

got bugs?

Enhanced In Situ Aerobic Bioremediation

Kevin Sharfe



CleanEARTH

S O L U T I O N S L T D.

Enhanced Aerobic Bioremediation

- Microbe supporting emulsification
 - Enhance bioavailability using an organic solution
 - Overcome abiotic molecular kinetics and physical considerations
 - Attenuation of microbe competition, crowding and the accumulation of toxic by products
- Boost microbial production
 - Solution containing cytokines – molecules that signal or “trigger” cell development in microbes
 - Sequence of events triggered splits a water molecules liberating oxygen and making it available for use
- Laboratory analysis by the Institute For Research, Inc.
Dr. Mosier
- Field results

Let's define a sharp knife

Let's define a sharp knife

- A tool with an edge so fine that it must break the fewest number of inter-atomic / molecular attractions



Microbe Supporting Emulsifiers and Surfactants

Emulsions

- Enhancing bioavailability to speed bioremediation
- Hydrocarbon droplets encapsulated by a colloidal film
- Should not phase out. Maintain the oil / water interface necessary for bioremediation

Microbe Supporting Emulsifiers

- Organic
- Contact emulsification
- Stable emulsion without biocide
- Not simply an aqueous carrier. Microbes are **metabolically active** while being carried, more easily, through matrices
- By adding microbes prior to application they begin repairing cell structures and preparing enzyme systems
- Rapidly initiate bioremediation once applied to impacted area

Dispersion vs. Stable Emulsion

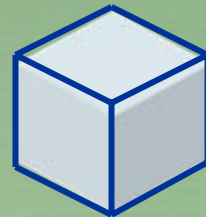
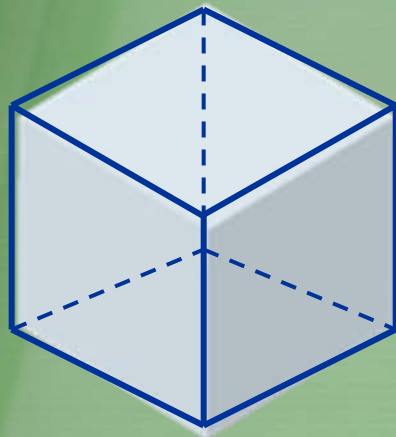
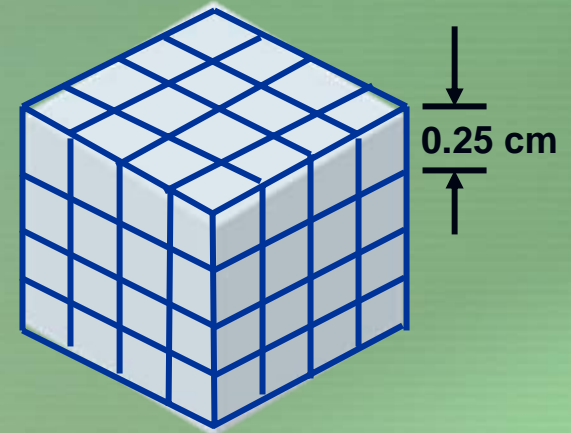
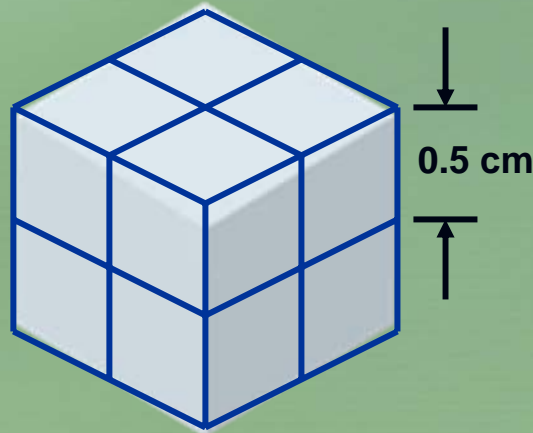
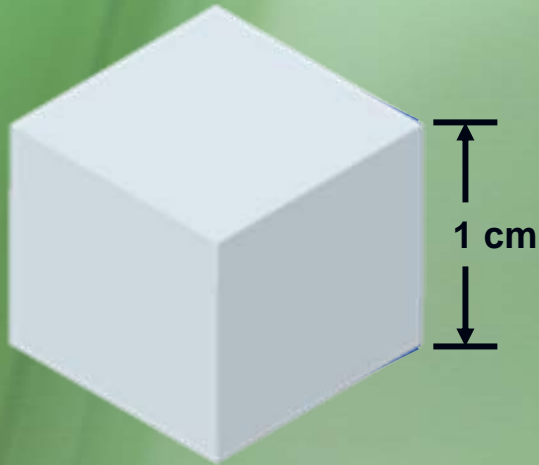


