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Monitoring & Maintenance Engineering, Inc.

PHOSTER PILOT TEST AND QPCR ANALYSIS PIKE ROAD, ALABAMA

Presented at:

26th UST Remediation and Assessment Conference

Montgomery, Alabama

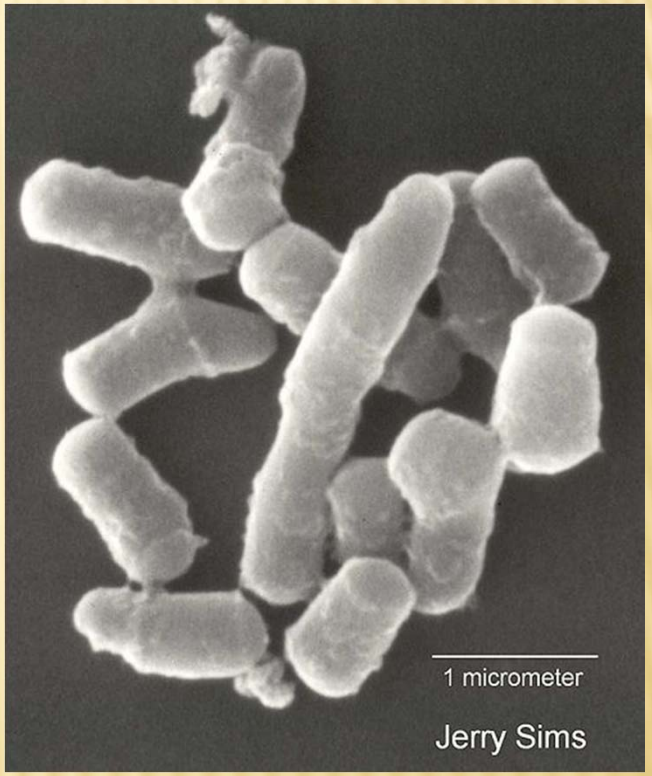
April 24 & 25, 2019

Presented by:

Richard B. Smith

PROCESS OVERVIEW

- Gas-phase nutrient injection to stimulate bacteria cell division and metabolism.
- Independently controlled pulsed air sparge. Flows from 0.5 to 2.0 cfm per injector.
- PLC controlled dosage:
  - Air
  - Nitrous Oxide
  - Triethyl-phosphate
- Bacteria Nutrient Molar Ratio: $C_{64}H_{85}O_{23}N_{13}P$



Rhodococcus

Aerobic bacteria active in the first stage of benzene oxidation.

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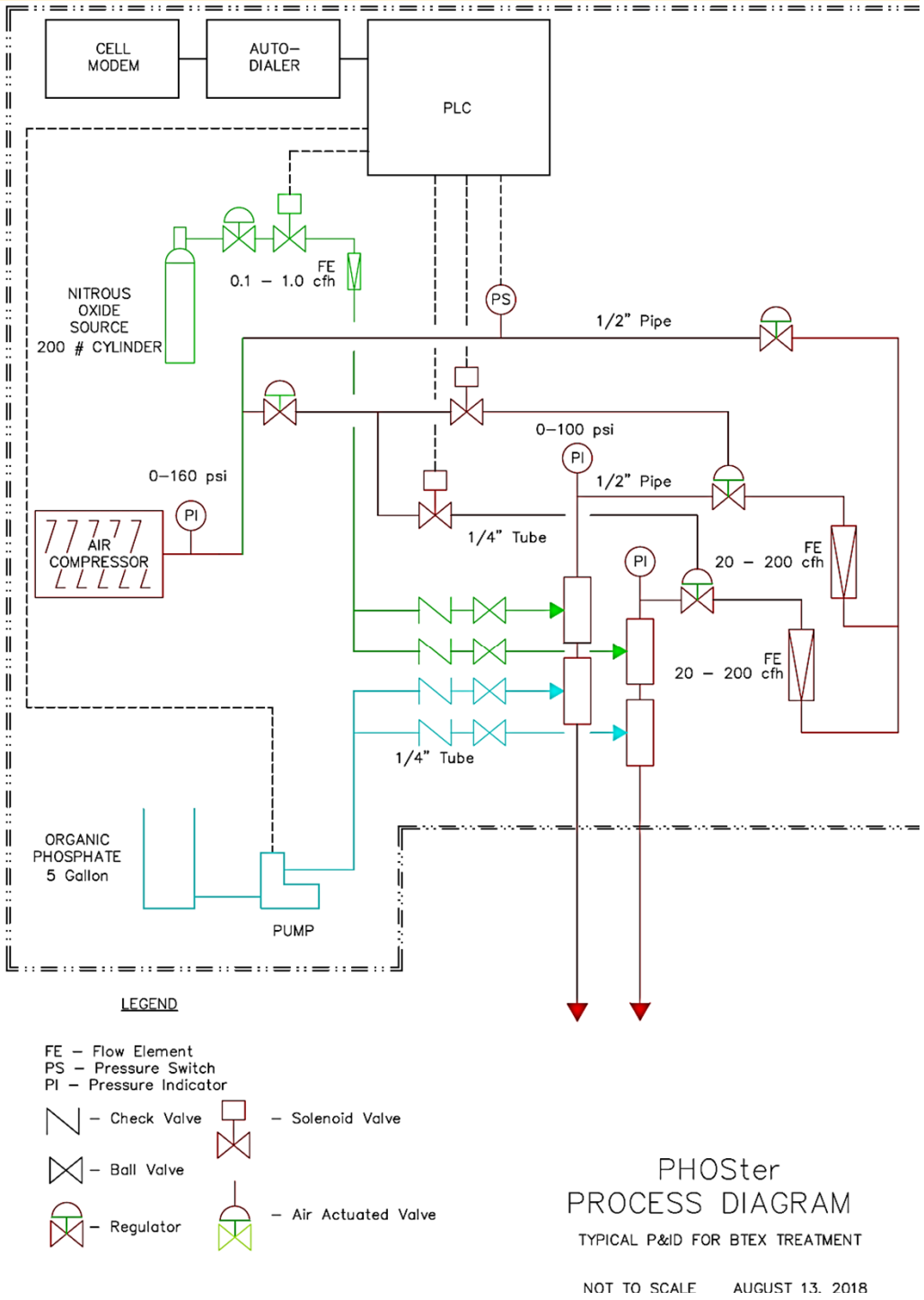
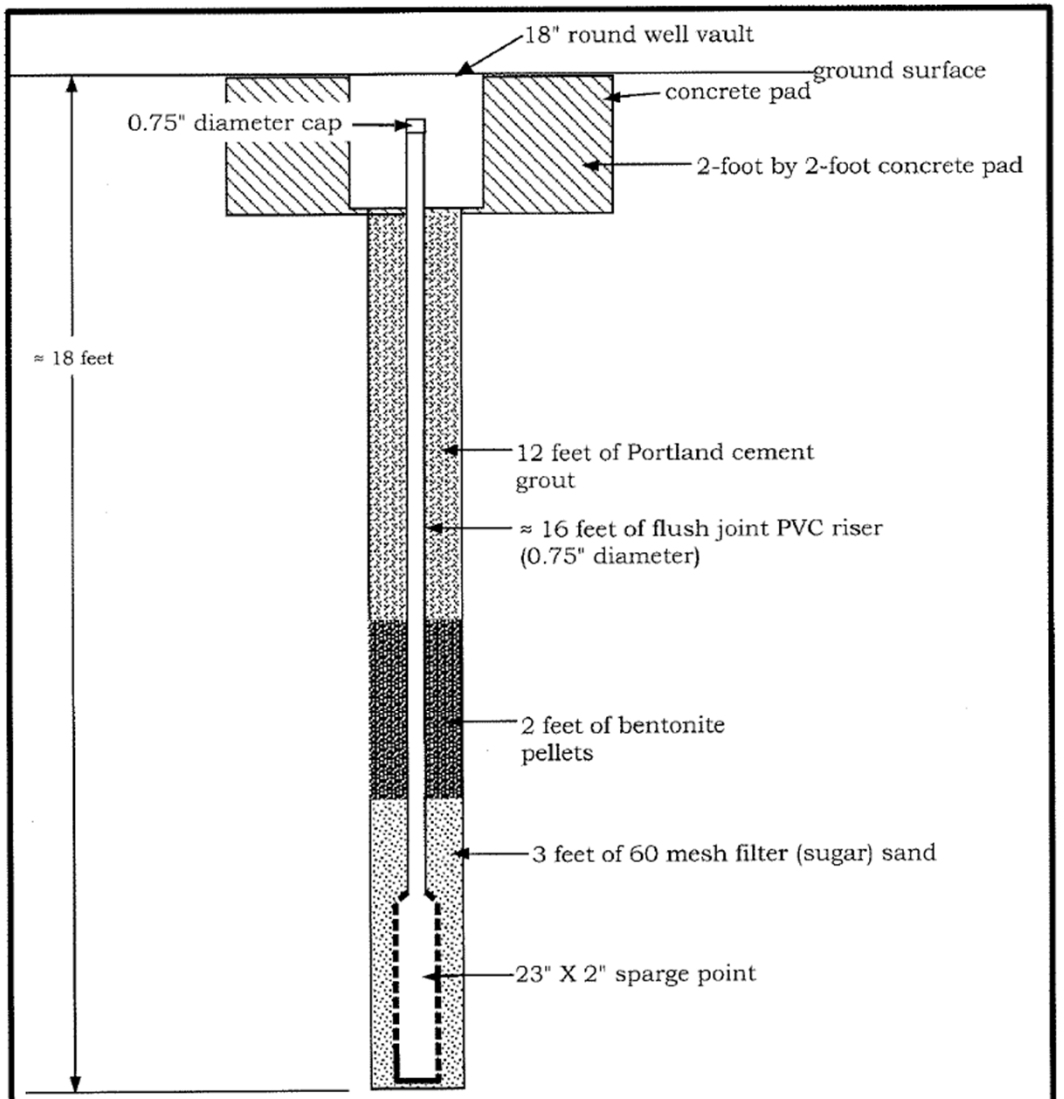
P&ID

- PLC Controlled.
- Independent gas flow regulation.
- Isolatable nutrient delivery.
- Cellular connection to auto-dialer.



SPARGE WELL

- ¾” PVC Riser.
- 2” x 23” Sparge Point.
- Supply Tubing is 3/8” LLDPE.
- Supply Tubing Installed Above Grade.



