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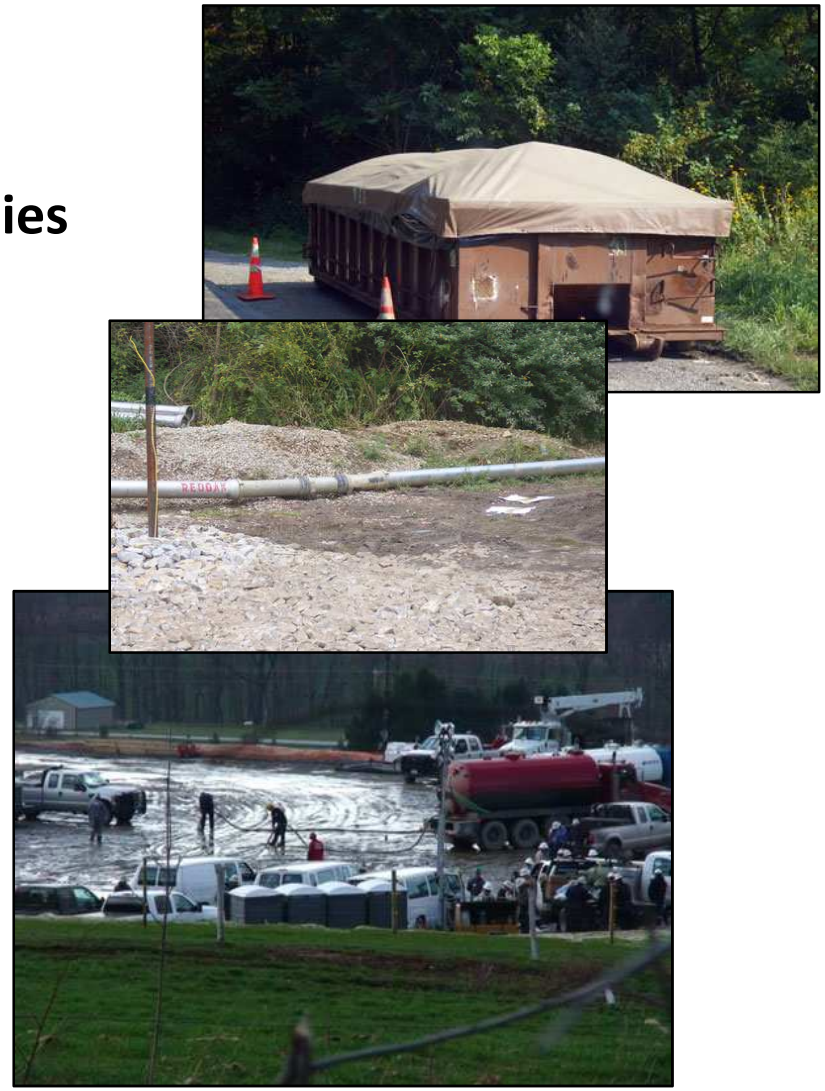
Recent Development of Cost-effective In-situ Bio-remediation in the Oilfield

Paul McKay, Bioxy Research
Dan Burdette, 3 Tier Technologies
Jason McMillan, Bioxy Research

Topics to be covered

- Discussion of the Technology
- Hydrocarbon Remediation Case Studies
  - Bio-Pile – Land Farming
  - In-situ Soil Remediation
  - Spill Response
  - Oilfield Wastewater Treatment
- Brine Spill Remediation Case Studies
  - China Saline Soil
  - Oklahoma Brine Spills
- Cost Comparison