Dispersion using surfactants
or phased out emulsion



Microbe supporting stable
emulsion

Increasing Available Surface Area



X 8 cubes

$$0.5 \times 6 \times 8 \text{ cubes} = 24 \text{ sq. cm}$$



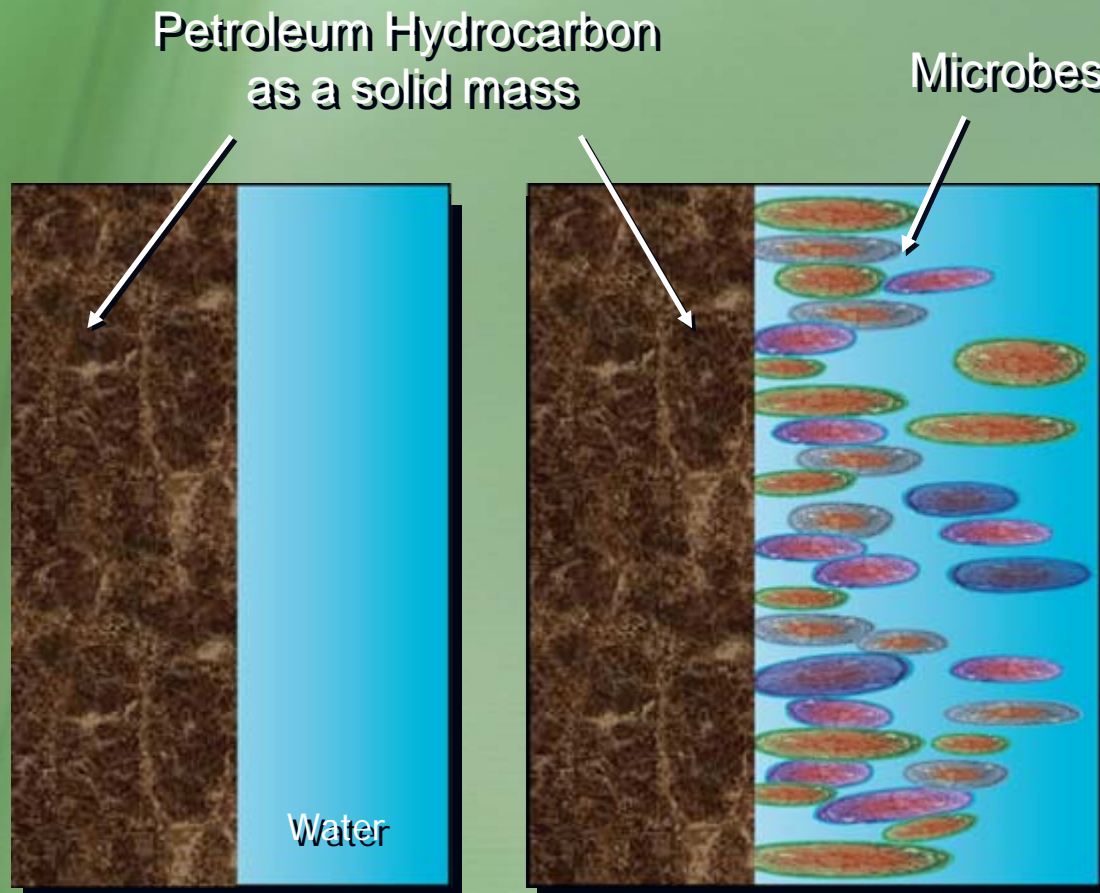
X 64 cubes

$$0.25 \times 6 \times 64 = 96 \text{ sq. cm}$$

$$1 \times 6 \text{ sides} = 6 \text{ sq. cm}$$

Exposed surface area increases exponentially as the hydrocarbon mass is divided. This greatly increases the water/substrate interface, where microbial action occurs.

