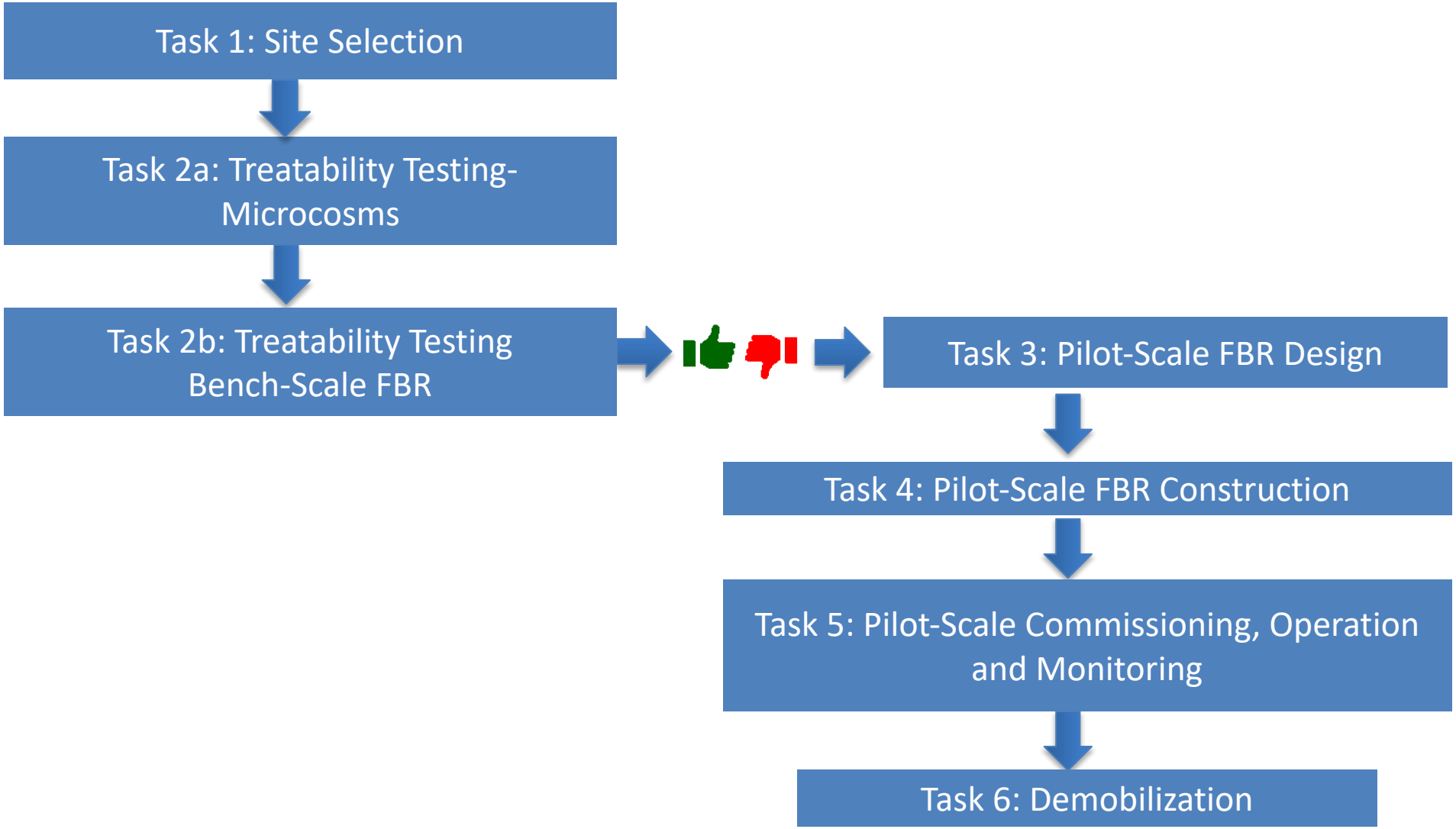


Biotreatment Approach



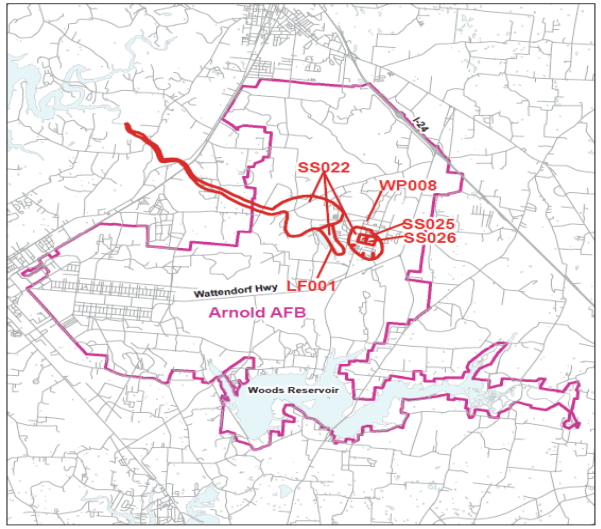
- Indigenous organisms grow aerobically on a supplied co-substrate
- These organisms have the ability to degrade numerous emerging contaminants and cVOCs
- Production of Short Chain Alkane Monooxygenases (SCAM) for 1,4-D and cVOC treatment
- Ability to treat contaminants to very low levels (low ppb achieved)