GENERAL INJECTED AIR-SPARGE POINT
CROSS-SECTIONAL CONSTRUCTION DIAGRAM

NOT TO SCALE

Former Spe-Dee-C Fuel Stop 7249 Troy Highway Montgomery County Pike, Road, Alabama Facility ID# 14537-101-004566 UST 99-07-40	JOB NUMBER: MA-1267	SCALE: Not to scale
	DRAWING NO: 1267-IAS	DRAWN BY: SPB
	DATE: 6/24/16	CHECKED BY: SPB

EMC
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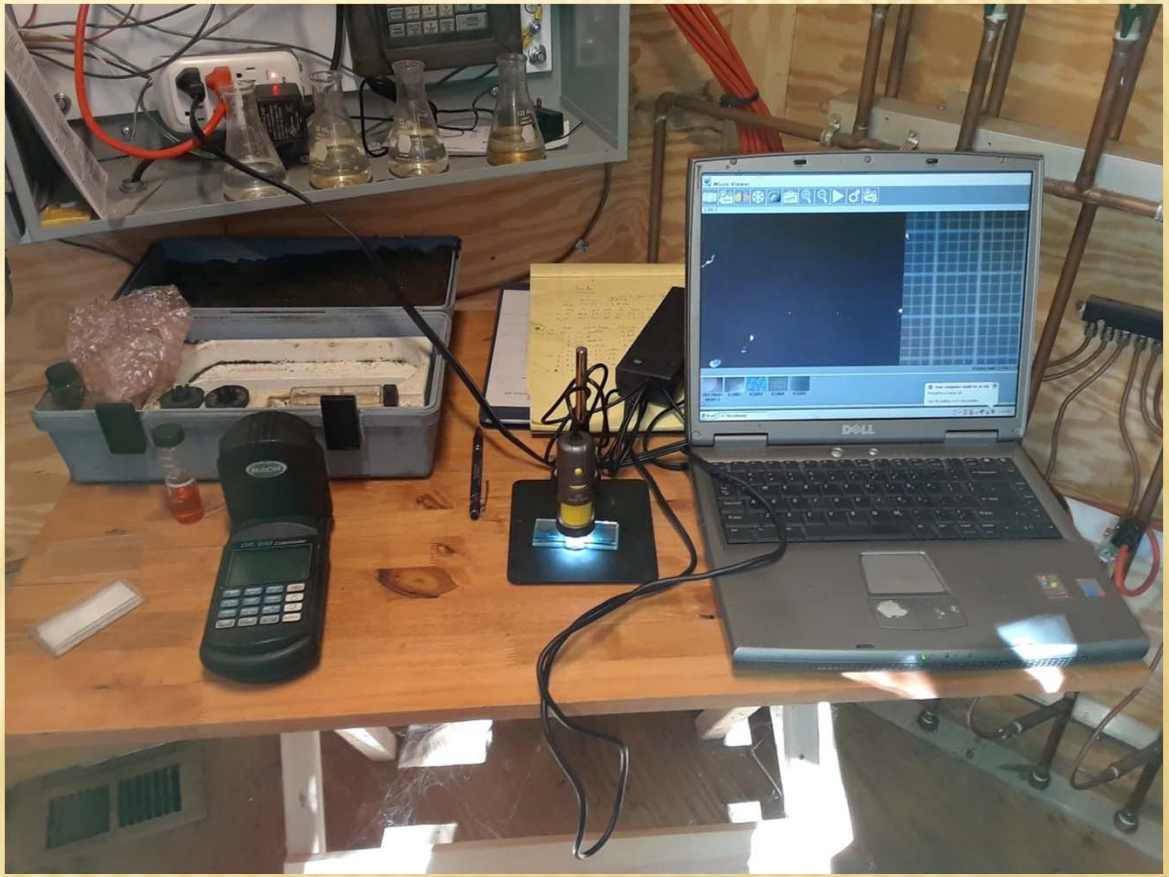
PROCESS CONTROL TESTING

- Bi-monthly review of biologically important parameters for process control.
- DO, Temp, pH, ORP, nitrate, phosphate and iron.
- Estimate bacteria cell density.



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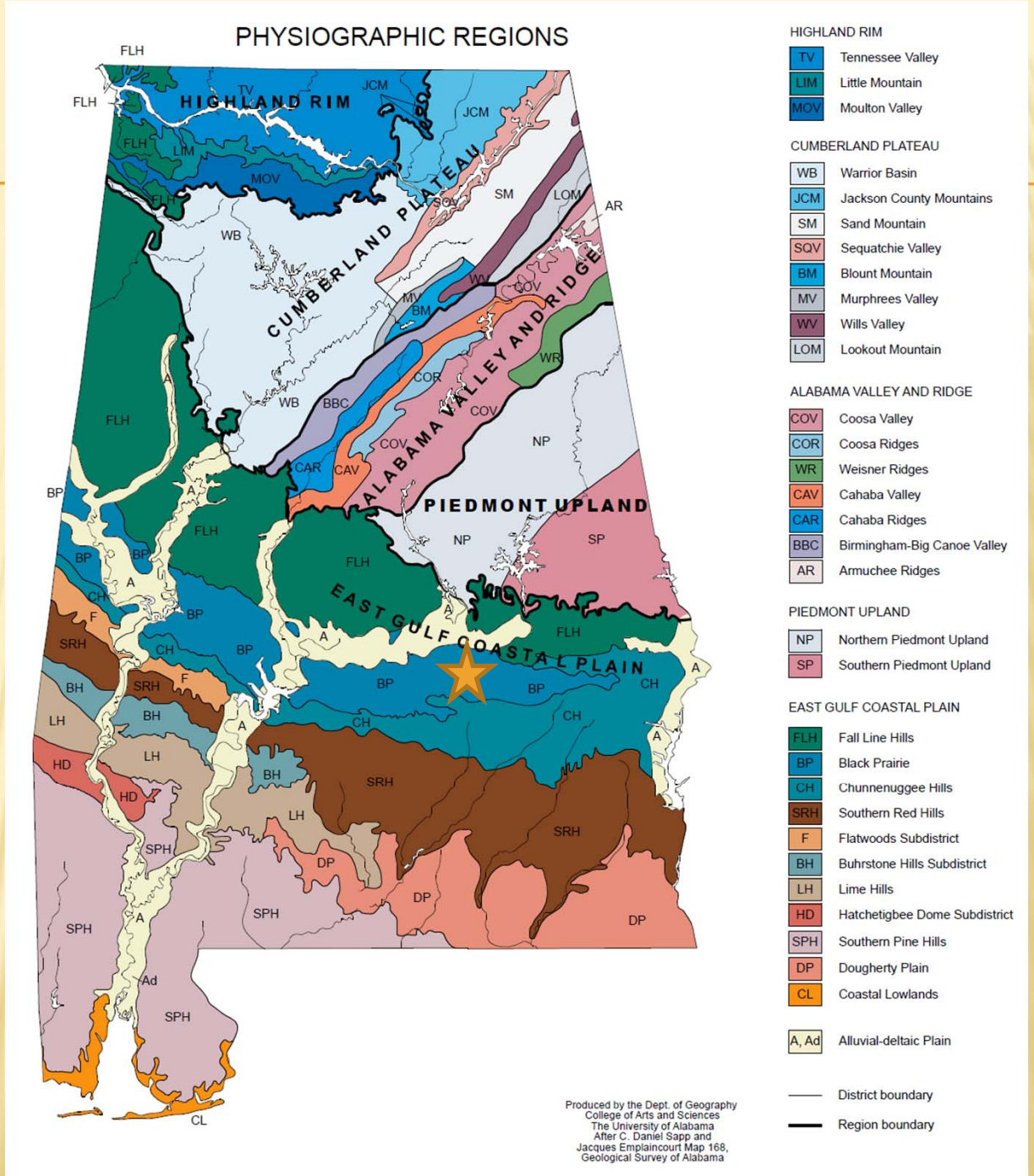
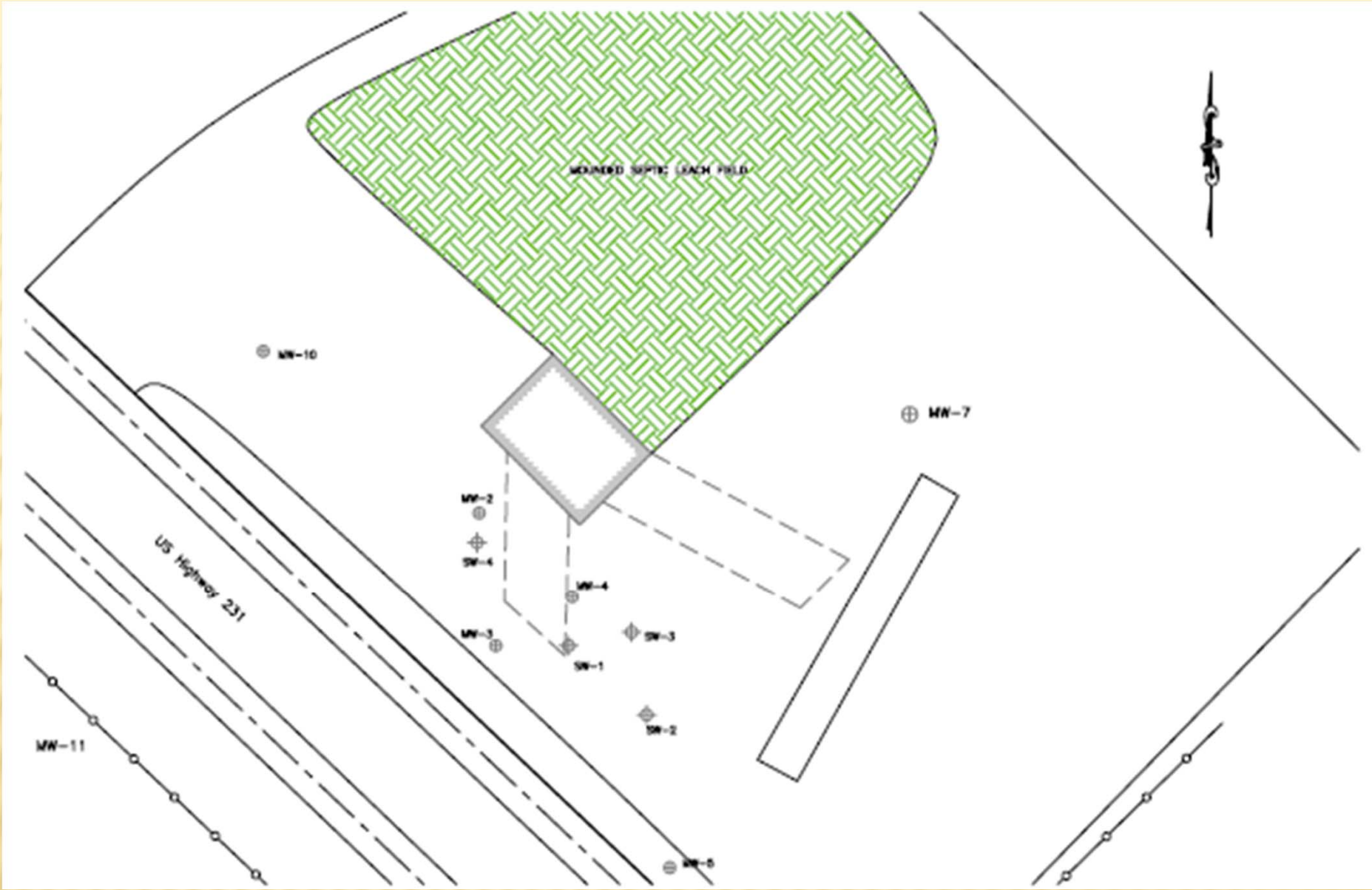
- Site is in Montgomery County.
- Black Prairie District.
- East Gulf Coastal Plain.





- Mounded Septic Leach Field.
- A Source of Nutrients/Bacteria?