“Normal” Bioremediation Illustration



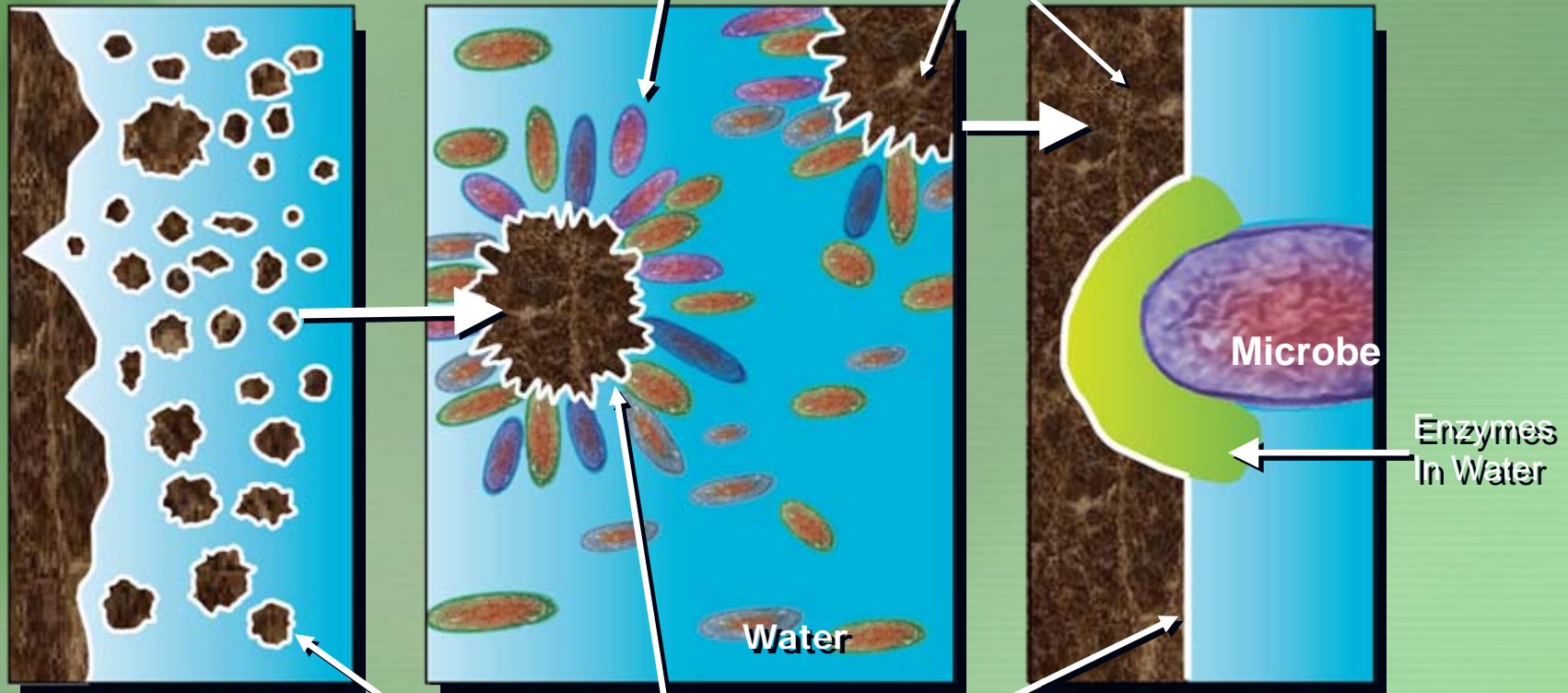
- Hydrocarbon inaccessible
- Microbe crowding and competition
- Limited reproduction

Bioremediation Using Microbe Supporting Emulsifiers

Hydrocarbon as droplets in water encapsulated by a colloidal film

Microbes

Hydrocarbon droplet



A

Protective Colloid

Attenuating

Crowding, Competition and Waste Accumulation

“Get outta my way!”