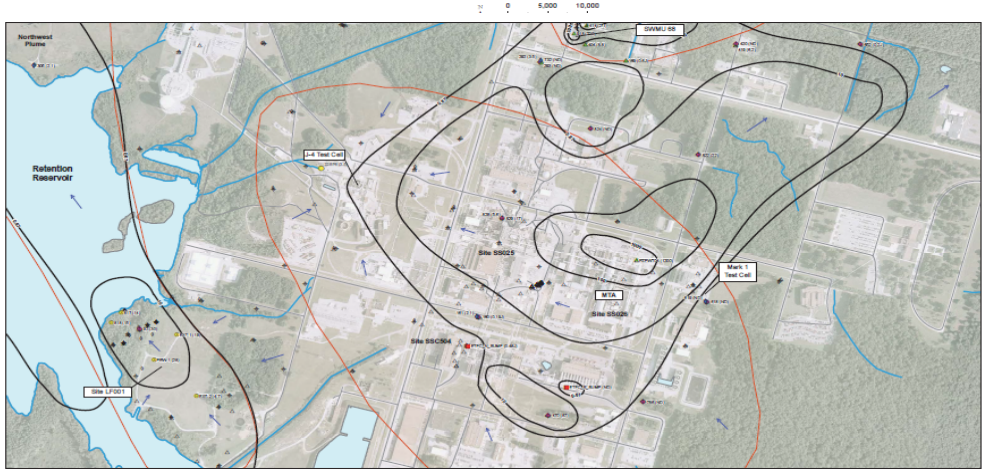
Test Design Overview



Site Selection-Arnold Air Force Base SS022

Parameter	Arnold Air Force Base (SS022)
Groundwater chemistry conducive to biotreatment	pH= 4.69-.6.49 Dis. Oxygen >3.54 mg/L ORP > 278 mV Temp.> 16 °C
1,4-D concentration	4.7-28 µg/L
Presence of cVOCs at low levels	DCA < 1 µg/L 1,1-DCE < 1 µg/L cis-DCE =1.2 µg/L PCE = 7.7 µg/L TCE = 55 µg/L VC < 1 µg/L
Basic infrastructure	Yes
On-site support of demonstration	Yes





Existing Air Stripper

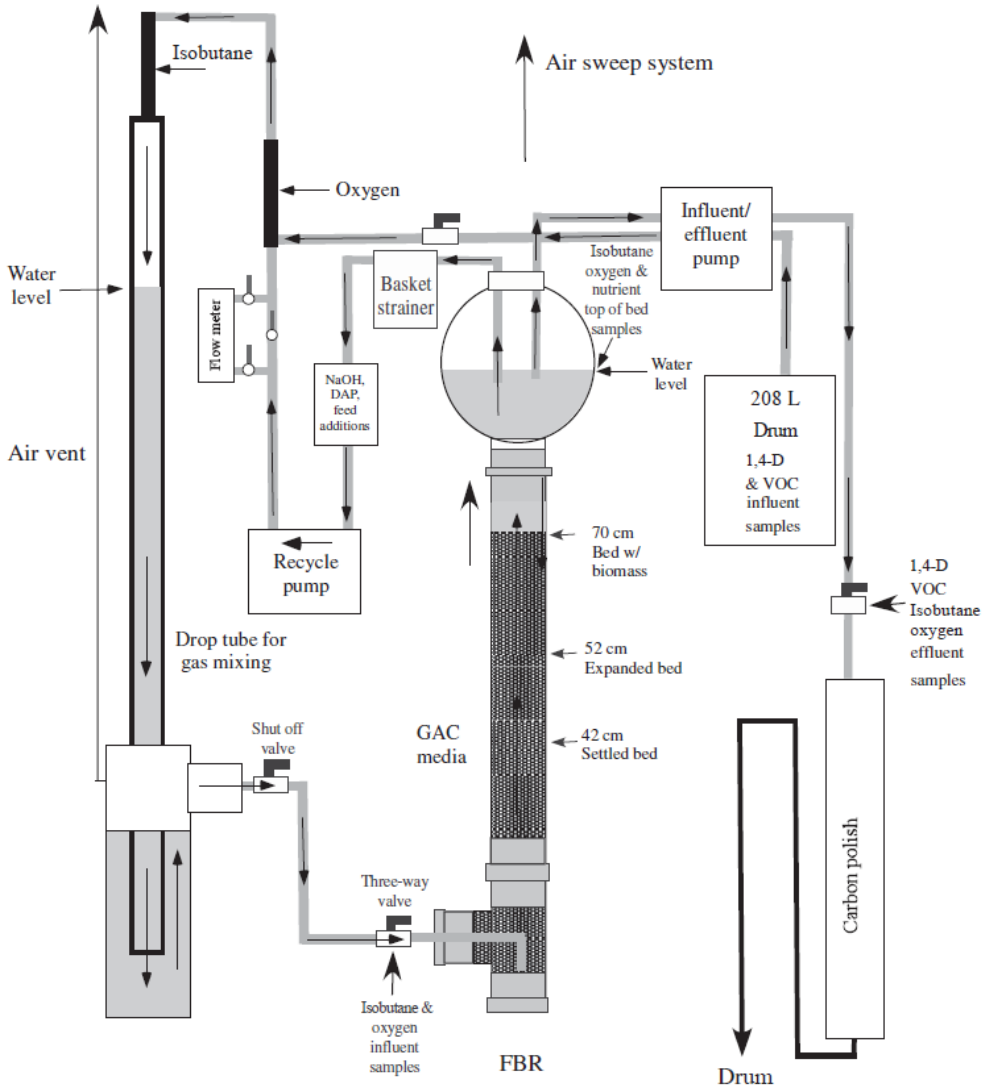
1,4-Dioxane Characterization Results for Site SS022 (CH2M HILL, 2017).