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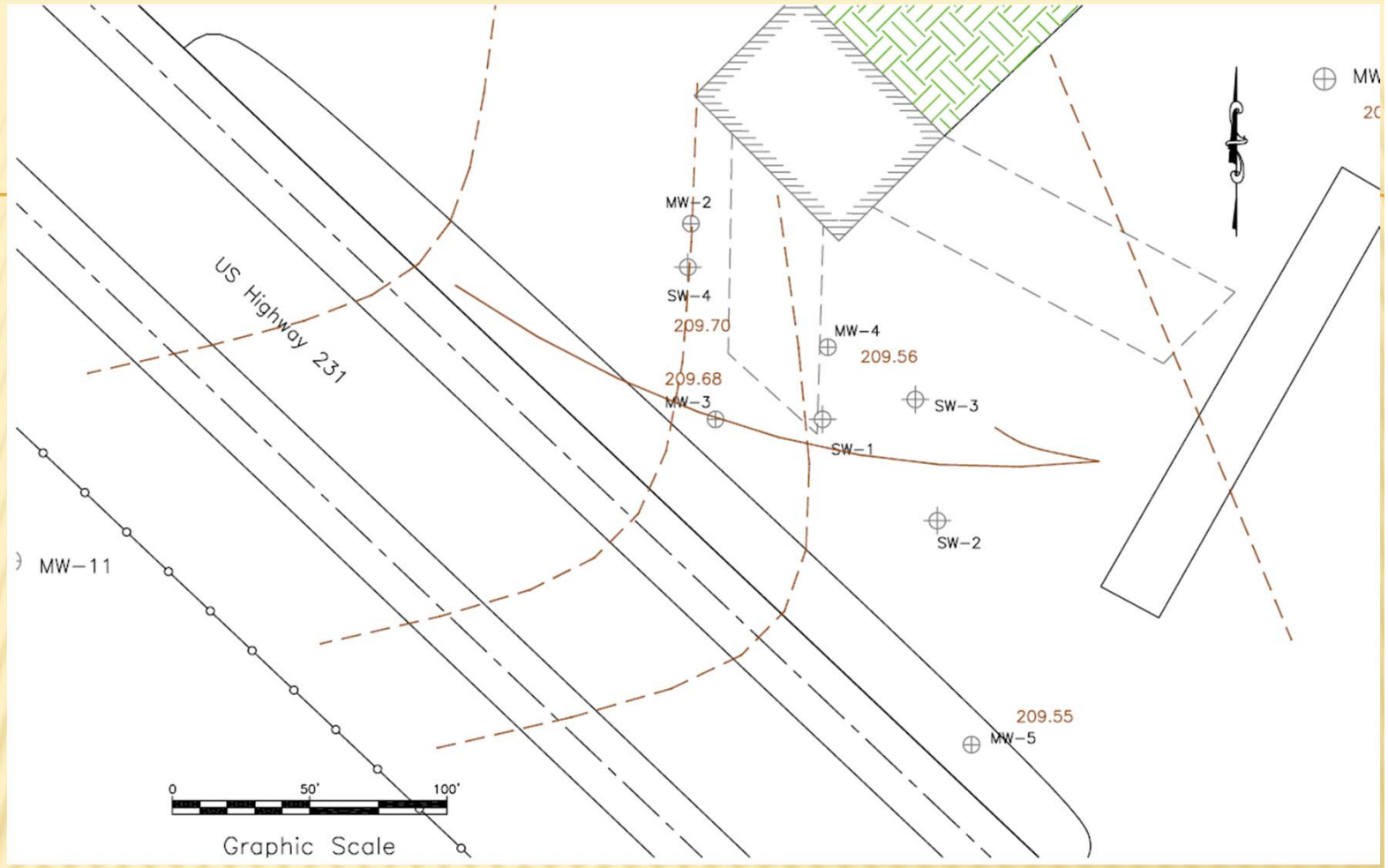

- Site Water Table Elevations During October 2018.
- Contours are 0.1'.
- Gradient is Less Than 0.1% to the South and East.

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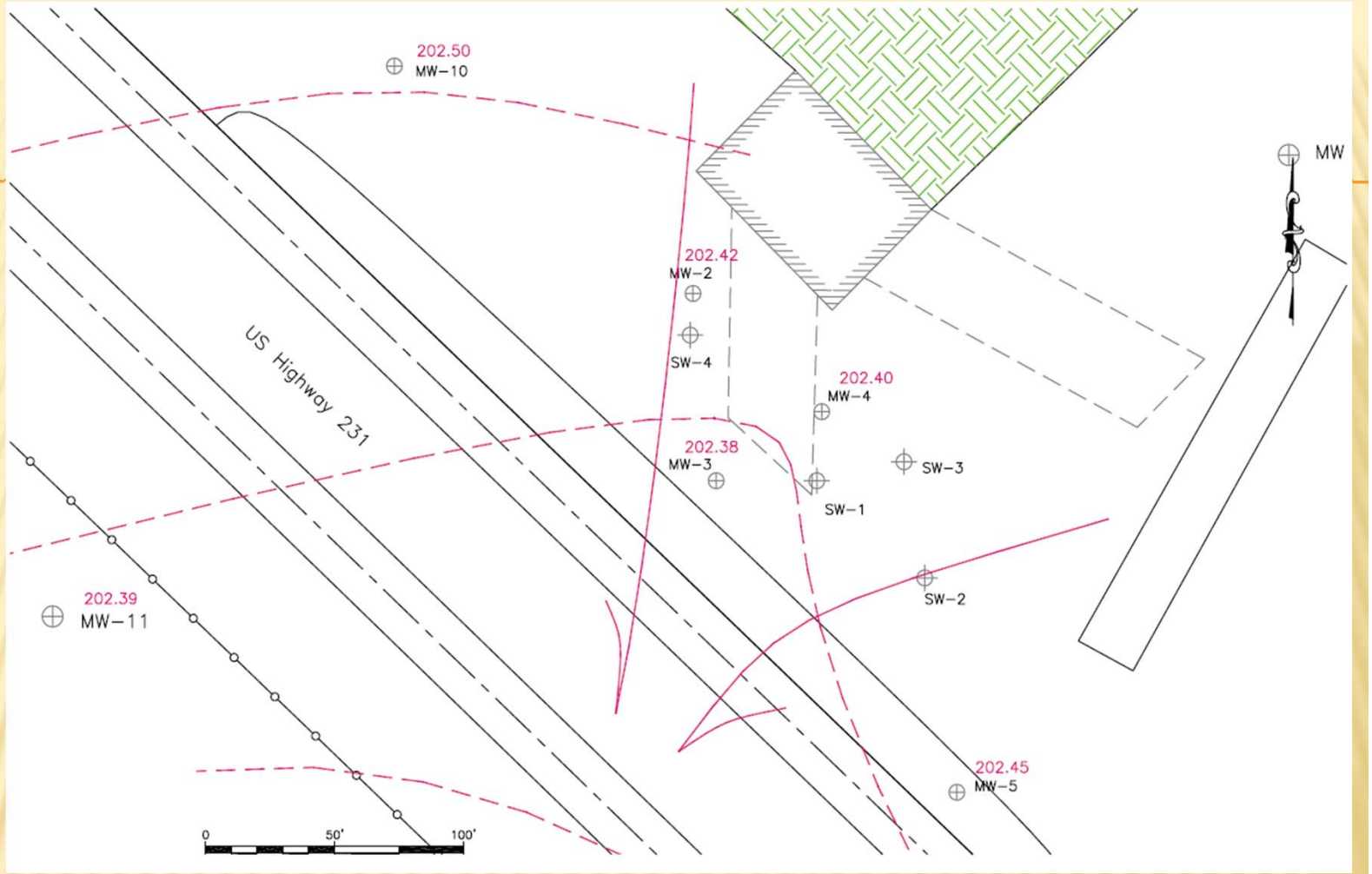
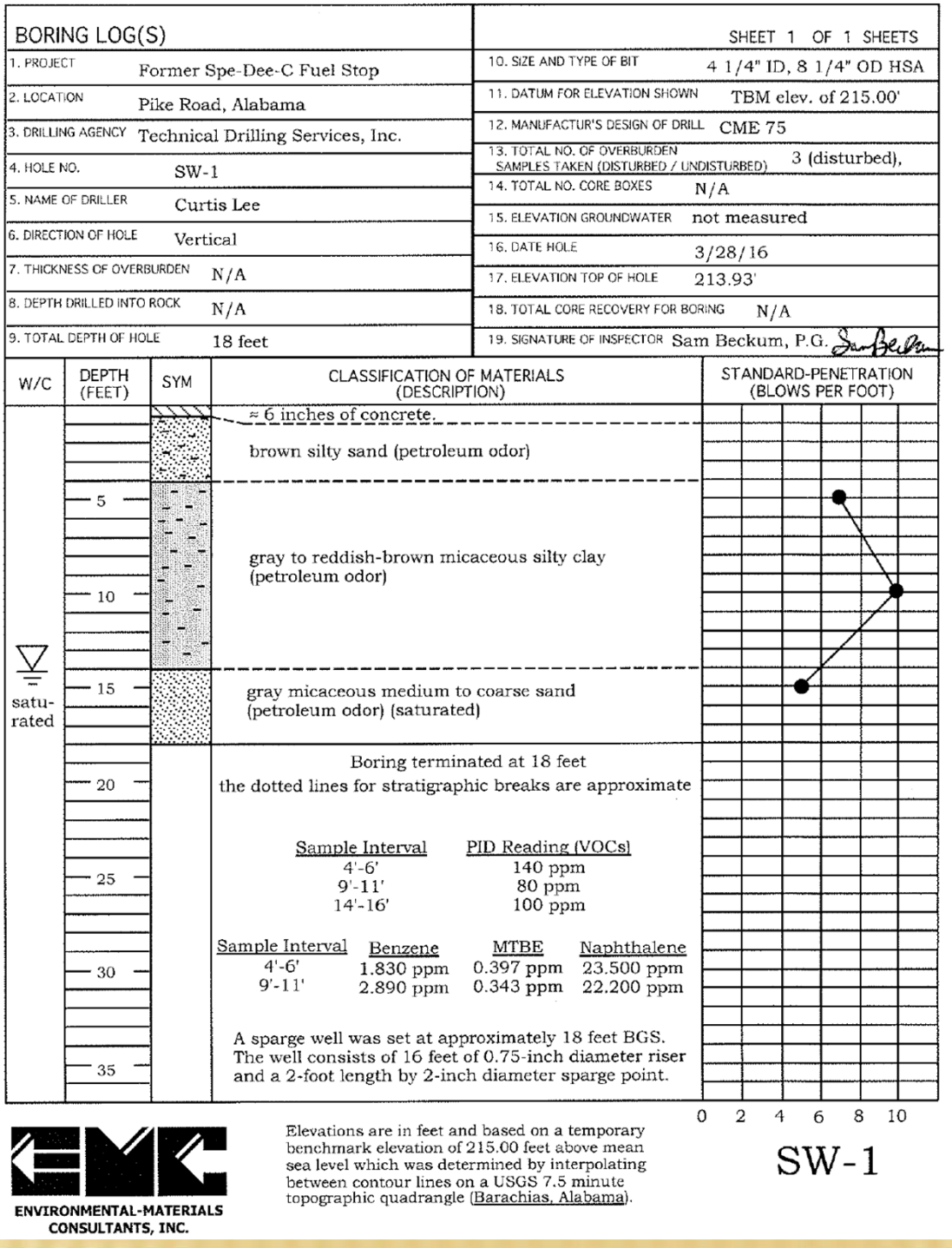


- Site Water Table Elevations During November 2016.
- Contours are 0.1'.
- Gradient is Less Than 0.1% to the South and West.