- When adequate nutrients are available and crowding and waste accumulation attenuated, the speed of growth and reproduction progresses exponentially
- Enhanced bioavailability creates an abundance of living space
- One bug’s toxin is another bug’s treasure... *or dinner*

Adsorption and Diffusion

Abiotic (**a = neg + bio = life**) process, an occurrence that is not dependent upon life forms for its actions

- Water carrying bacteria, enzymes and nutrients will be adsorbed to the soil particle's surface then diffuse between particles.
- Microbe supporting surfactants carry bacteria etc. through pores, particle voids and interstitial spaces ***before molecular kinetics take hold***

bacteria, enzymes and nutrient → hydrocarbons

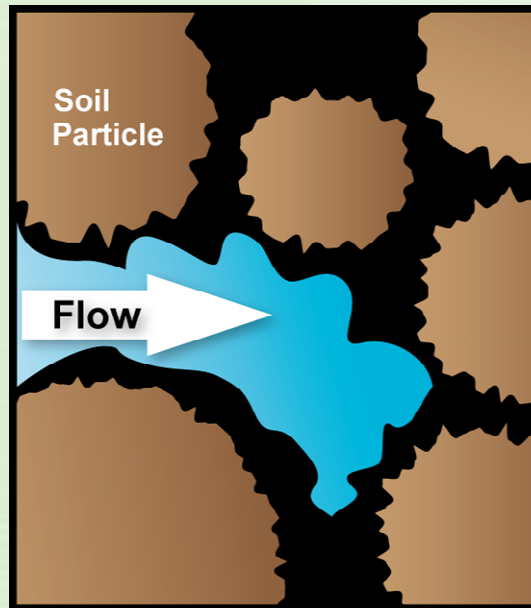
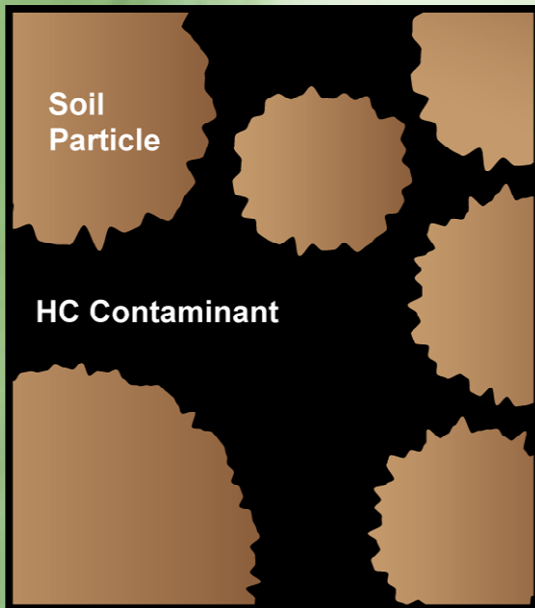
Diffusion of Hydrocarbon

- Hydrocarbon (hydrogen) affinity to the oxygen lining the sand (silicon dioxide) particle
- Bind to particles and diffuse inside particle spaces
 - Protected from microbial attack when sequestered between pores, voids and interstitial spaces
- Surfactants carry enzymes through
- Sequestered hydrocarbons are emulsified, removed and made available for bioremediation