Treatability Testing-Task 2a Microcosms

- Task 2a-Microcosms
  - Confirm that isobutane-degrading strains are capable of degrading mixed cVOCs and 1,4-D in site water
  - Confirm low levels (MCLs) achievable
  - Evaluate options to optimize degradation rates
    - Stimulation of multiple oxygenases
    - Multiple cometabolic organisms/substrates
  - Design
    - Small and large volume batch studies

Treatability Testing-Task 2b Bench-Scale FBR

- **Task 2b- Bench-Scale FBR Testing**
  - 9-month duration
  - Bench-scale FBR designed and installed
    - GAC media used
    - Isobutane mass flow controller system utilized
  - **Phase I: Initial loading with 1,4-D for abiotic removal**
  - **Phase II: Inoculation/Recycle**
  - Use of simulated and actual site water
    - **Phase III: 1,4-D treatment alone (30 ppb)**
    - **Phase IV: 1,4-D and cVOCs together (TCE at 40 ppb and PCE at 10 ppb)**
    - **Phase V: Site water with 1,4-D/cVOCs**
  - 1,4-D, cVOCs-EPA 8260 and 8270-SIM ID
  - Go/No Go decision to the pilot study
    - ≤ 60-70 minute HRT
    - Treatment of 1,4-Dioxane and cVOCs >80-90+%



