Bio-Regen: Triple Action Technology

Overview

Effective bio-remediation requires multiple systems to work as an orchestrated ballet to achieve maximum performance. If one of the key systems is compromised in the process the entire process will be affected and the required results will be inconsistent. These inconsistencies often reduce the performance that limits the desired results. For this reason, 3 Tier has spent several years researching the bio-remediation industry to identify the various challenges and create solutions.

3 Tier first identified that microbial and enzyme based products used for bioremediation of hydrocarbons, PCB's and organic waste constituents can be successful though it also identified that these processes are often inconsistent, slow, and can be affected by a variety of variables. To overcome these challenges, 3 Tier Technologies has discovered that current products offered in today's markets offer a single solution, either microbes or enzymes. The development of a complete regeneration solution would not only enhance the remediation of the organic challenges, it would also regenerate contaminated soils or wastewater streams to a healthy and active state for effective and safe release or re-use.

3 Tier achieves a complete regeneration system through the use of a special recipe of three distinct yet synergistic components; a blend of activated Polyelectrolyte Enhanced Biopolymers (PEB), a ultra high concentration of live, target-specific, naturally occurring bacteria, and a readily biodegradable natural enzyme product consisting of a nutrient-rich extract with a broad-spectrum package of identifiable enzymes, coenzymes, amino acids and other proteins. This next generation of bio-remediation products create a triple action solution while providing a unique stimulation to all bioremediation processes; bio-stimulation, bio-augmentation, and natural attenuation.

These hybrid products were created to be self-supporting while overcoming the various performance challenges that most of these environments present. High performance products must be complete with the ability to work in various temperature ranges, pH ranges, soil types, contamination levels, high salt environments, and various organic materials.

The Technology Behind the Solutions

Polyelectrolyte Enhanced Biopolymers (PEB): 3Tier's Environmental products are in a class by themselves and to see why, you have to look all the way down to the molecular level, to the very building blocks of natural remediation techniques. With a revolutionary, patented manufacturing process, 3Tier has bio-engineered very stable, organic compounds found in brown and oxidized black coal to produce **PEB™** – an activated long chain carbon based molecule that multiplies its benefits and removes the traditional limitations of bio-based products while introducing them to the world of bioremediation for the first time. **PEB™** is the cornerstone for all 3 Tier Technologies Environmental Products.

PEB™ is a unique Activated Biopolymer, ultra-pure balance of beneficial carbon groups that provides an unprecedented capacity to capture and absorb a wide array of contaminants found in soil, water, and waste water. **PEB**™ creates a platform for accelerated natural and augmented decomposition of organic compounds found in any soils or water columns. **PEB™** unique formulation also provides a stable remediation environment and protects the process from inconsistent results by reversing the effects of high or low pH levels, excessive salts, high clay compacted soils, or limited oxygen. **PEB**™ under the trade name Carboxx is OMRI Listed – Organic.

Our Activated Biopolymer differs from traditional biopolymers in several ways. The key difference is in the required steps to process the specific natural raw materials into this organic and reactive biopolymer. This proprietary process utilizes several additional steps that breaks very stable, organic compounds found in brown and oxidized black coal down to its molecular structure, removes all potential contaminants, releases and defines all the specific acid/functional groups including carbon, fulvic acids, and an abundance of hydroxyl and phenolic groups. The combination of all these components provides for a highly reactive solution that when added to contaminated sites stabilizes the inconsistencies and provides a balanced environment and support to critical biological activities.

PEB™ is a chemically, biologically and geologically active material. In contaminated soils, sodium, chemical pollutants, metals, and pH instability contribute significant obstacles to proper biological activities. The addition of **PEB™** adsorbs and coordinates sodium cations and chlorine anions which allow excessive amounts of salt to become more mobile in terms of sodium cations and chloride anions that have a natural ability to flush through the soil. Any sodium residue creates a new soil mineral formation resulting in sodium, chlorine, cation and anion conversion into physically and mechanically bound status, thus eliminating salt toxicity resulting in soil desalination and salt toxicity reduction/ elimination. **PEB™** will naturally stimulate toxic organic and mineral pollutants decomposition into neutral compounds. **PEB™**, with an abundance of hydroxyl and phenolic groups, provides these functional groups that are key to the metal complexation resulting in the binding of various metals. The presence of **PEB™** also establishes a balanced pH zone in which maximum biological activities can occur. All these processes generally require long periods of time, but **PEB™** accomplishes these results in short time periods, often detectable in weeks.