WELL LOG

- Typical of the 4 Sparge Wells.
- 4’ Silty Sand.
- 10’ Silty Clay.
- >14’ Medium to Coarse Sand.



BORING LOG(S) — SHEET 1 OF 1 SHEETS

1. PROJECT: Former Spe-Dee-C Fuel Stop	10. SIZE AND TYPE OF BIT: 4 1/4" ID, 8 1/4" OD HSA
2. LOCATION: Pike Road, Alabama	11. DATUM FOR ELEVATION SHOWN: TBM elev. of 215.00'
3. DRILLING AGENCY: Technical Drilling Services, Inc.	12. MANUFACTUR'S DESIGN OF DRILL: CME 75
4. HOLE NO.: SW-1	13. TOTAL NO. OF OVERBURDEN SAMPLES TAKEN (DISTURBED / UNDISTURBED): 3 (disturbed),
5. NAME OF DRILLER: Curtis Lee	14. TOTAL NO. CORE BOXES: N/A
6. DIRECTION OF HOLE: Vertical	15. ELEVATION GROUNDWATER: not measured
7. THICKNESS OF OVERBURDEN: N/A	16. DATE HOLE: 3/28/16
8. DEPTH DRILLED INTO ROCK: N/A	17. ELEVATION TOP OF HOLE: 213.93'
9. TOTAL DEPTH OF HOLE: 18 feet	18. TOTAL CORE RECOVERY FOR BORING: N/A
	19. SIGNATURE OF INSPECTOR: Sam Beckum, P.G.

W/C	DEPTH (FEET)	SYM	CLASSIFICATION OF MATERIALS (DESCRIPTION)
			≈ 6 inches of concrete.
			brown silty sand (petroleum odor)
	5		gray to reddish-brown micaceous silty clay (petroleum odor)
	10		
▽ satu-rated	15		gray micaceous medium to coarse sand (petroleum odor) (saturated)
	20		Boring terminated at 18 feet the dotted lines for stratigraphic breaks are approximate
	25		
	30		
	35		

STANDARD-PENETRATION (BLOWS PER FOOT): 0 2 4 6 8 10

Sample Interval	PID Reading (VOCs)
4'-6'	140 ppm
9'-11'	80 ppm
14'-16'	100 ppm

Sample Interval	Benzene	MTBE	Naphthalene
4'-6'	1.830 ppm	0.397 ppm	23.500 ppm
9'-11'	2.890 ppm	0.343 ppm	22.200 ppm

A sparge well was set at approximately 18 feet BGS. The well consists of 16 feet of 0.75-inch diameter riser and a 2-foot length by 2-inch diameter sparge point.

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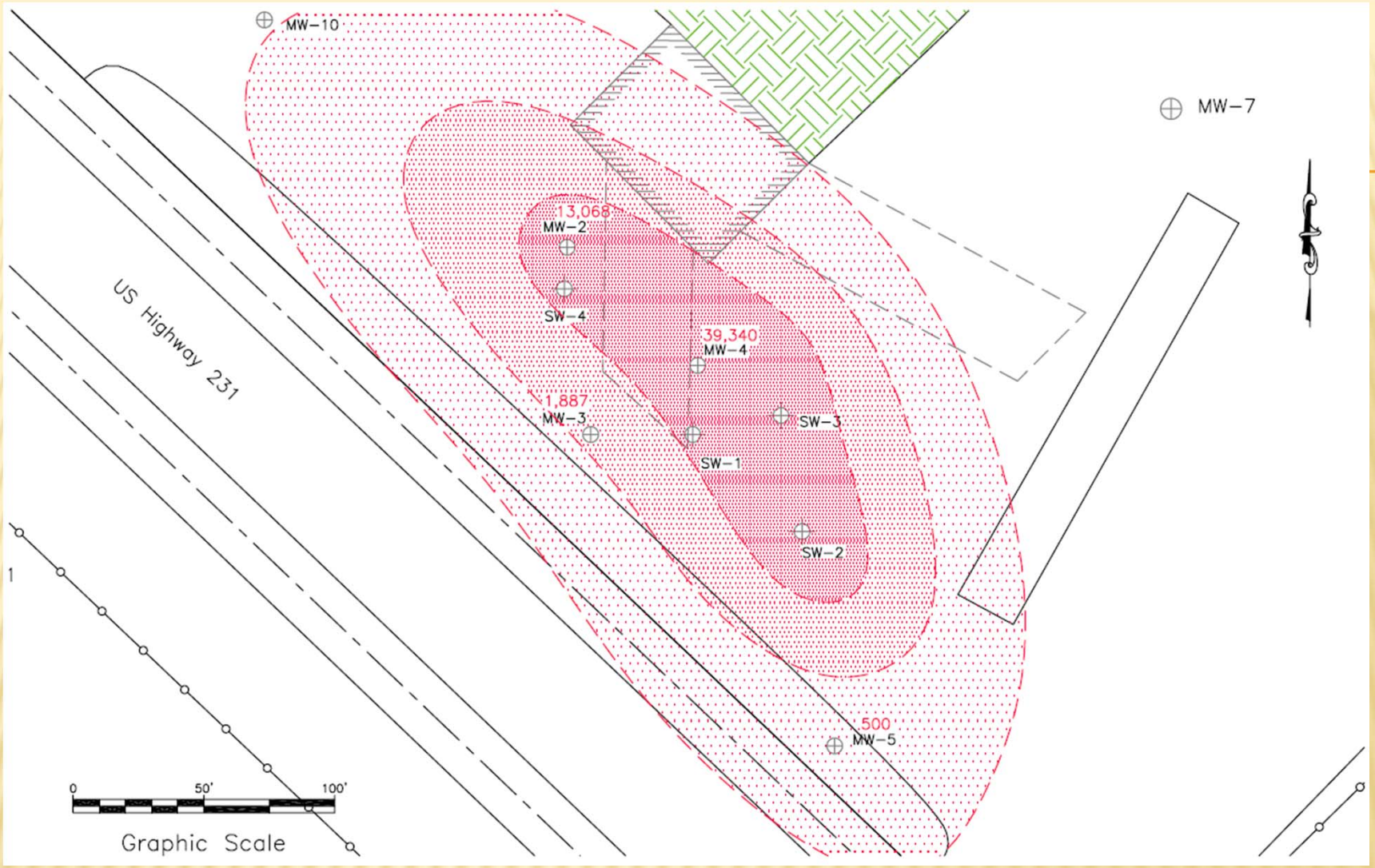
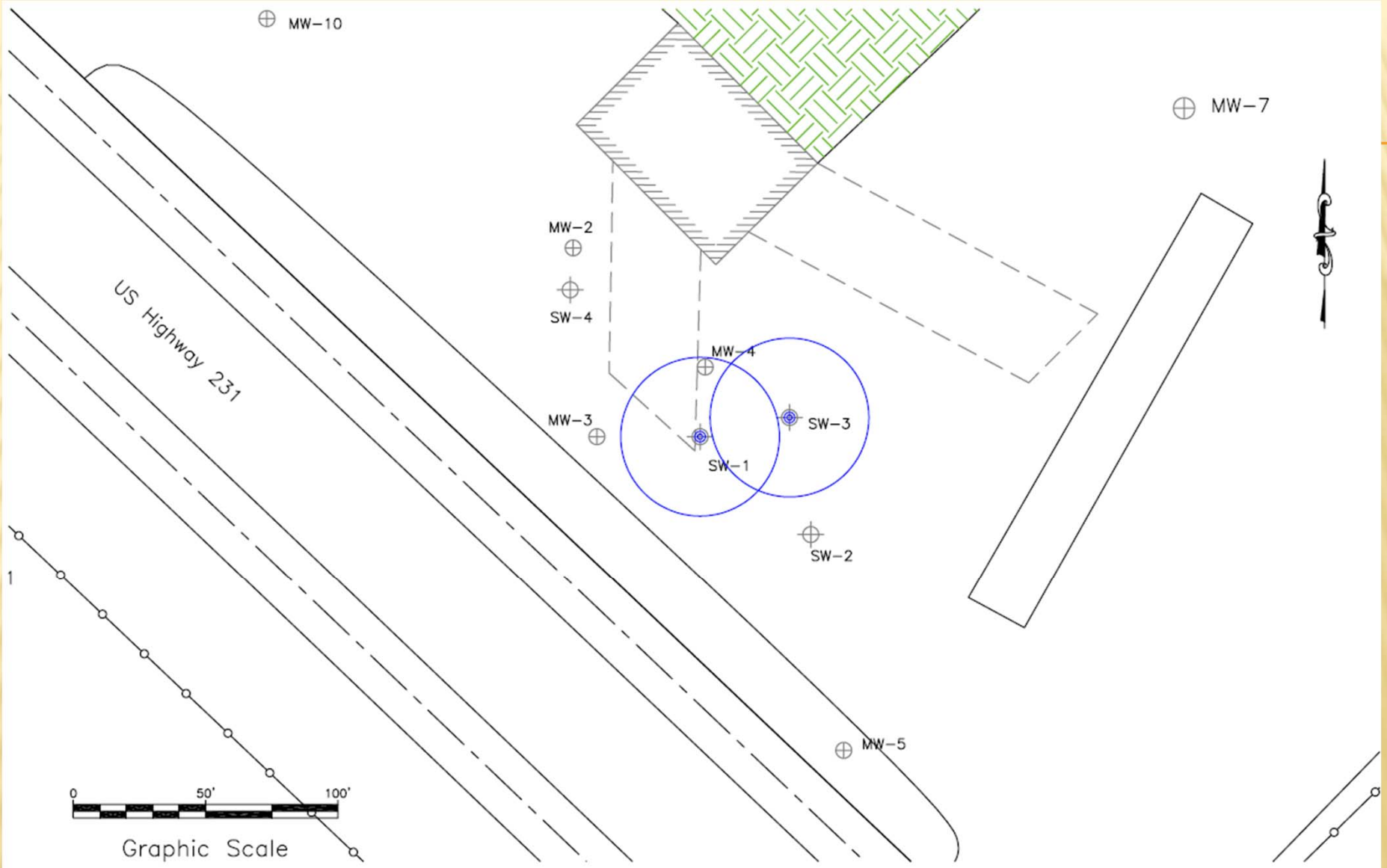
Elevations are in feet and based on a temporary benchmark elevation of 215.00 feet above mean sea level which was determined by interpolating between contour lines on a USGS 7.5 minute topographic quadrangle (Barachias, Alabama).