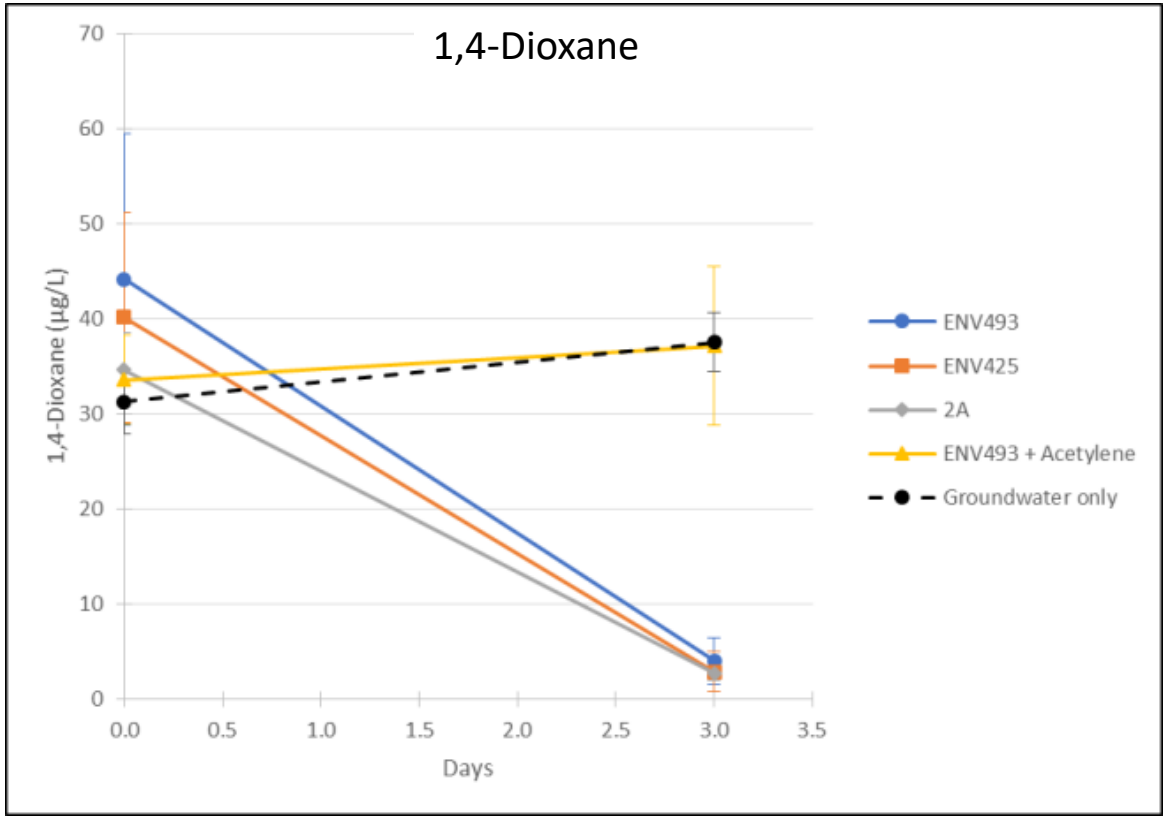
Performance Assessment

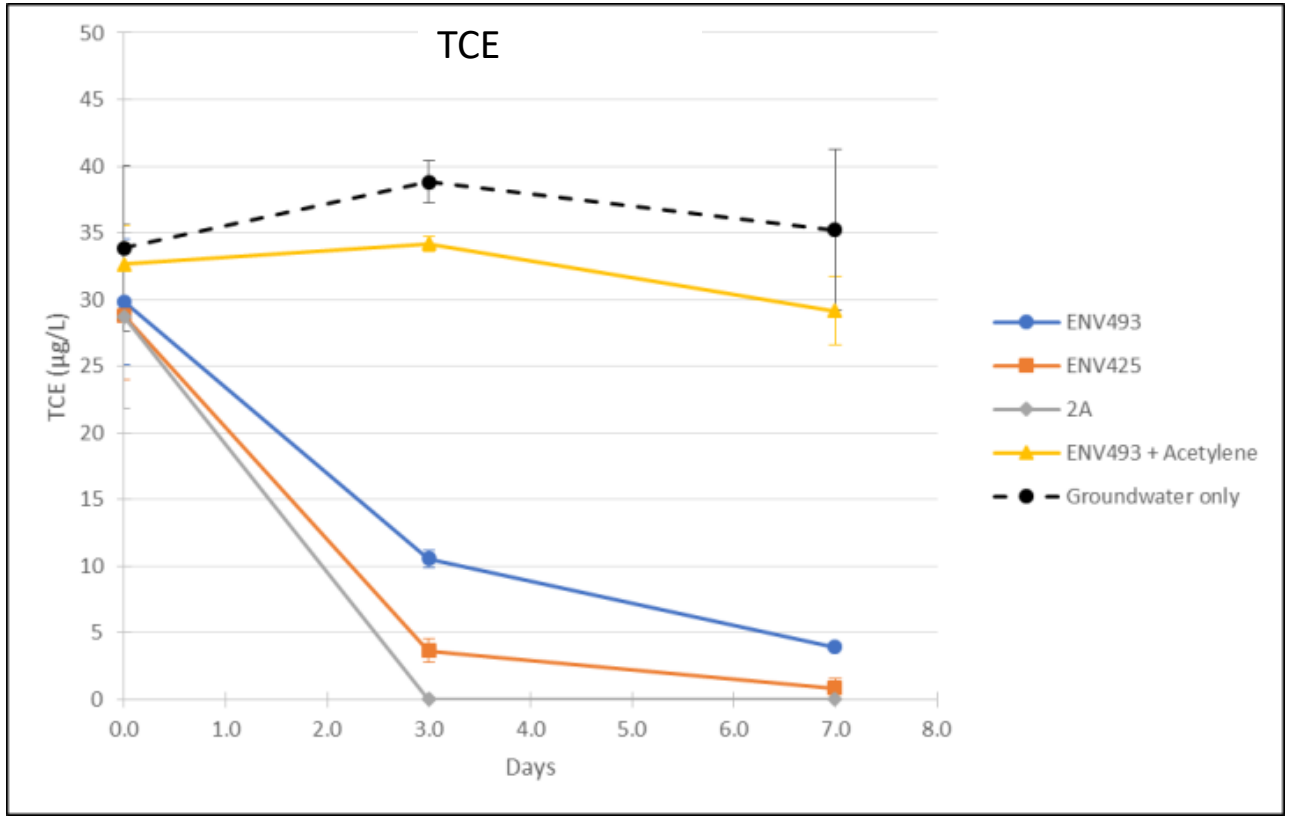
- **Task 2A-Batch Studies**
  - **Evaluating isobutane-degrading bacteria *R. ruber* ENV425, R. *aetherivorans* ENV493, Mycobaterium 2A**
  - **Microcosms with (1) growth media and (2) actual site groundwater**
  - **cVOC analysis via EPA 8260 and 1,4-D analysis via EPA 8260 (APTIM) or EPA 8270 SIM (outside lab)**
  - **Site SS022 groundwater concentrations**

Contaminants	Concentrations (µg/L)
1,4 Dioxane	6.37
TCE	81.97
PCE	8.85
Other CACs	< 2.0

Performance Assessment – Small Volume Batch Studies

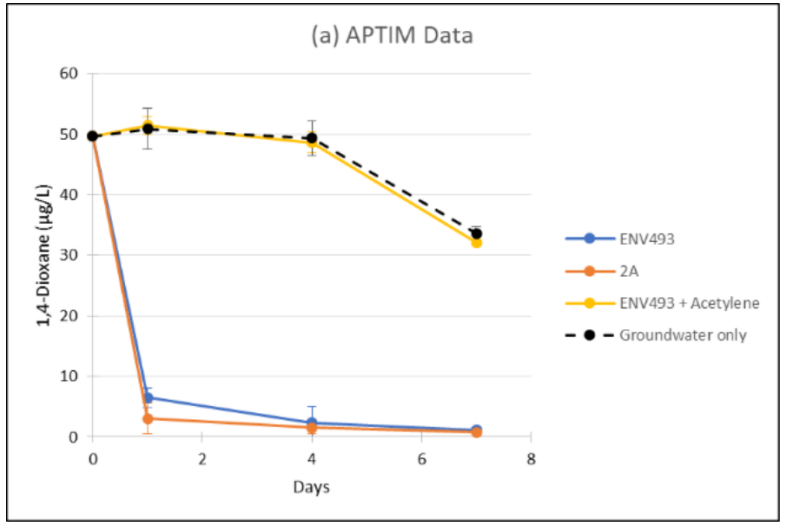
Biodegradation of 1,4-dioxane and TCE by ***Rhodococcus ruber*** **ENV425, *R. aetherivorans*** **ENV493, and Mycobacterium 2A** in Site SS022 Site Water