The Triple Play Technology

- Core Technology
- Microbial Component
- Enzymatic Component

The Core Technology

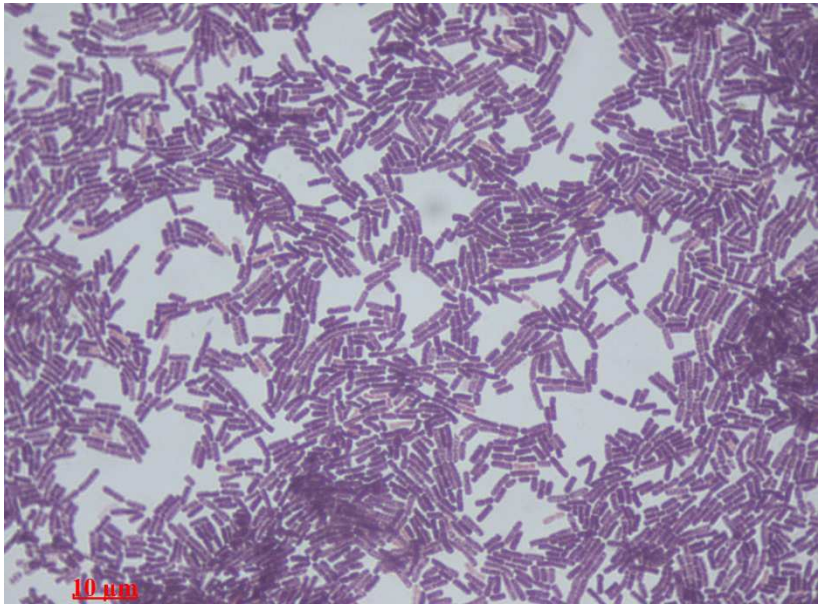
The Core Technology
The Advanced Organic Polymer (AOP)

- Originally…
  - Designed to immobilize heavy metals and prevent uptake into plants
  - Raw feedstock 48.6% carbon, 30.9% oxygen, 2.9% hydrogen, 1.1% nitrogen
  - Ion exchange reduced ash content
  - Contains an abundance of hydroxyl and phenolic groups which are key to metal complexation
  - Also applied to radionuclides in soil

- Now - The Advanced Organic Polymer (AOP)
  - Patent-pending polymer manipulation process that unfurls the long-chained molecule and releases activated carbon, fulvic acid and the hydroxyl/phenolic groups
  - Enhanced with polyelectrolytes
  - Variations developed for specific applications (specific heavy metals salts, H2S, facilitating microbe- and enzyme-based processes)

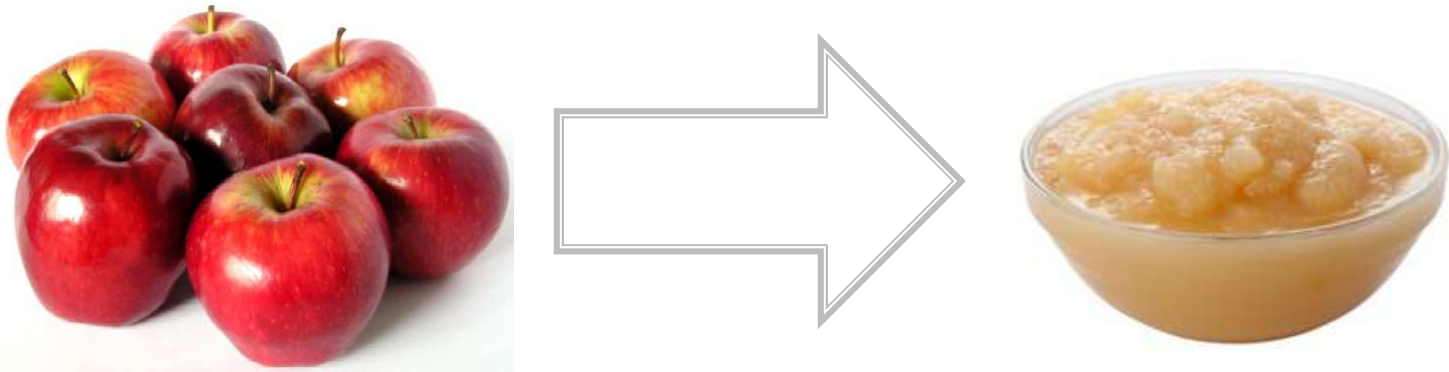
The Microbe Component
Keys to our success

- **Specialized** - Microbes selected for specific contaminant
- **Stable** - Sugar-based solution preserves microbes in dormant state for up to 2 years and stimulates exponential growth when activated
- **Tolerant** to most contaminants due to the AOP except chlorine
- **pH tolerant** - (4-10)



The Enzyme Component

- Contaminant-specific enzyme package
- 19 of the 20 most common amino acids
- Catalyzes the hydrolysis of complex molecules
- Decomposes hydrocarbon chains into more digestible components for microbes
- Also effective in H2S abatement
- Careful protein selection for thermal stability



What are the limitations?