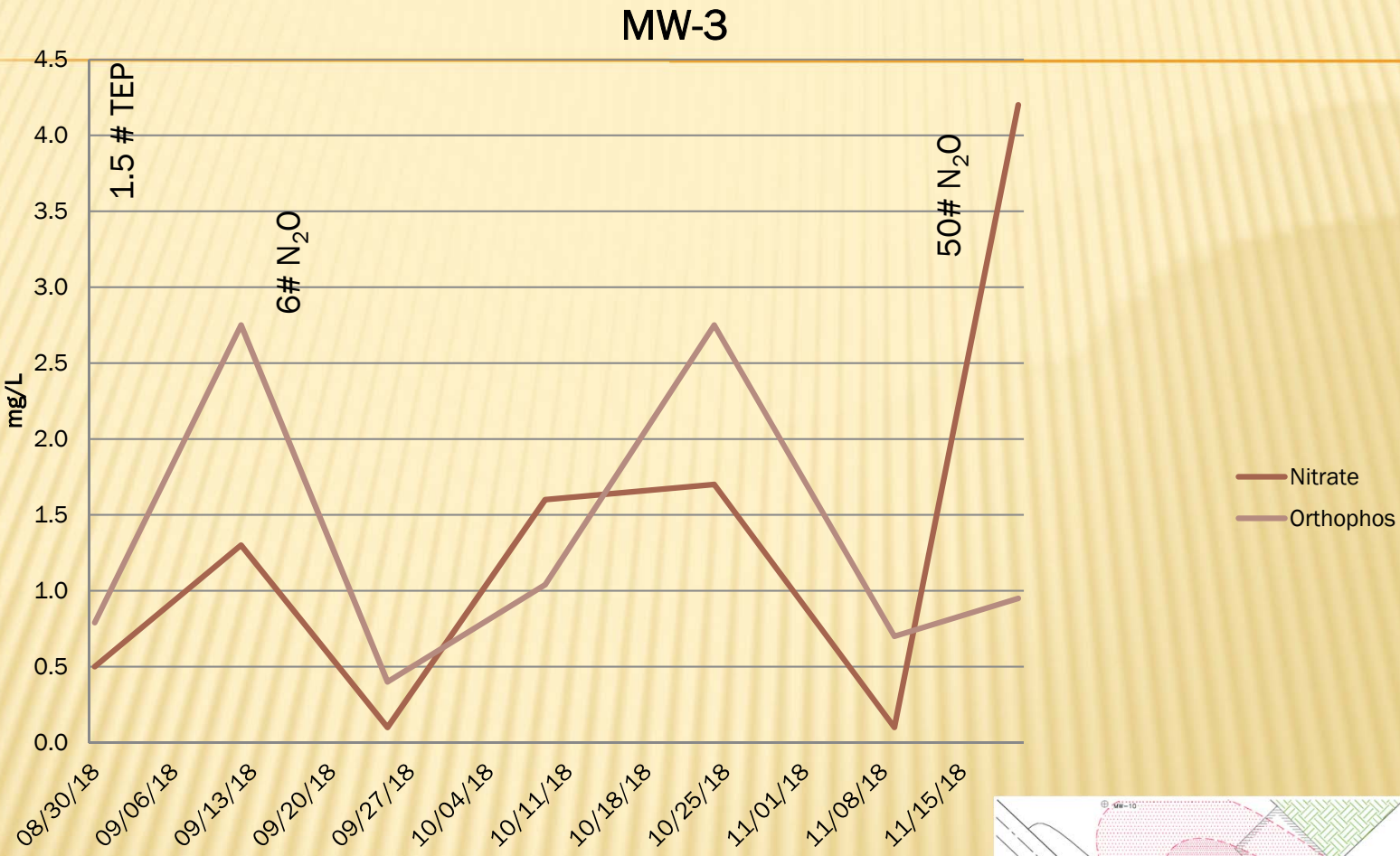
SW-1

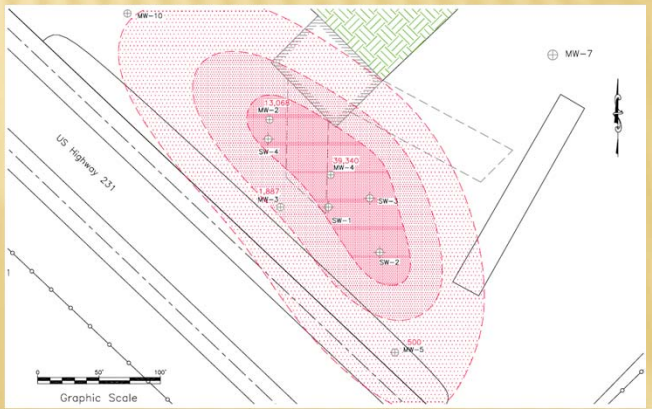


- Contamination was due to piping leaks below the pumps in the middle auto pump bay.
- Total BTEX from Oct. 2018 sampling.

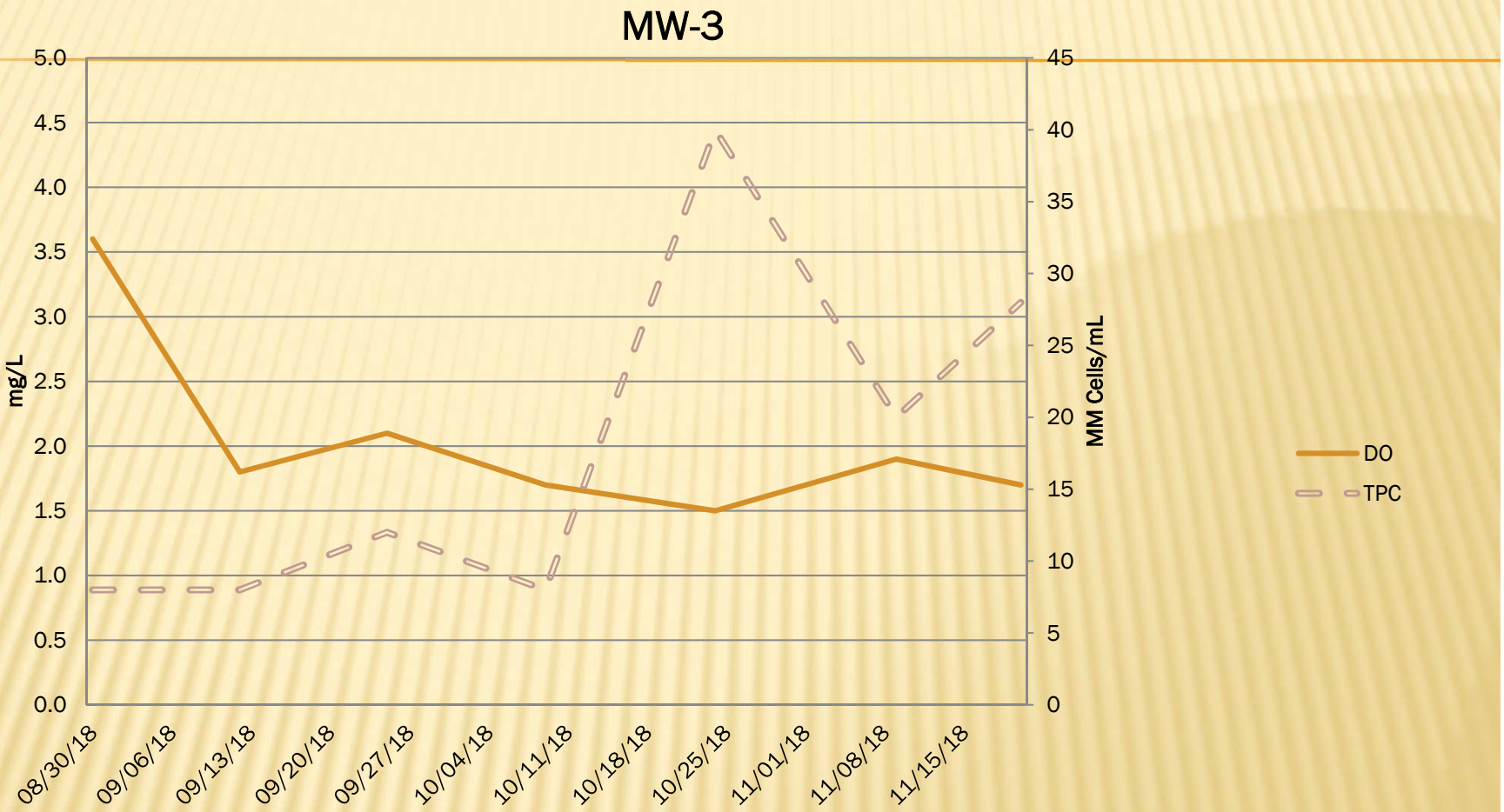


- Four sparge wells were installed.
- SW-1 and SW-3 were selected for the pilot test.

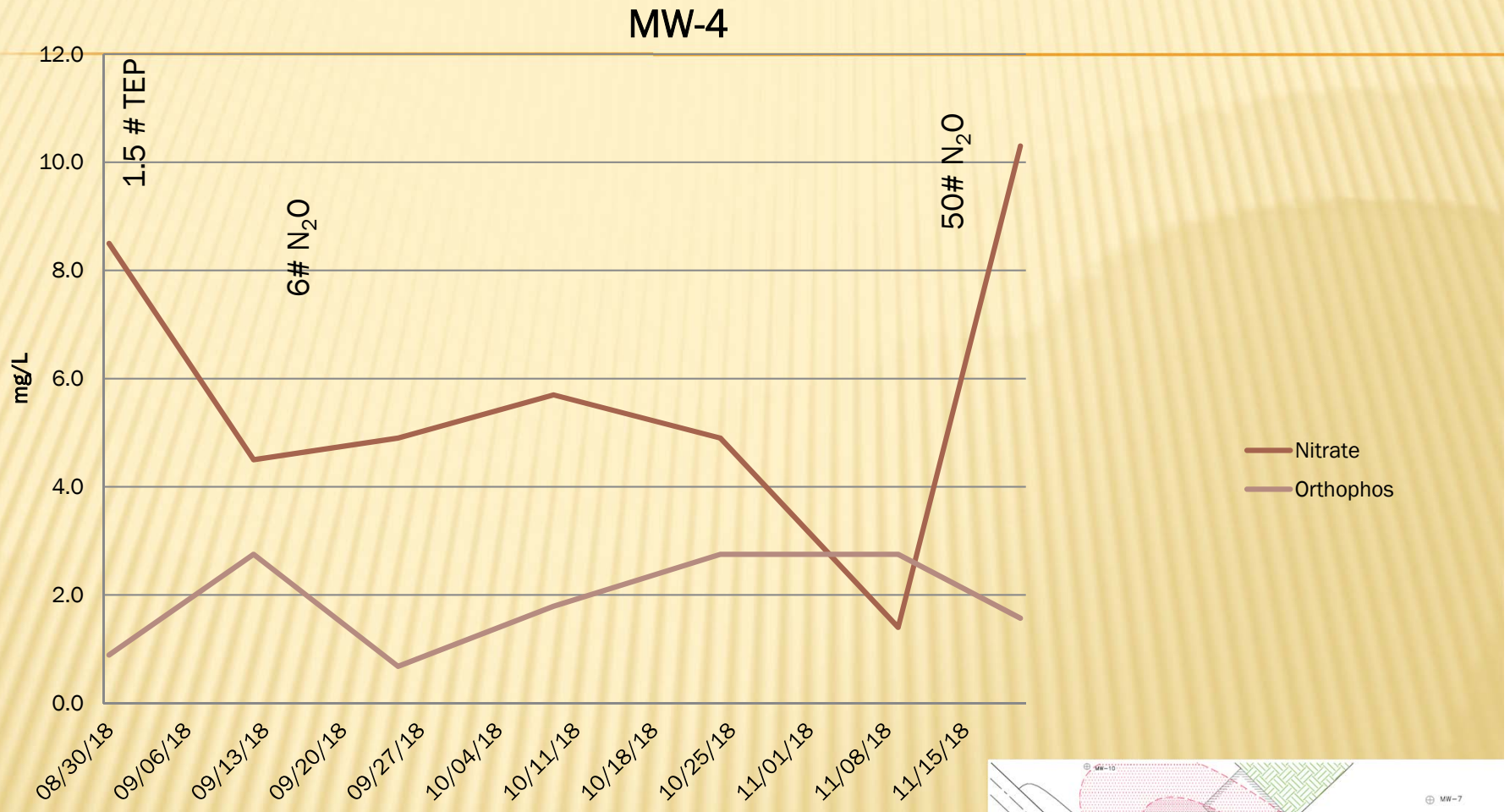




- Bi-Weekly Nutrient Analysis.