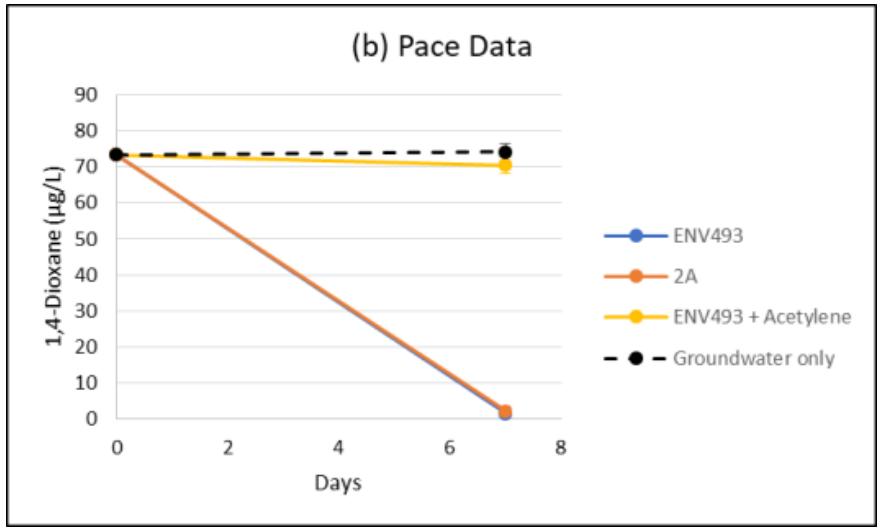




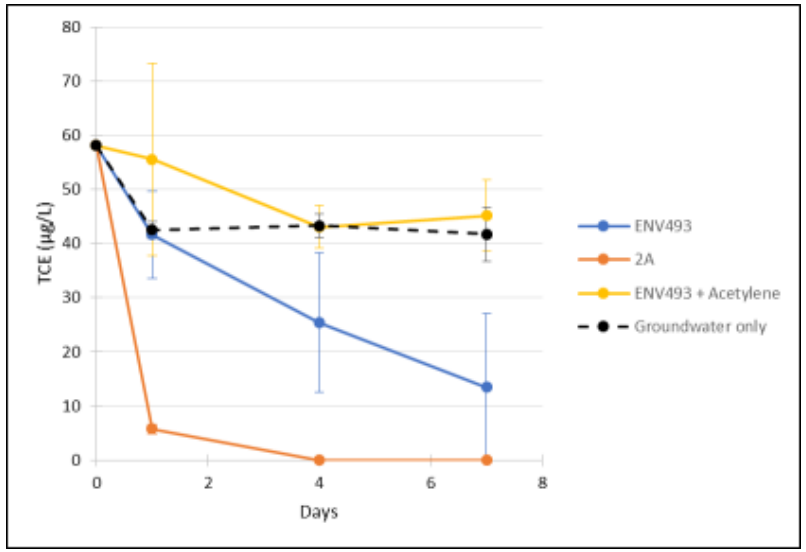
Performance Assessment – Large Volume Batch Studies

Biodegradation of 1,4-dioxane and TCE by ***R. aetherivorans*** **ENV493** and **2A** in Site SS022 Site Water





(a) Aptim: PQL of ~ 10 ppb for 1,4 D and cVOCs
(b) Pace: PQL of ~ 1-2 ppb for 1,4 D and cVOCs