- Must have contact with the contaminant
- Residence Time – Although results are typically seen in 30-90 days, longer is better; contingent on rate, methodology, etc.
- Capillary movement (wicking) can occur for sodium chlorides in arid environments; so maintenance dosage could be required
- Extreme acid events can break the biopolymer bonds
- pH must be between 4 and 10 (pretreatment?)

Land Farming - Argentina

- After 14 years, no vegetation was growing on this oilfield waste landfarm
- Need – Establish effective hydrocarbon reductions immediately
- 30-day results
  - Treated area grew vegetation
  - Sampling validated 65% reductions in all hydrocarbon levels.
- Entire site has been remediated in less than one year.

Hydrocarbon Contamination Soil Remediation

- High Clay Content Soils with 2% by weight each – crude oil, diesel, & gasoline (w/10% ethanol)
- Need – Determine reduction potential and time required on high clay soils. Benzene was primary target. Site is located in Nova Scotia Canada with 6 month season.
- Result – Benzene eliminated in 30 days with significant overall reductions in 60 days. Trial completed after 47 Day results. Entire site treated in August, 100% Benzene, Toluene, Ethylbenzene, and Xylene reduction after 30 days, all other BTEX ranges reducing faster in full scale treatment.

Sample Description				**Untreated 1**	**Treated 2T**	**Treated 3T**	**Treated 4T**	**Change**
Date Sampled				**04/17/2015**	**04/29/2015**	**05/18/2015**	**06/02/2015**	
Parameter	**Unit**	**G / S**	**RDL**	**6494946**	**6494949**	**6535218**	**6638857**	
Benzene	mg/kg		0.03	13.4	1.08	<0.03	<0.03	-100%
Toluene	mg/kg		0.04	858	192	2.28	<0.04	-100%
Ethylbenzene	mg/kg		0.03	270	122	7.98	<0.03	-100%
Xylene (Total)	mg/kg		0.05	1720	882	71.2	<0.05	-100%
C6-C10 (less BTEX)	mg/kg		3	4560	2340	17	5	-99.80%
>C10-C16 Hydrocarbons	mg/kg		15	6540	4620	4640	2250	-66.60%
>C16-C21 Hydrocarbons	mg/kg		15	5960	6570	7180	5590	-6.20%
>C21-C32 Hydrocarbons	mg/kg		15	4070	5090	8220	7490	84%
Modified TPH (Tier 1)	mg/kg		20	21100	18600	20100	15300	-27.50%
Return to Baseline at C32				Y	Y	Y	Y	
% Moisture	%			14	18	29	11	
Isobutylbenzene - VPH	%			118	126	96	66	-44.10%
n-Dotriacontane - EPH	%			125	136	79	N/A	-100%

Hydrocarbon Contamination Immediate Spill Response

- Petroleum spills occur daily at refineries, tank farms, well heads, and exploration sites. These results are from a refinery that had spills both inside the refinery and at the loading heads.
- Need – Immediate remediation and cleanup of two different spills
- Result – 100% cleanup of gasoline in 30 days and significant reductions on the crude oil.