- Bi-Weekly DO and Cell Count Analysis.



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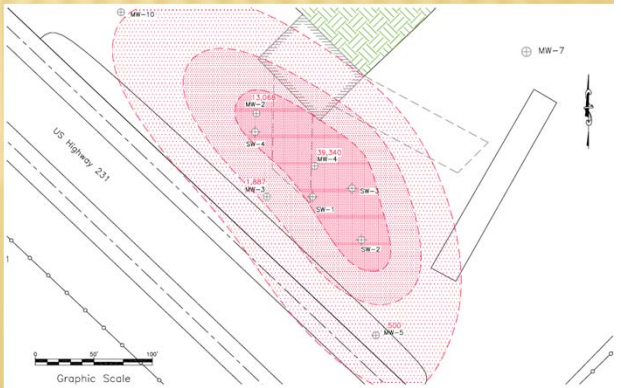

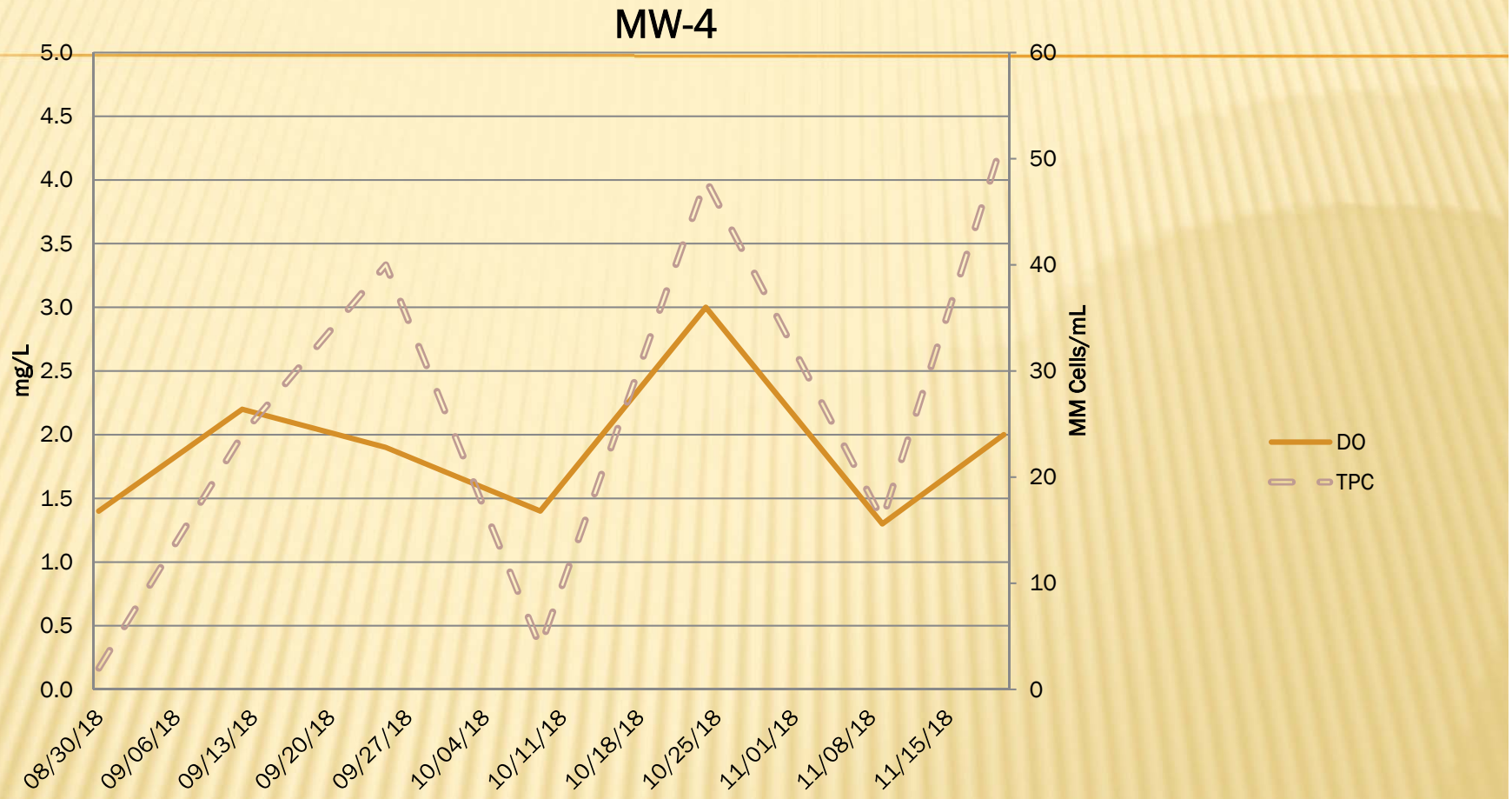

- Bi-Weekly DO and Cell Count Analysis.

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METRICS

- Applied 270,000 CF Air;
- 931 # O_2; (OTE – 20%)
- 56 # N_2O;
- 1.8 # TEP;
- 2,000 kWh/ $ 260
- Operation Eff.: 99.2%
- Analysis: $ 350/sample.

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CENCUS: QPCR

- Quantitative Polymerase Chain Reaction.
- Analysis to determine the quantity of bacteria that can express specific enzymes or types of enzymes.
- Target enzymes were toluene mono-oxygenase and phenol hydroxylase.

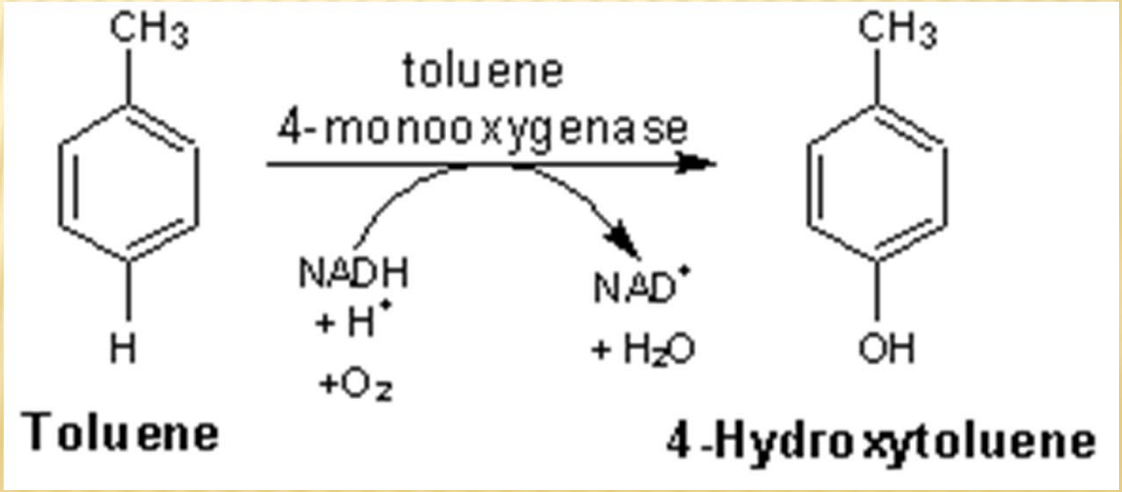

Image: http://eawag-bbd.ethz.ch/index.html

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POLYMERASE CHAIN REACTION

- Kary Mullis won the Nobel Prize in Chemistry in 1993 for PCR development.
- Discovered during treatment studies of Sickle Cell Anemia.
- The process is used to amplify segments of DNA to larger quantities.

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POLYMERASE CHAIN REACTION

Elements:

- DNA Template (Sample).
- Primer – Short sections of DNA used to initiate PCR reaction.
- DNA Nucleotide Bases (dNTP).
- Taq polymerase enzyme.
- Buffer to maintain pH of 8.
- Fluorescent Dye.

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THE REACTION

Phases:

Denaturation

15 – 30 seconds

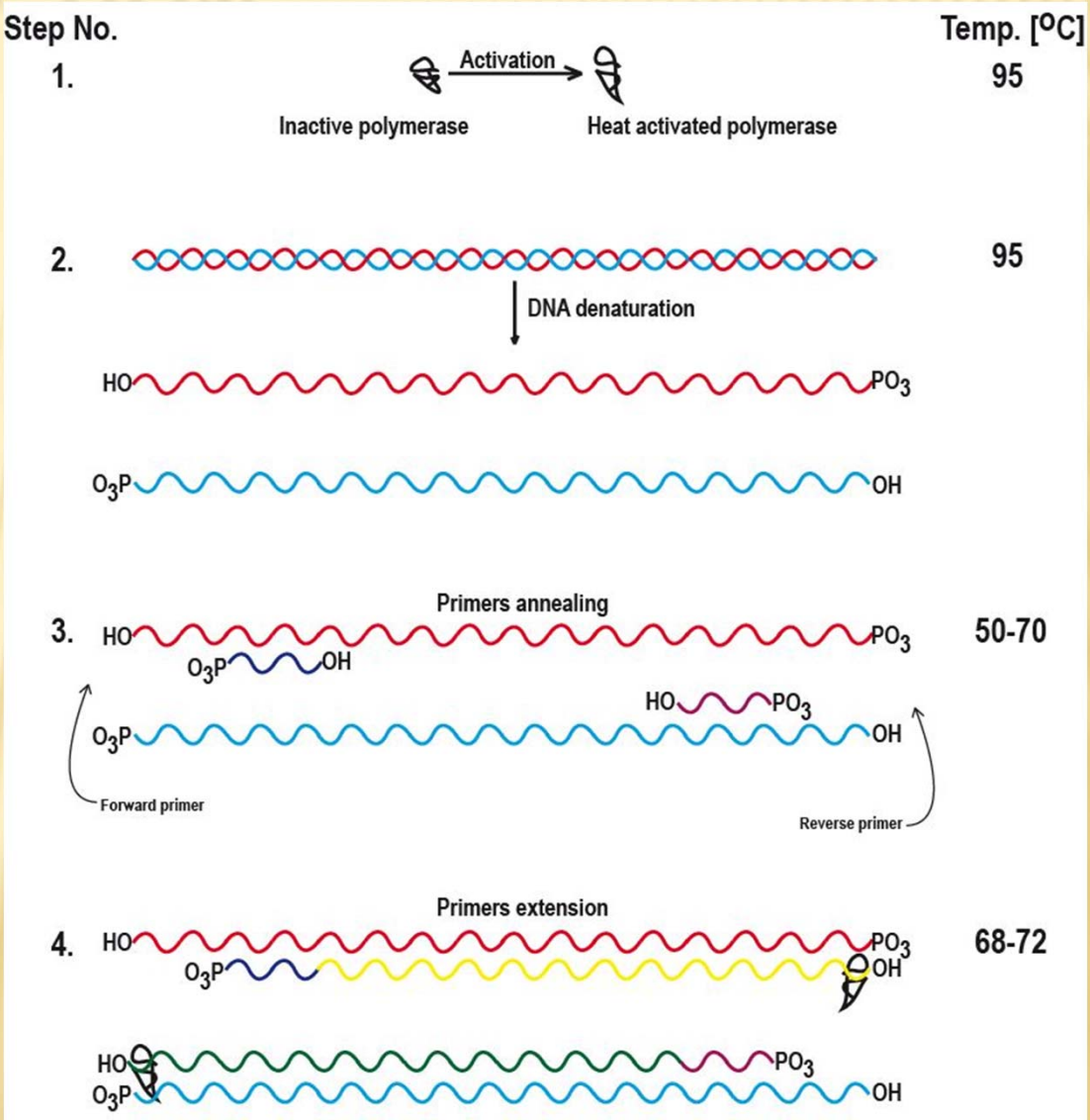
Primer Annealing

10 – 30 seconds

Extension

1 minute/1 Kb

Image from BioFreaks Blog



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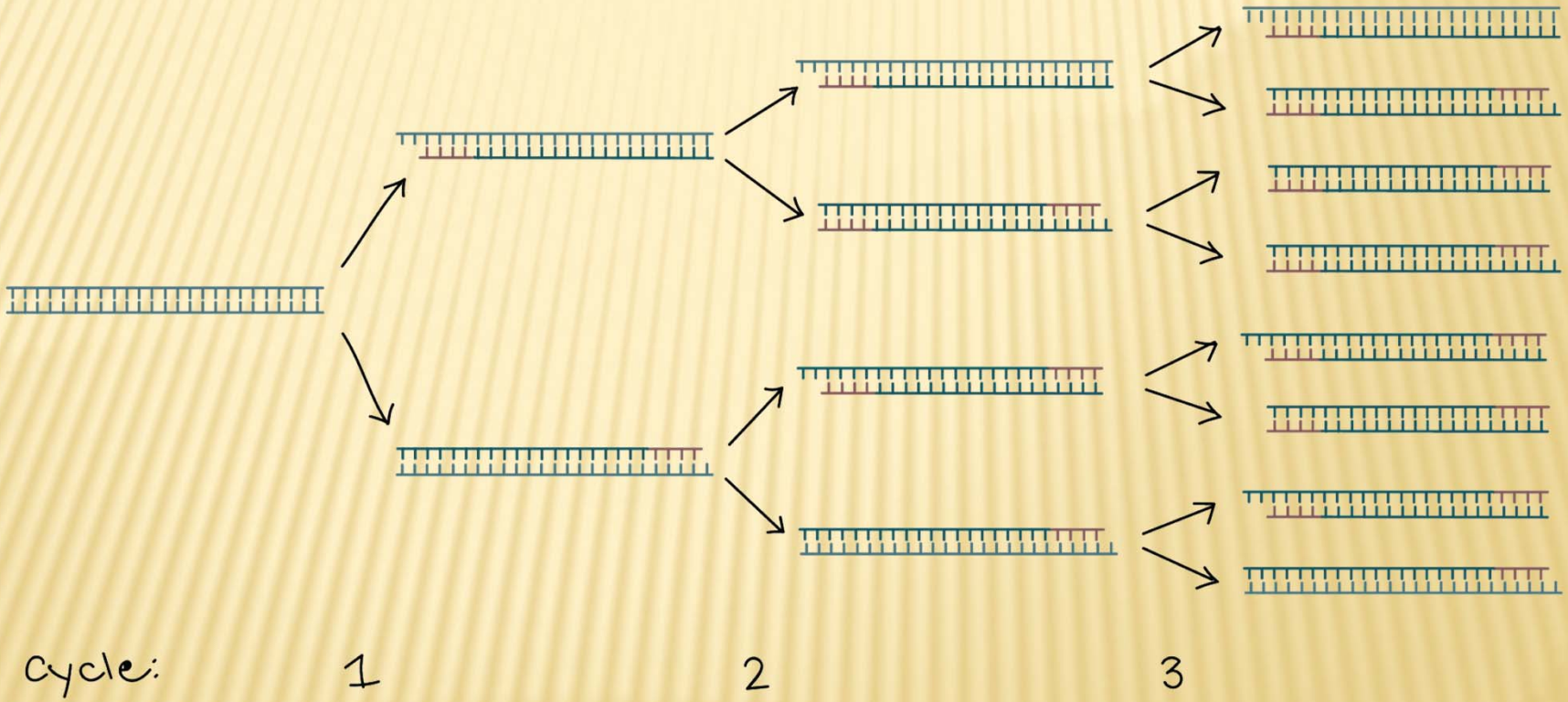
THE REACTION



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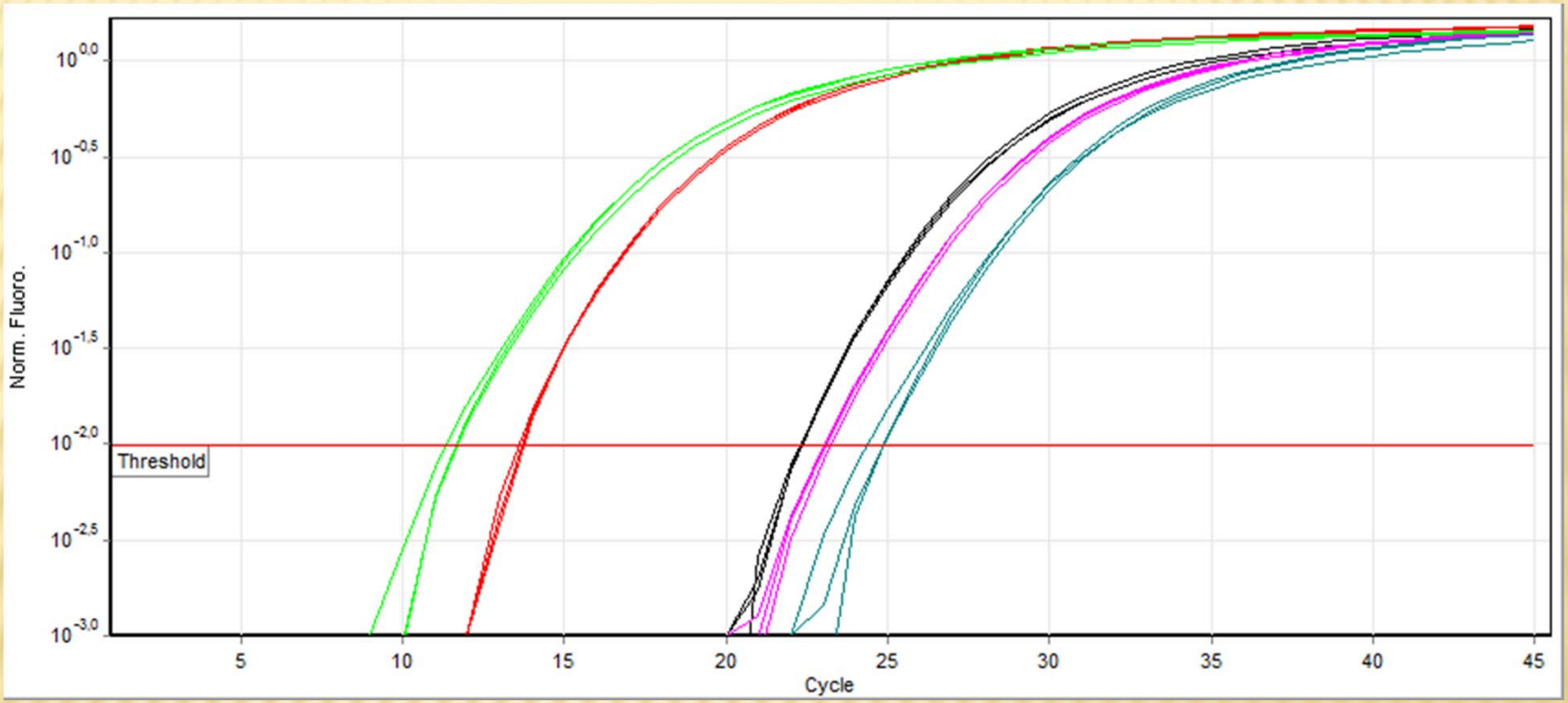
THE REACTION



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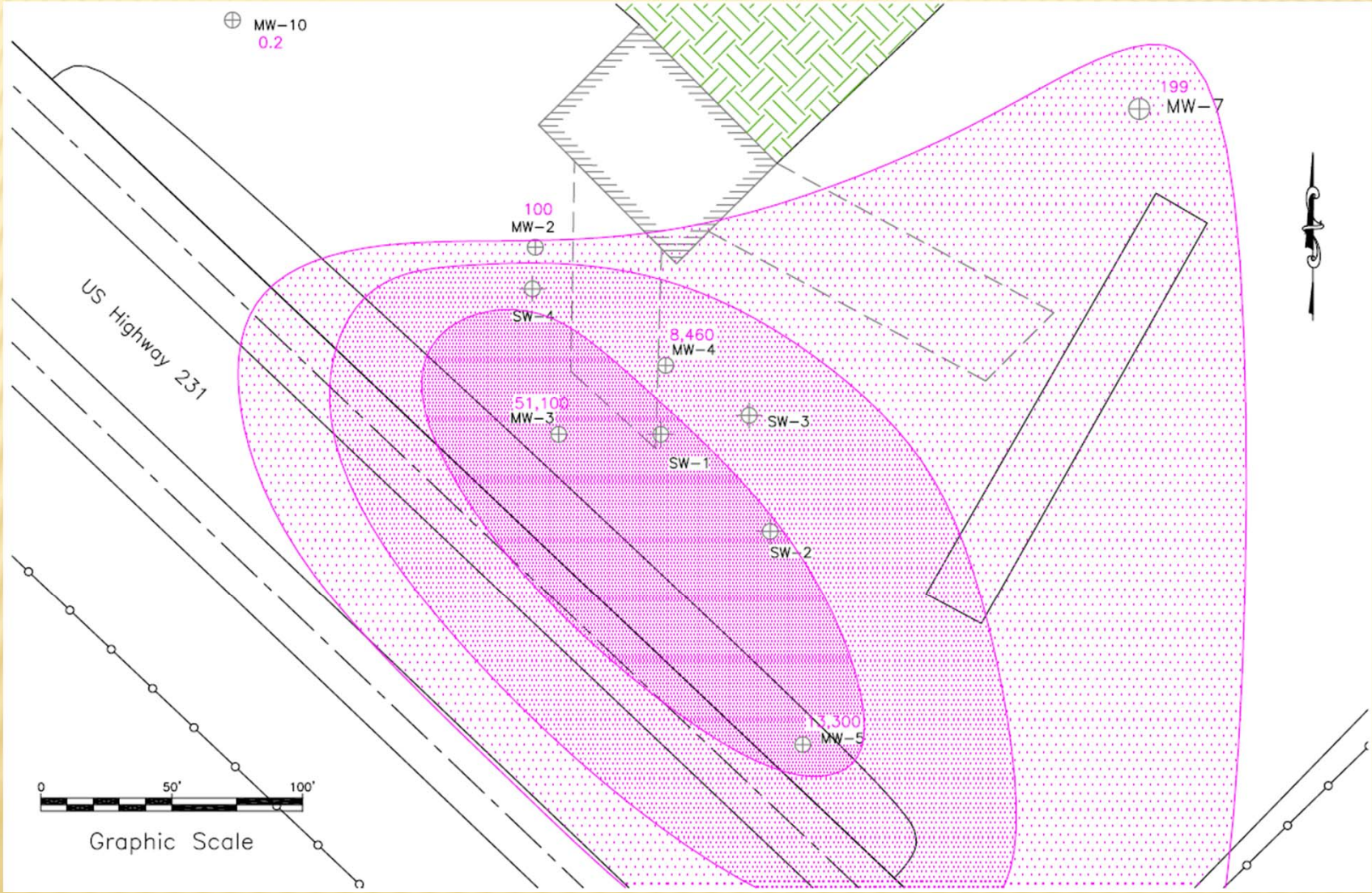
PHENOL HYDROXYLASE



