

## **Expedited Petroleum Hydrocarbon Destruction via Biostimulation Alone under Baseline Conditions Considered Unsuitable for Bioremediation Without Augmentation**

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**Background:** Concept suggesting results historically obtained during bioremediation projects designed to enhance the destruction of organic contaminants may be serendipitous, with too much focus on planktonic densities and the genomic makeup present, and too little focus on the development and importance of biofilm.

Biofilm comprises any syntrophic consortium of microorganisms where cells stick to each other and/or surfaces. These cells embed within slimy extracellular matrices composed of extracellular polymeric substances (EPS). Cells within biofilm produce polysaccharides, proteins, lipids and DNA which is mixed and matched. Biofilm is three-dimensional, structured, and represents a community of microbes working in unison.

**Approach:** Regardless of additive input results, pro/con, often misrepresent the success/failure of the input due to a lack of appreciation for the nutritive capacity of the treatment zone and its ability to raise and subsequently support a microbial ecosystem capable of supporting organic contaminant destruction.

We suggest sites where baseline biogeochemical conditions *prior* to the introduction of an input are biologically viable; capable of raising/supporting a microbial ecosystem to include growth, communication, and the development of biofilm, more likely than not realize expedited levels of performance. Conversely, sites incapable of raising/supporting a microbial ecosystem prior to an input often fail to realize performance objectives. Blame/praise is given to the additive. What should matter is baseline conditions of the microbial ecosystem and its ability to support biofilm formation. We suggest neither the baseline density of planktonic bacteria nor the type of bacteria present matter.

In Dr. William Costerton's 1965 classic 'The Biofilm Primer' he states on page-65 'First, we discovered that starved bacterial cells are converted to very small dormant 'ultramicrobacteria' (UMB) that retain their full genomic complement and resuscitate to full size and metabolic activity when *nutrients* again become available'.

He further states '...numbers of these dormant prokaryotes are present in virtually every ecosystem in which nutrient content varies from feast to famine. Therefore, in all ecosystems, a vast library of genomes is available....can be mobilized and mixed and matched....to capitalize on any nutrient opportunities that are presented.'

**Process:** A field treatability study evaluated the need for minimal baseline heterotrophic plate count (HPC) densities and the influence biostimulation alone has on enhancing natural attenuation of petroleum hydrocarbon (PHC) contaminants. At a former trucking facility, where USTs were removed in the 1980s, diesel/oil range organics (DRO/ORO) in saturated soils/groundwater are  $\approx 100$   $\mu\text{g/L}$ . Residual source mass was suspected adjacent to the tank grave and downgradient under an abutting roadway. A solution of biostimulant **TPEnhanced** with water and surfactant (615-pounds additive to 1,200-gallons with 16-gallons surfactant) was

gravity fed three times (400-gallons  $T_1$ ,  $T_2$ ,  $T_3$ ) into a recovery well (RW-2) generating  $\approx 15$ -20 ft AOI. Groundwater was monitored from a network of six wells and RW-2.

**Conclusion:** We suggest biostimulation alone restored the supportive capacity of the microbial ecosystem allowing HPC densities to increase to a 'quorum' level ( $T_2$ ). As growth increased the level of communication also increased such that the microbial population collectively changed their phenotype from planktonic to sessile ( $T_3$ ) initiating secretion of EPS and biofilm formation. Within the biofilm they collectively expedite liberation of residual mass ( $T_4$ ) and collectively expedite PHC utilization ( $T_5$ ). As the PHCs (energy) are exhausted planktonic bacteria re-emerge ( $T_6$ ) from the mature biofilm. These 'scout' bacteria have evolved in real time to site conditions such that the density of the re-emerging population is five orders of magnitude greater than  $T_0$  and consist of a greater bulk fraction of degraders.

**Summary:** Support of the microbial ecosystem and biofilm formation is paramount to consistent and sustainable performance regardless of input, who, and how many bacteria are there to start. We need more evaluation into what factors limit nutrient(s) availability and how the mass flux of nutrients, growth, and biofilm formation/distribution affect microbial performance.