CRUDE OIL

	Method	8/31/2009 Result	9/23/2009 Result	10/2/2009 Result	Change
Benzene	8021B	15	0.15	0.13	**-99.10%**
Toluene	8021B	54	2.5	1.8	**-96.70%**
Ethylbenzene	8021B	33	3.8	4.4	**-86.70%**
Total Xylene	8021B	190	68	45	**-76.30%**

GASOLINE

	Method	8/31/2009 Result	9/23/2009 Result	10/2/2009 Result	Change
Benzene	8021B	140	BDL	BDL	**-100%**
Toluene	8021B	2000	BDL	BDL	**-100%**
Ethylbenzene	8021B	810	0.52	0.64	**-99.90%**
Total Xylene	8021B	5500	7.6	8.4	**-99.80%**

Recent Test Results (September 2015) China Oilfield Wastewater Stream

EXPERIMENT SUMMARY

- A lab-scale test was performed on real-world oilfield wastewater sample
- Very difficult waste stream.
- Some of the best and fastest hydrocarbon remediation results we have ever recorded.
- Field results expected to be even better in larger biological system.

DRILLING MUD TREATMENT RESULTS

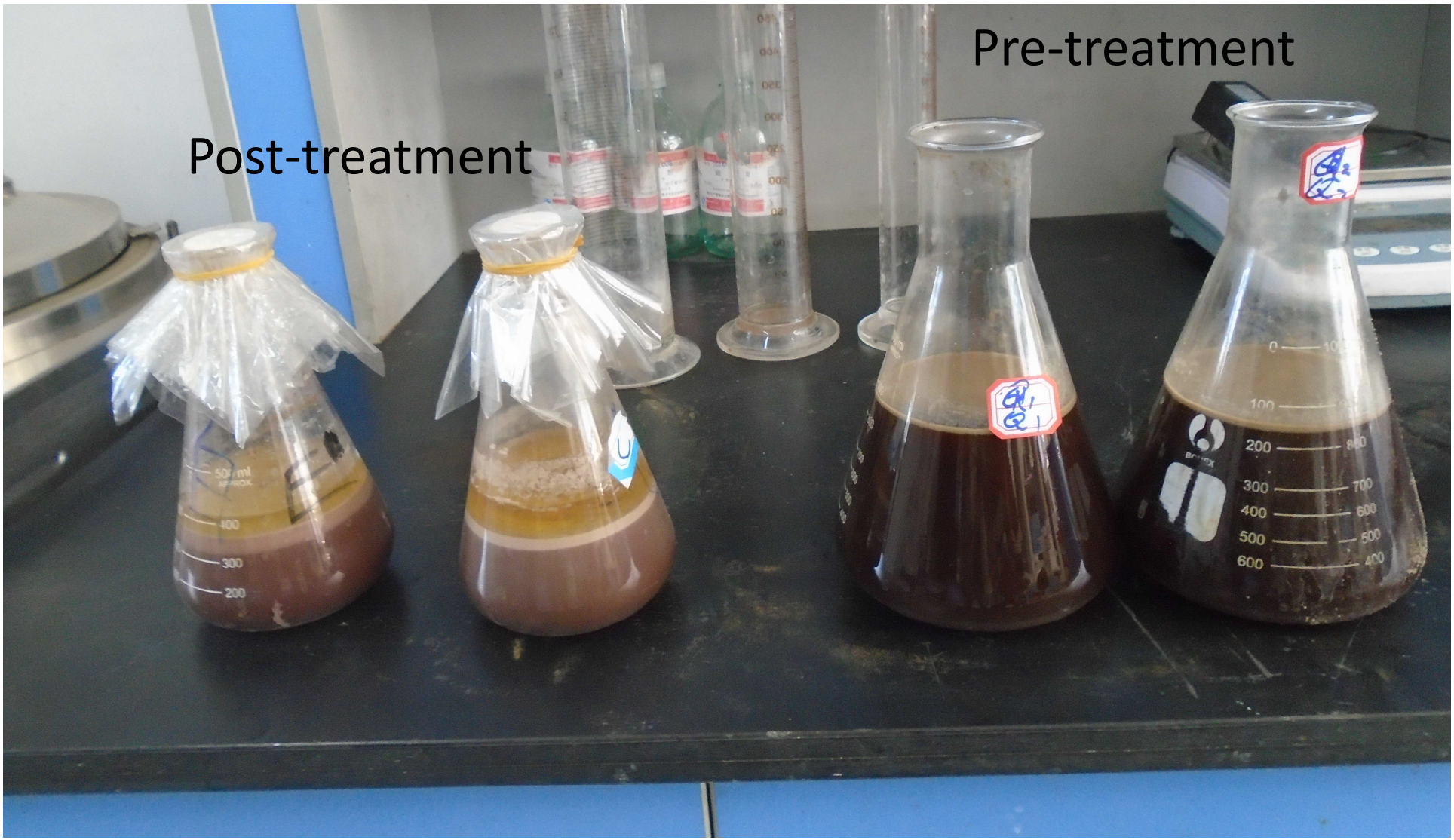
	Initial	Final	% Reduction	No. of Days
TPH %	0.98	0.10	**90%**	**16**
COD mg/l	42,777.50	2,940.8	**93%**	**23**
BOD mg/l	10,532.00	915	**91%**	**23**
Total C g/kg	12.56	1.26	**90%**	**16**
NH3-N mg/l	10.34	0.01	**100%**	**9**
SS mg/l	2,135.00	944	**56%**	**23**