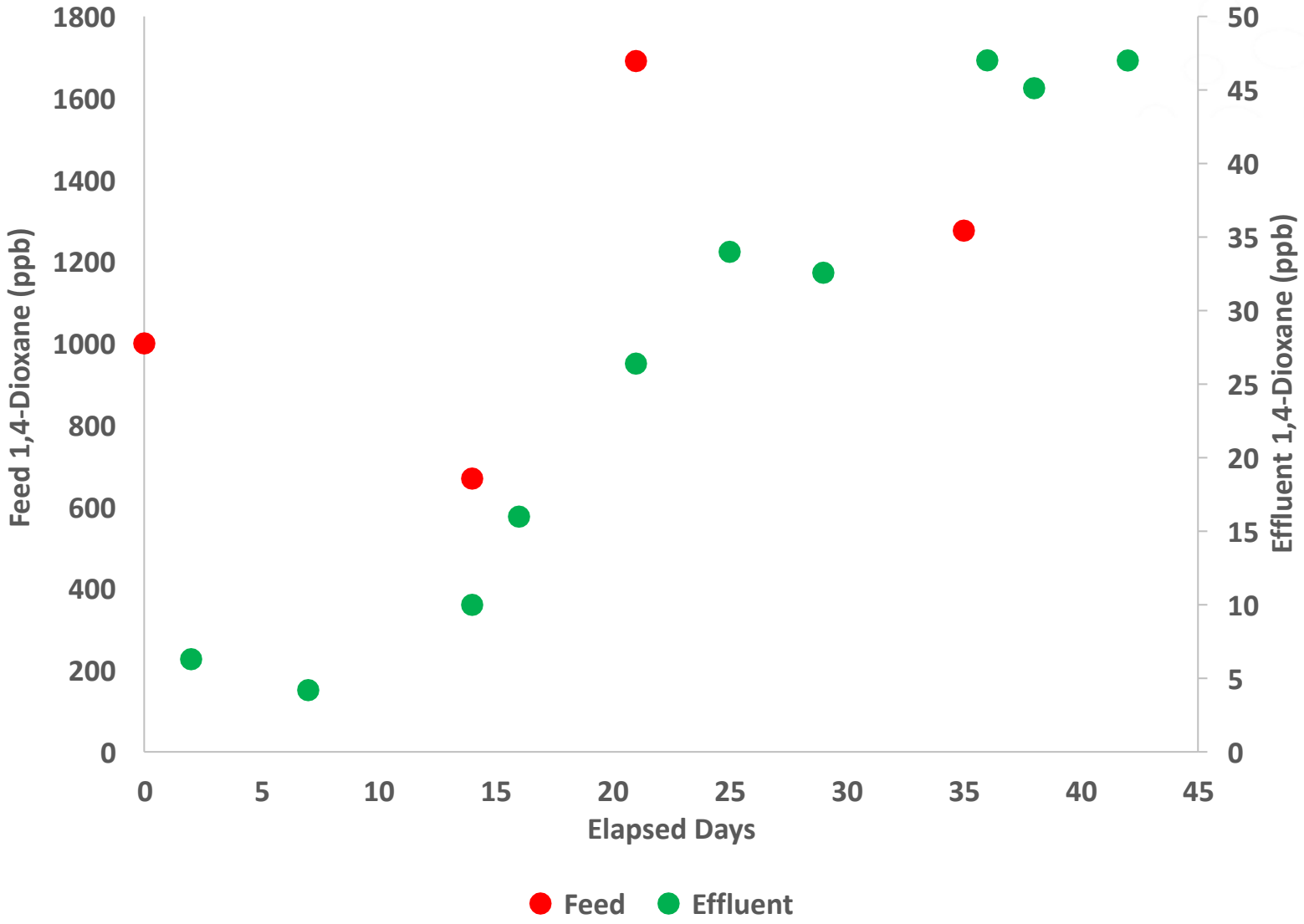


Performance Assessment-Bench-Scale Testing

- **Task 2b: Laboratory-Scale FBR Testing**
  - System set up in late April, 2023
  - Continuous Stir Tank Reactor vs. Plug-Flow kinetics
    - Media creates this condition
    - Data achieved is somewhat binary
    - Not entirely used for scale-up design
  - General Operating Conditions
    - 15 ml/min of feed flow
    - 60-70 minute hydraulic residence time
    - Oxygen addition rate of 35 ml/min (46.5 mg/min)
      - Set to be not limiting
    - Isobutane addition rate of 0.2 ml/min (0.4 mg C/min)
  - Phase I: Began initial 1,4-Dioxane abiotic loading in early May, 23.

Performance Assessment-Abiotic Testing

- Task 2b: Phase I- Abiotic Testing
  - Conducted for 42 days
  - Achieved 45-50 ppb of 1,4-D breakthrough at effluent
  - Allows abiotic/biotic treatment to be differentiated in ensuing phases