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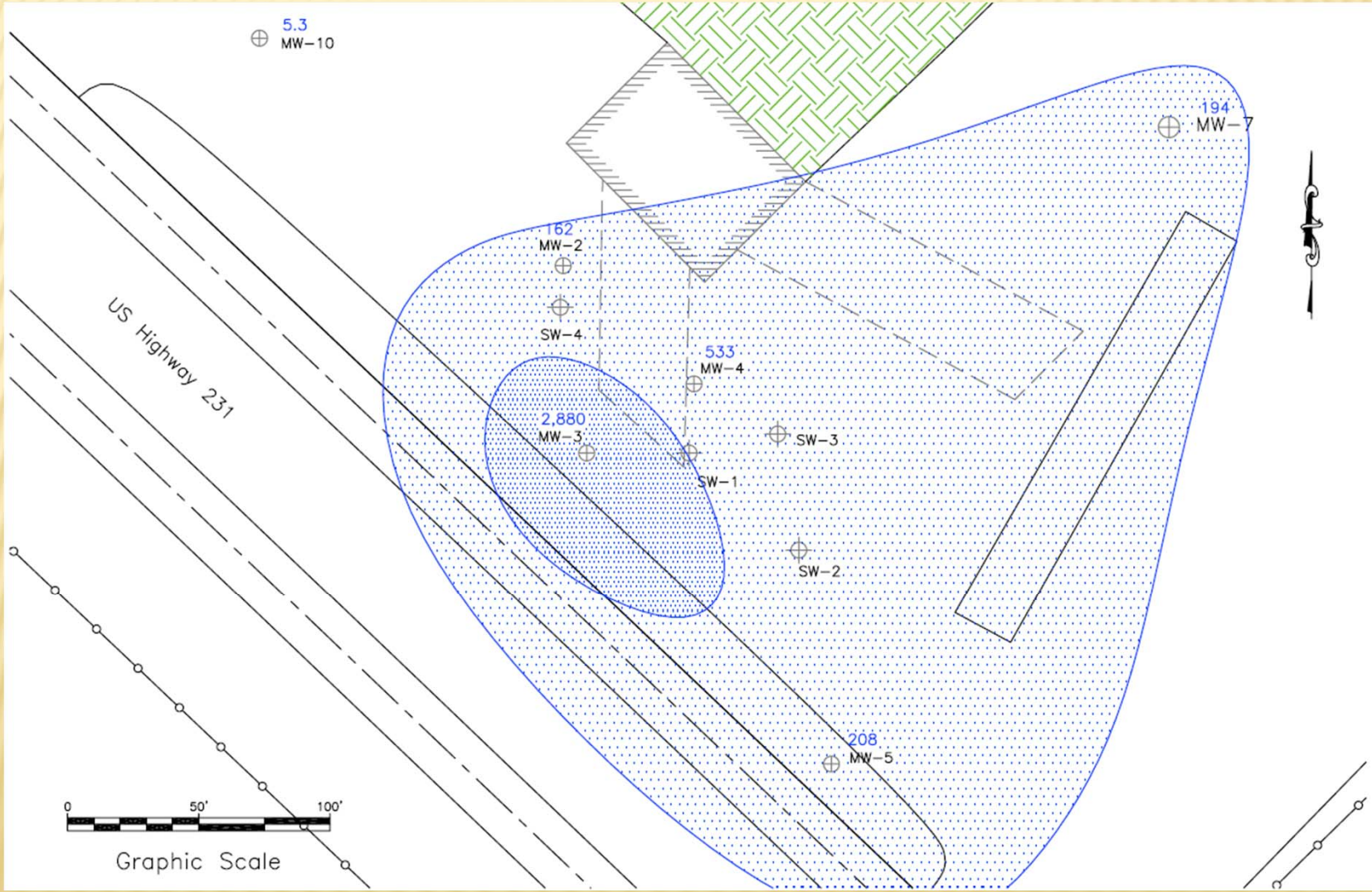
RMO PRIMER



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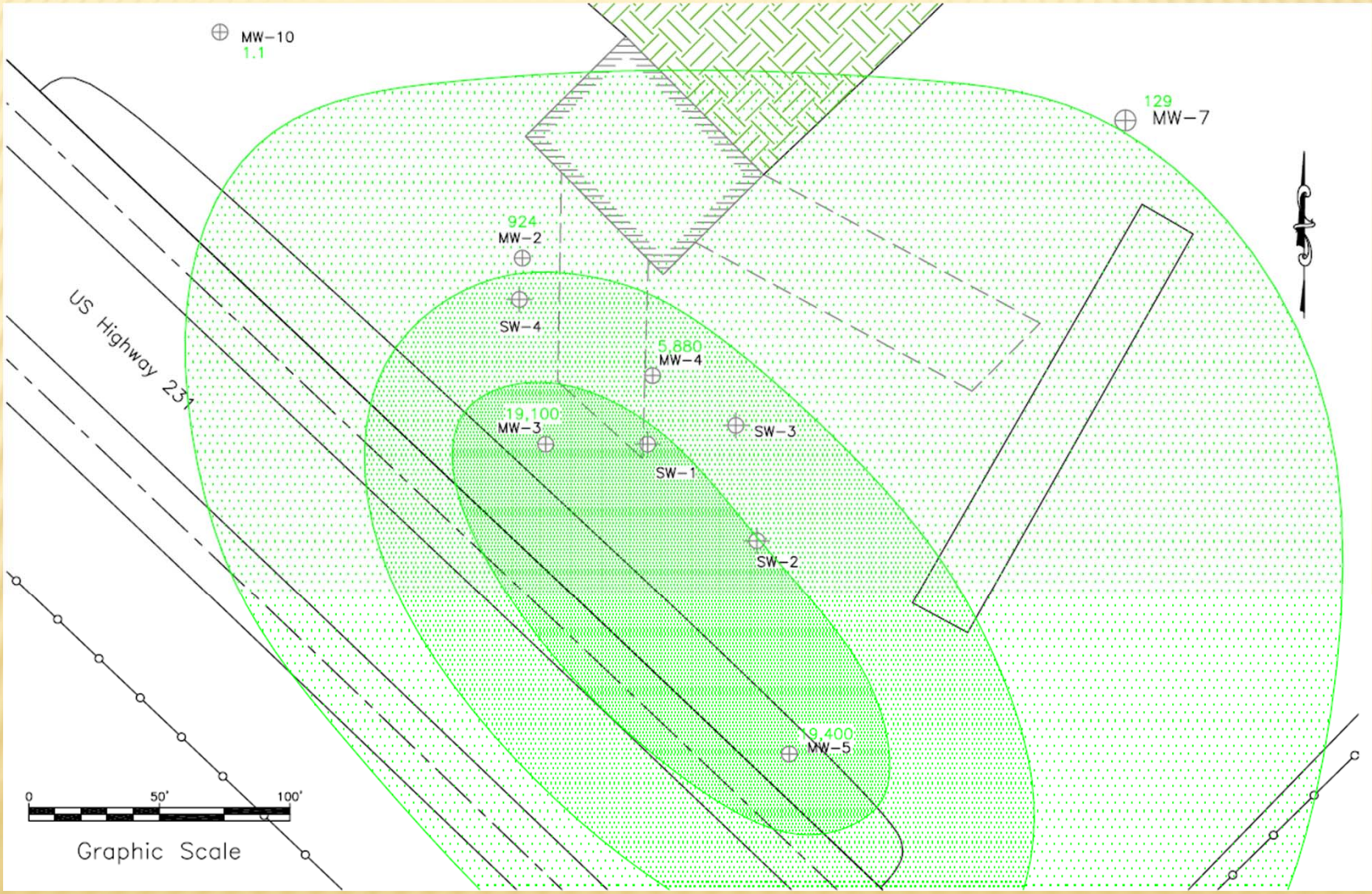
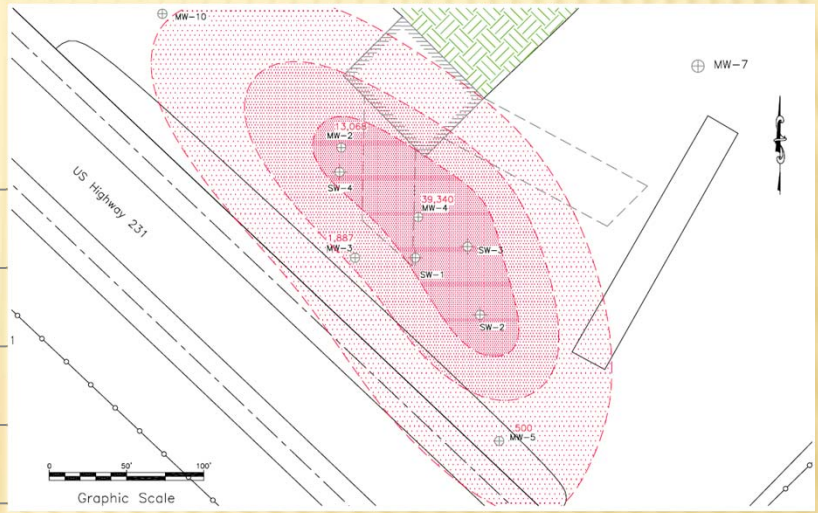
RDEG PRIMER



COMPARITIVE RANKING



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QUESTIONS?

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