Microbes: Through our relationships with leading bio-remediation companies around the world, we discovered that current microbial products had similar challenges and limitations. The common challenges that needed dramatic improvement was stability, consistent performance in a variety of conditions, ease of use, and established application guidelines. 3 Tier's state-of-the-art microbial packages have been created to overcome all of these challenges.

Stability: 3 Tier developed a patented sugar based preservative package that neutralizes the bacteria spores in a chemical-free environment. This concentrate remains stable for over two years when stored under normal conditions. The most significant advantage to this natural preservative package is that it also provides the microbes the required carbohydrates and sugars needed for exponential growth and support after dilution. High temperature above 50° C (120° F) is the only danger to microbial products. Should the product freeze, this will not damage the integrity of the product unless heat is used to thaw.

Consistent Performance in a Variety of Conditions: The key to consistent results require a target-specific blend of microbes that are in a quantity significant enough to overcome and consume the contaminant while benefiting and promoting the indigenous biological environment. 3 Tier's microbiologist understands the various contaminants we are targeting and have developed microbial packages they are specific to the task with compatibility to the existing biological environment. These microbes use the specific contaminant as their food source and will remain active until the food source is depleted or remediatiated. After they have completed their task they will die and become another natural organic compound.

In addition to the microbes being specific to the task, they must also be in tremendous numbers to complete the task in a reasonable period of time. Through a proprietary fermentation process and the use of our natural preservative packages, 3 Tier has developed a process of maximizing the available microbial counts in every concentrate. Not only will every concentrate be guaranteed for viability, we also insure exponential growth once the concentrate is diluted. The combination of the diversity of makeup with guaranteed quantities and viability insure peak performance in a variety of conditions.

Ease of Use: A common practice of competitive microbial products in the market require the addition of special water, some type of carbohydrate to help the microbes grow, and often need to be prepared well in advance of use and then have a short time span when they are to be used before the solution goes bad. 3 Tier microbial products simply require dilution with a variety of potable and non-potable water to activate. The only limitation to the water source is high chlorine which will damage the microbial viability. Water can be drawn from creeks, lakes, oceans, tankers, wells and municipal water supplies without fear of hurting the microbial viability. We do recommend that given a choice, clean, fresh water will provide maximum results every time. After dilution, the product will provide maximum results for up to three days in a vented tank. After this time the microbial viability will reduce due to limited food sources and exponential growth.

Established Application Guidelines: 3 Tier and its trained distributors work closely with our customers to ensure that you are successful from the start. We understand that every project has its own set of unique challenges that must be considered when using this type of technology. Though we will continue to learn from every application, we do know the base parameters of each product and how to successfully apply and use the products. We can make recommendations on the best way to use the products and our staff will remain involved in the process as long as you request. We do not ship product and hope that you figure out the way to use it, we will guide you through the process and work with your staff to ensure the product has the best opportunity to perform.

Microbial Performance Parameters: During bioremediation, microbes utilize chemical contaminants in the soil or water column as an energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. Microorganisms also have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are pH, temperature of the soil/water matrix, and moisture content. Though 3 Tier has developed an advanced formulation to increase the performance ranges of our products and overcome these limiting factors, science has shown that the following performance ranges must be achieved or maximum microbial performance will be reduced.

- pH: Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons for example is optimal at a pH 7 (neutral); the maximum performance range is pH 5 – 9 Acceptable performance of our microbial products will be achieved in pH ranges from 4 to 10. The microbes will have an incremental reduction in performance in environments below 4 and above 10. Should the pH ranges of the treatment fall outside these ranges, methods for neutralizing the pH will need to be considered prior to treatment.

- Temperature: **The scientific rule is this**: With every 18 degree rise in temperature, from 32°F (0°C) degrees to 95°F (35°C) degrees, there is a 1.5 to 3.0 % increase in microbial activity. Optimum performance temperatures range from 40°F (5°C) to 98°F (36°C). Many factors influence soil temperature including lay of the land, vegetation covering the soil, length of solar exposure, and natural color of the soils. Each of these characteristics will influence the speed and overall ability of the soils to reach maximum beneficial temperatures.