Hydrocarbon Contamination Bio-Piling (Soil Recycling)

- Bio-Pile Location – Site land-locked for growth
- Need – Turn material faster, save on treatment cost
- Result – 33% faster turn-around time, $2.50 per cubic meter savings (28%), 31% increase in annual volume with same footprint.

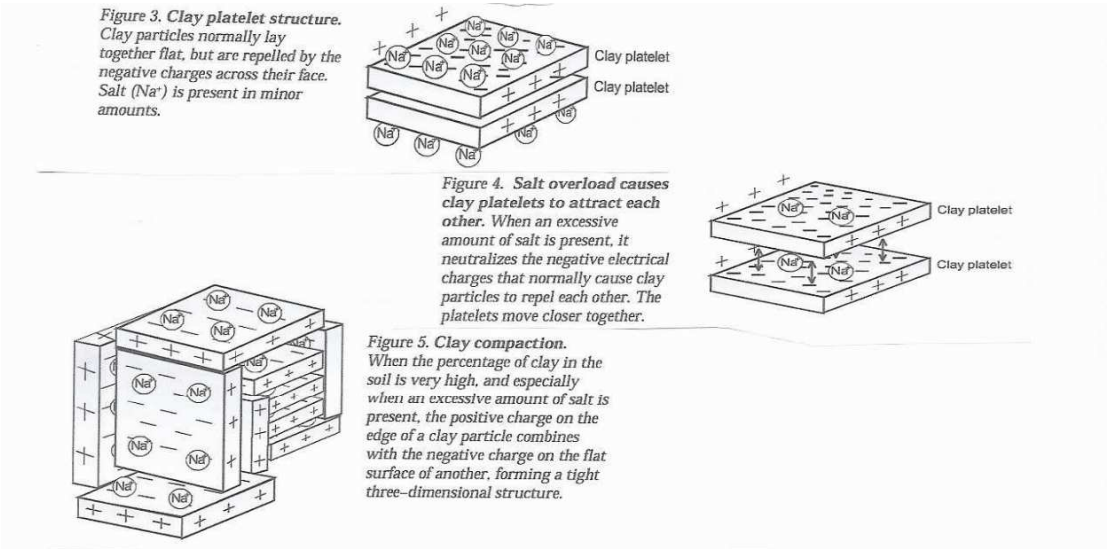
Parameter	Unit	RDL	July 9 Level	July 23 Treated	Treated Change
Benzene	ug/g	0.02	0.13	N/A	-100.00%
Toluene	ug/g	0.08	32	N/A	-100.00%
Ethylbenzene	ug/g	0.05	11	N/A	-100.00%
Xylene Mixture (Total)	ug/g	0.05	100	N/A	-100.00%
C6 – C10 (F1)	ug/g	5	440	N/A	-100.00%
C6 – C10 (F1 minus BTEX)	ug/g	5	300	N/A	-100.00%
C>10 – C16 (F2)	ug/g	10	5900	19	-99.67%
C>16 – C34 (F3)	ug/g	10	4700	130	-97.23%
C>34 – C50 (F4)	ug/g	50	<50	<50	
Moisture Content	%	0.01	11.3	6.1	-46.01%