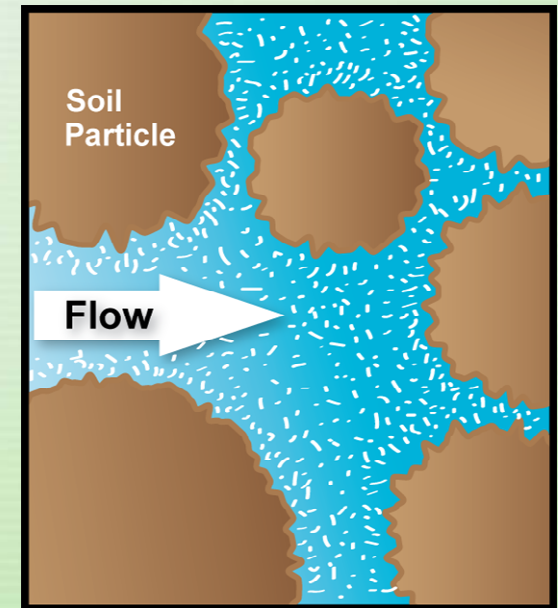
bacteria, enzymes and nutrient ← hydrocarbons

Physical Considerations

Partial Displacement
with immiscible material



Miscible Displacement with
microbe supporting emulsifiers



BioActive, a Biological Catalyst Increasing Microbial Activity

Cytokines

Cyto- (cell) + -kine (kinetic/action)

- Aqueous solution containing cytokines
- Cytokines are signal molecules used in cell to cell communication. Cytokines in BioActivate trigger both general and specific responses which are capable of triggering anabolic responses in microbes, stimulating microbes to great activity.

Biocatalyst Solution Analysis by the Institute For Research, Inc., Dr. Mosier

- BioActivate vs. tap water
- Dynamic sealed closed system
- Gas Chromatograph study
- Energy source, 5% dextrose – Lactose Ringer's
- Samples of gaseous matter extracted from the headspace of the sealed vessels of the Hach BODTrak manometer apparatus

End of Test, Headspace Gasses

Substance	Dry Air % By Volume	End BioActivate Test Vessel Headspace % by Volume
Nitrogen, N ₂	78.08	22.060
Oxygen, O ₂	20.95	< 1.019
Argon, Ar ₂	0.93	< 1.019
Carbon dioxide, CO ₂	0.033	27.563
Hydrogen, H ₂	0.00005	49.358
Plus other gasses (Neon, Helium, Methane, Nitrogen oxide, Krypton)	0.00367	Not Measured

- Carbon Dioxide increase – normal respiration product in aerobic microbes
- Decrease in nitrogen as gaseous matter. Needed and used due to formation of cell structures and reproduction
- Presence of hydrogen as gaseous matter
 - The possible breaking of water molecules by enzyme induced catalysis
 - The enzymatic electrolysis whereby the molecular hydrogen is separated from the water. Oxygen is liberated from its attachment to hydrogen.

Biocatalyst + Microbe supporting Emulsifiers

Continuous Cycle Of:

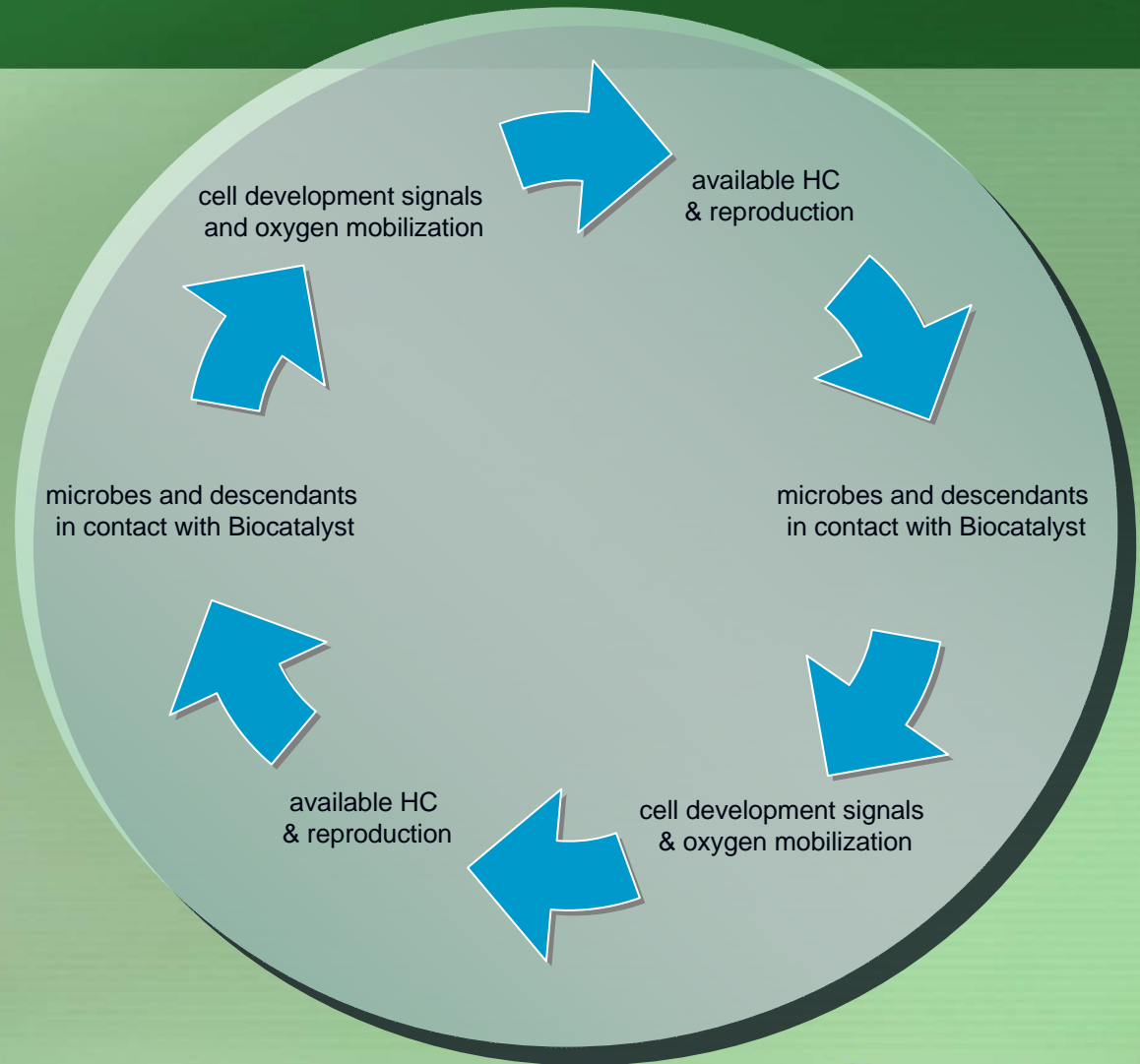
highly accessible hydrocarbons

+

cell development catalyst and available oxygen

=

enhanced aerobic bioremediation



Site Examples

Parameter	Initial Soil Samples	Sample 1 6 months	Sample 2 6 months	Sample 3 6 months	Sample 4 6 months
PHC F1 (C06-C12)	12,000	12	ND	ND	ND
PHC F2 (>C10-C16)	19,000	700	ND	210	ND
PHC F3 (>C16-C34)	11,000	770	ND	250	23
Benzene	ND	ND	ND	ND	ND
Toluene	0.41	ND	ND	ND	ND
Ethylbenzene	0.86	0.07	ND	ND	ND
Total Xylenes	32	0.17	ND	ND	ND

- Source: UST, Petroleum Hydrocarbons (measured in ppm)
- Depth: 10 to 15 feet below asphalt parking lot and apartment
- Medium and fine textured soil

Site Examples

Parameter	Initial Soil Sample 1	Initial soil Sample 2	Initial soil Sample 3	5.5 weeks Sample 1	5.5 weeks Sample 2
PHC F1 (C06-C12)	2,300	2,400	280	400	540
PHC F2 (>C10-C16)	27,000	36,000	13,000	12,000	11,000
PHC F3 (>C16-C34)	6,600	12,000	4,800	3,800	3,400
Benzene	ND	0.5	ND	ND	ND
Toluene	6.0	8.9	ND	ND	1.1
Ethylbenzene	7.4	8.0	ND	ND	1.5
Total Xylenes	67	75	1.1	3.1	15

- Source: UST, Petroleum Hydrocarbon (measured in ppm)
- Depth: 3 m below grade
- Fine sand and silt



Thank you for attending

www.cleanearthltd.com