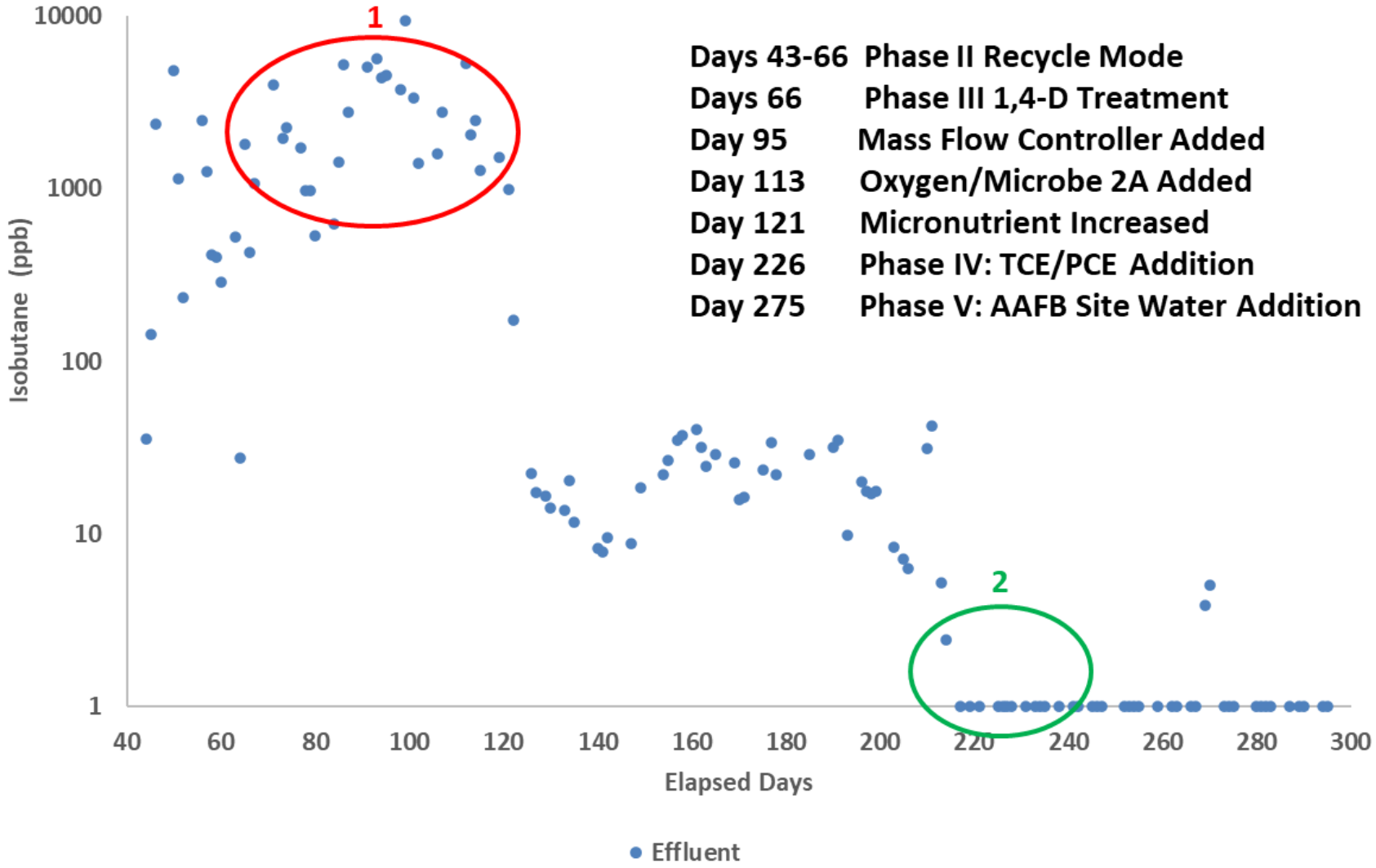


Performance Assessment-Inoculation/Recycle

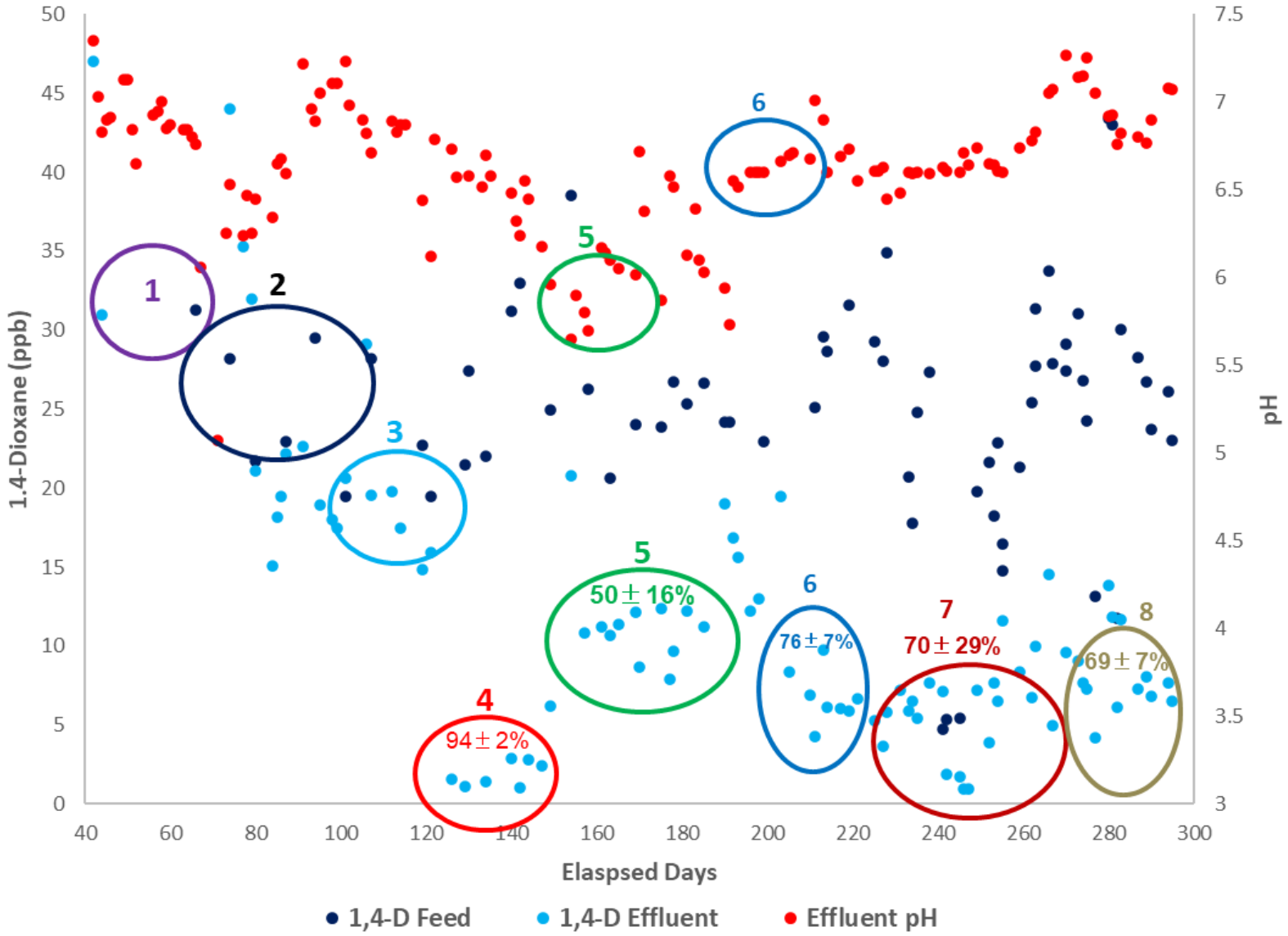
- **Task 2b: Phase II- System Inoculation/Recycle**
  - Day 43: Addition of ENV493 (2.4 Liters of ENV493 at an OD600 of 12.9), BSM nutrients, isobutane, and isobutanol
  - Recirculated through at 28 mL/min
  - Air: 700 mL/min, Isobutane: 200 mL/min (reduced to 40 mL/min)
  - Several mechanical issues with the mass flow controller and air addition system occurred
  - Vigorous growth was visually observed on the media
  - Uptake of isobutane demonstrated
  - System operated in recycle through Day 66
  - Phase III-1,4-Dioxane treatment-Continuous flow

Performance Assessment-Phases III-V (Isobutane Uptake)

- Note 1: Issues with control of isobutane
  - Electronic mass flow controller not functional
  - Required rote addition of isobutane at 30-40 mL/min
  - Created situation from Day 66-110 where minimal 1,4-D treatment observed.
  - Saturation of the active sites of the produced SCAM enzymes
- Mass flow controller replaced on Day 95 at an isobutane flowrate of 0.2 mL/min (0.4 mg C/min).
- Note 2: By Day 220, complete isobutane uptake-Net Zero Addition/Consumption.