Drilling Mud Wastewater Treatment
Unexpected Benefit - Separation

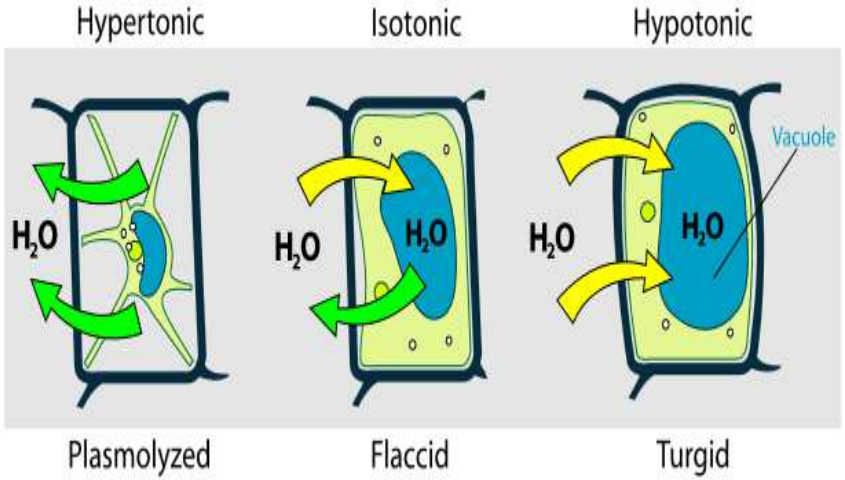


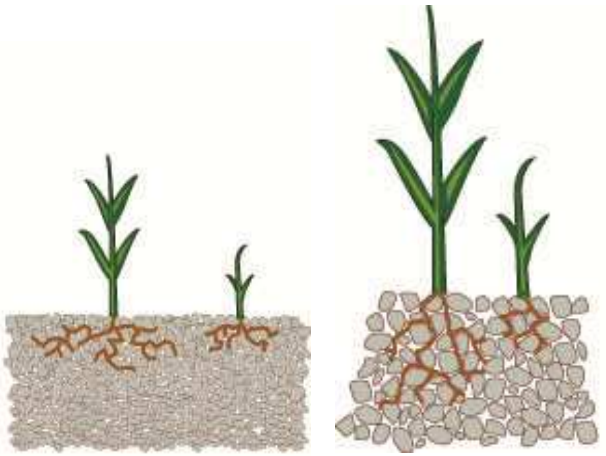
Brine Spill Remediation
The Effects of Salts/Chlorides on Soil

Sodicity
Creates
Compaction
And Dispersion



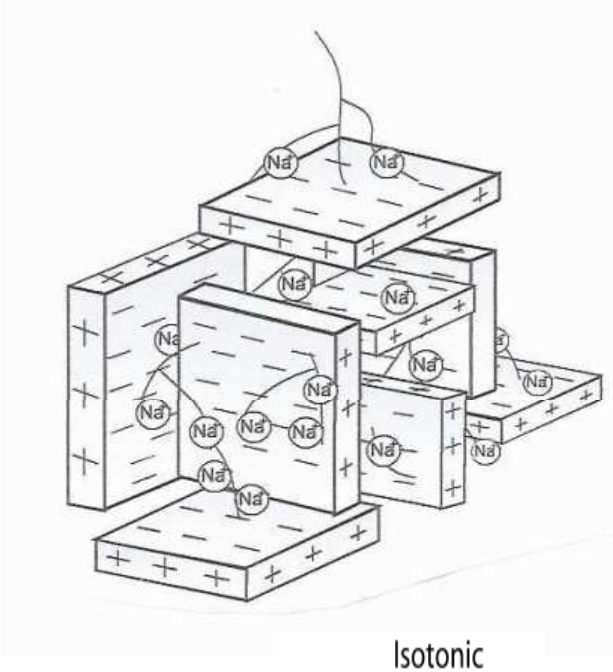
Salinity
Creates
Osmotic
Pressure
Issues