- Moisture Content: All soil microorganisms require moisture for cell growth and function. Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of

biodegradation, becomes the predominant process. Soil moisture content "between 45 and 85 percent of the water-holding capacity (field capacity) of the soil or about 12 percent to 30 percent by weight" is optimal for petroleum hydrocarbon degradation and most organic contaminant decomposition.

Enzymes: Soil microorganisms are surrounded by organic matter that is rich in carbon and nutrients that are required for growth and cell maintenance. However, microbes cannot directly transport these macromolecules into the cytoplasm. Rather, they rely on the activity of a myriad of enzymes that they produce and release into their environment. These enzymes are proteins that catalyze reactions by lowering the activation energy of biochemical reactions and depolymerize organic compounds or contaminants that generate soluble oligomers and monomers that are then recognized by cell wall receptors and transported across the outer membrane and into the cell. Thus, the activities of extracellular enzymes are critical to soil functioning and for maintenance of the vast biodiversity of organisms in soils.

The enzymes found in Bio-Regen Products are made up of 19 out of the 20 known Amino acids. Amino Acids are often considered the basic building blocks of enzymes that form chains which sometimes are referred to as "Random coils". Although the enzyme coils may contain hundreds of amino acids, only a few of the amino acids of which it is composed are involved in the work of the enzyme. The poly peptide chains easily fold or twist so amino acids from different parts of the chain come together to perform various tasks with greater efficiency. These activities are the principal steps in regaining ecological balance within contaminated soils and water columns. 3 Tier specially blends our enzyme formulations to help in catalyzing the hydrolysis of the complex molecules present in a variety of contaminated environments, transforming them into substances that are easier to biodegrade. In short the enzymatic reactions turn apples into applesauce for easier consumption.

As with microbes, enzyme activity is also temperature sensitive. Enzymes possess greater thermal stability or their ability to maintain their structure across a wide range of temperatures. The structure and variations of proteins is the main determinant of their thermal stability. Enzymes have a natural ability to have a greater climatic adaptability and often work in a wider range of temperatures than microbes. All enzymes are not equally sensitive to temperature and even the same class of enzyme exhibits a variety of temperature sensitivity. It is widely assumed that enzyme activity roughly doubles with a 10°C increase in temperature.

Triple Action Performance Summary: 3 Tier's unique product formulation introduces the bio-remediation industry with the first complete multi-action solution that supplies all the critical components to effectively manage all levels of contamination, in a variety of environments. All aspects of effective bio-remediation are addressed through a product that delivers the critical components needed for complete remediation while promoting the natural and indigenous environment. The natural approach produces no harmful by-products which continue to work until the targeted contaminant food source is gone.

The process, in simple terms, starts with the activation of our concentrates to fully mobilize a target-specific microbial package with a complete support system. Once the product is added to the contaminant zone, the humic and enzyme components go to work to bind the contaminants and start the initial remediation process by breaking down the contaminants into simpler forms that promotes increased microbial consumption of the contaminants. At the same time, these same components are minimizing the various site, soil, and water challenges that can inhibit proper bio-remediation techniques. From this point, both the supplemented microbes and the indigenous biological environment can aggressively consume the organic contaminants is an effective manner.

The next question to answer is how do these products work on heavy contamination? We have discovered that heavy contamination is usually difficult to define due to the fact that everyone who has contaminated locations wants it cleaned up safely and effectively and all feel that any contamination is heavy to their situation. Knowing that any contamination is not acceptable to the client, our approach is more in working with all the various remediation techniques to provide cost effective recommendations that will provide the solution within the timeframes required by the customer for success. Factors that drive our solutions are:

- Type, level, and age of contamination
- Location/depth of the contaminants – soil or water
- Soil Type
- Conditions of the site – environmental/public safety, access, regulatory challenges
- Available remediation techniques – in-situ or ex-situ
- Time required to complete the process
- Regulatory standards

The key to a successful solution is adapting our products into all situations with scientific recommendations and techniques that will provide a positive result every time. We have developed application guidelines that are modified according to the information provided. Heavy contamination is merely a time/product/frequency equation that is part of the solution development process.

For additional information, contact 3 Tier Technologies LLC or an authorized distributor.