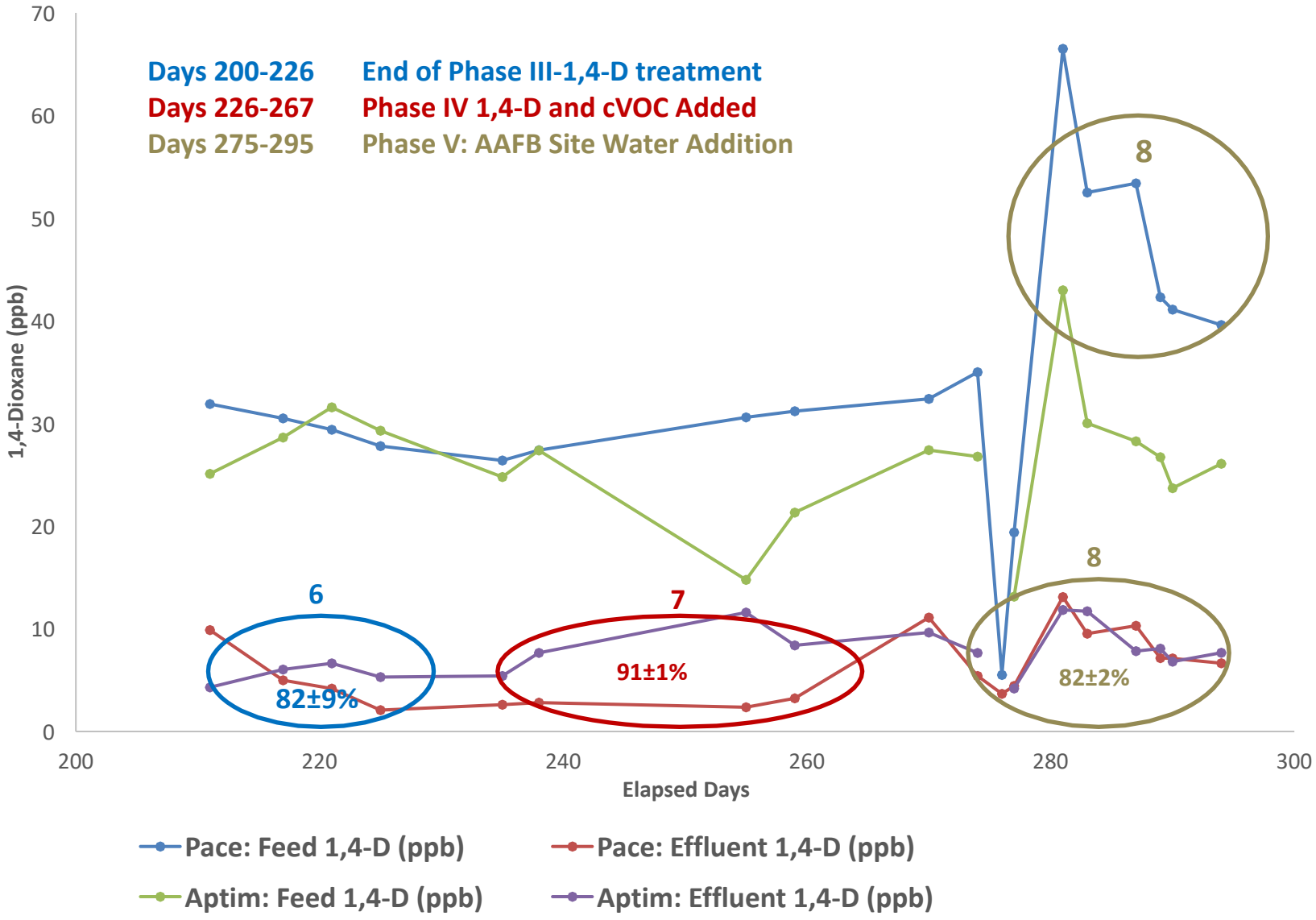


Performance Assessment-Phases III-V (On-Site Lab Data)



1: Days 43-66 Phase II Recycle Mode
2: Day 66 Phase III 1,4-D Treatment
3: Day 95 Mass Flow Controller Added
3: Day 113 Oxygen/Microbe 2A Added
4: Day 121 Micronutrient Increased
5: Days 143-190 HRT reduced to 52-54 minutes
6: Days 191-226 HRT increased to 65-70 minutes
7: Day 226- 267 Phase IV: TCE/PCE Addition
8: Day 275-295 Phase V: AAFB Site Water Addition

Performance Assessment-Phases III-V (Off-Site Lab Data)



Day 300

Short Chain Alkane monooxygenase SCAM: **2.04E+08**

Aldehyde Dehydrogenase ALDH: **3.40E+05**

Propane Monooxygenase PPO: **6.35E+07**

Ammonia Monooxygenase AMO: **6.27E+07**

Key Findings and Summary

- Site SS022 chosen as test site based on a number of factors
- Water samples from SS022 have been tested - no biological inhibition concerns
- Batch studies identified several microbial strains available for 1,4-Dioxane/cVOC treatment
- ENV493 and 2A used to inoculate bench-scale FBR
- At 60-70 minute HRT, 85-90+% treatment demonstrated for 1,4-Dioxane using isobutane as co-metabolite
- Lower HRT(52 minutes) combined with nutrient addition impacted pH
    - Ammonia oxidizers produced acid-lowering pH
    - Nitrate> 20 mg/L observed at effluent, ammonia oxygenase present
    - Direct correlation between declining pH (below 6.0) and lower 1,4-Dioxane treatment
    - Therefore, operating system optimized at 60-70 min HRT
- Sufficient and regulated nutrient addition for pilot system will be critical
- cVOC additions did not negatively impact 1,4-Dioxane treatment in FBR
- Abiotic/biotic removal of cVOCs demonstrated

Next Steps

- **Bench results demonstrate effective treatment of 1,4-Dioxane and cVOCs**
- **Move forward to pilot-scale system**
- **Tasks 3/4-Design and Fabrication**
  - **0.5 to 5 gpm flow**
  - **Isobutane, oxygen addition and pH control**
  - **Computer automated for process control and data collection**
  - **5-6 Months**
- **Tasks 5/6- Commissioning, Operation and Monitoring**
  - **Nine(9) month operational period**
  - **The capital and operational costs of the pilot-scale FBR system closely tracked to compare to competitive alternative**
  - **Develop full-scale FBR design**

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QUESTIONS?