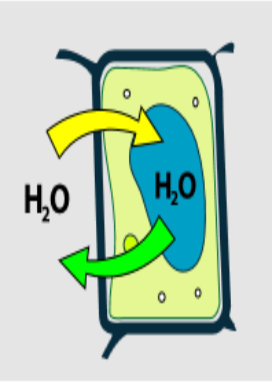


Nullifying the effects of salts

- Reversing Compaction
  - The two biopolymers and calcium bind the sodium cations and return the repelling negative charges to the clay platelets
- Osmotic Pressure
  - The binding of the salts returns the plant's root system to an isotonic state and allows water to penetrate the cell walls readily
- We have found that a blend of both ionic and non-ionic biopolymers plus calcium is ideal to battle the effects of brine.



Isotonic



Flaccid

High-clay/High Salt Soil Treatment

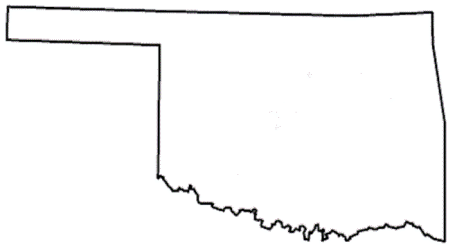
China Saline Soil Remediation
Reclaimed salt ponds (Oct 2015)

Salt-contaminated Field (6500 ppm)
Never supported crops prior to treatment

10 days after SaltBinder treatment and
winter wheat planting

Brine Spill Remediation with SaltBinder – Site #1

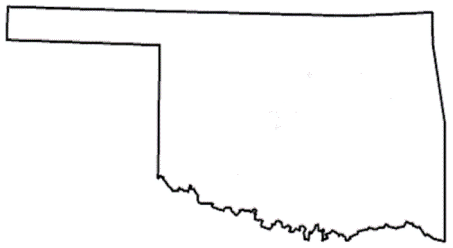
- Location and Date: Oklahoma; July 2013
- Contaminant: Produced water (brine)
- Impact Area: .58 acres by 8” deep
- Sample Analysis: Environmental Testing, Inc. (OKC)
- Testing Methods:
  - Total Soluble Salts: SM 2520A
  - Chlorides: EPA 300.0



Contaminant	Initial	30-day	60-day	+/-
Total Soluble Salts (ppm)	13,900	2920	1960	**-85.9%**
Chlorides (ppm)	6080	1080	674	**-88.9%**
Conductivity (um/cm)	21,545	4536	1235	**-94.3%**

Brine Spill Remediation with SaltBinder – Site #2

- Location and Date: Oklahoma; July 2013
- Contaminant: Produced water (brine)
- Impact Area: 1.74 acres by 8” deep
- Sample Analysis: Environmental Testing, Inc. (OKC)
- Testing Methods:
  - Total Soluble Salts: SM 2520A
  - Chlorides: EPA 300.0



Contaminant	Initial	30-day	60-day	+/-
Total Soluble Salts (ppm)	15,000	3530	3990	**-73.4%**
Chlorides (ppm)	6410	1170	1410	**-78.0%**
Conductivity (um/cm)	36,540	5472	6240	**-82.9%**

Basic Cost Comparison
Typical Salt or Hydrocarbon Spill

<u>Dig & Haul</u> - Rates vary dramatically by region and disposal costs. Cost for excavation, hauling, tipping fees, and soil replacement range from **$55 to $175 per cubic yard.** Client may still have liability issue with disposed material.

<u>Bioxy In-Situ Soil Remediation cost per cubic yard:</u> Product Cost - $8.00 to $17.85
Application (Labor and Equip.) $2.50 to $7.50
Excavation (if required) - $2.50 to $7.50
Average total cost is $10.50 to $33.00 per cubic yard.

<u>Factors That Influence Cost</u> – Loading, age of spill, time required to complete, method of application, soil conditions, site access, region, regulatory standard, and testing requirements.

Questions?

www.bioxyresearch.com

info@bioxyresearch.com