

The Triple Play Technology Behind Bioxy's Products

Effective bio-remediation requires multiple systems to work as a team effort 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 hamper performance and limit the desired results. Bioxy's product set are the result of a decade of research of the bio-remediation industry and its toughest challenges. These creative solutions that were developed (and continue to evolve) improve the efficacy, consistency and efficiency of the bio-remediation process.

It was first discovered that microbial and enzyme-based products used for bioremediation of hydrocarbons, PCB's and organic waste constituents *can* be successful, though these processes are often inconsistent, slow, and are affected by many variables.

Our mission is to apply a more reliable, consistent bio-remediation solution, removing the impact of the variables encountered.

Current products offered in today's markets usually offer a single solution, either microbes or enzymes. The development of a complete regeneration solution with the right blend of reagents would not only enhance the remediation of organic contamination, but it could also regenerate soils, water and wastewater polluted with salts, heavy metals, chlorinated hydrocarbons and other contaminants.

Our solution achieves a complete regeneration system through the contaminant-specific blending of three distinct yet synergistic components:

- 1) a blend of **Advanced Organic Polymers (AOPs)**,
- 2) an ultra-high concentration of live, target-specific, naturally occurring **microbes**, and
- 3) a readily biodegradable natural **enzyme** package consisting of a nutrient-rich extract with a broad spectrum of identifiable enzymes, coenzymes, amino acids and other proteins.

This next generation of bio-remediation technology creates a triple-play solution while providing a unique stimulation to all bioremediation processes; bio-stimulation, bio-augmentation, and natural attenuation.

Bioxy's next-generation environmental remediation products were created to be self-supporting while overcoming the various performance challenges that contaminated 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. It is the collaboration of three distinct components that sets us apart and usher in a new era in bio-remediation.

Core Component #1: Advanced Organic Polymers (AOPs)

Bioxy'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, Bioxy has bio-engineered very stable, organic compounds found in a rare deposit of oxidized black coal to produce the AOPs – an activated long-chain, carbon-based molecule. Our AOP is the core for all Bioxy environmental and agricultural products.

AOP is a unique activated biopolymer, an 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. AOP creates a platform for accelerated natural and augmented decomposition of organic compounds found in any soils or water columns. AOP's 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 anaerobic conditions. The product is OMRI Listed – Organic.

Key Differentiator: Patent-pending Polymer Manipulation Process

Our organic polymer 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, provides a balanced environment and supports critical biological activities.

AOP is a chemically, biologically and geologically active material. In contaminated soils, sodium, chemical pollutants, metals, and unstable pH create significant obstacles to proper biological activities. The addition of AOP adsorbs and coordinates sodium cations and chlorine anions allowing excessive amounts of salt to naturally flush through the soil. Any residue results in the formation of a new soil mineral through the physical and mechanical binding of sodium and chlorine cations and anions. This eliminates salt

toxicity and desalinates the soil. AOP will naturally stimulate toxic organic and mineral pollutants' decomposition into neutral compounds. AOP, with an abundance of hydroxyl and phenolic groups, also binds and immobilizes various metals. The presence of AOP also establishes a balanced pH zone in which maximum biological activities can occur. These natural processes normally require long periods of time, but AOP accelerates the process, usually detectable in weeks.

Additionally, the hydrophobic and hydrophilic sites and open receptors in the AOP make it a molecular scaffolding, providing microbes better access to hydrocarbon molecules and accelerating their consumption. AOP also scavenges H₂S, converting it to elemental sulfur and adjusting the oxidation reduction potential, inhibiting the formation of H₂S.

Component #2: 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 include a lack of stability, inconsistent performance in a variety of conditions, difficulty to use, and a lack of established application guidelines. Bioxy's state-of-the-art microbial packages have been created to overcome all of these challenges.

Key differentiator: **Stability**

Bioxy utilizes 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 excessive heat is used to thaw.

Key Differentiator: **Consistent Performance in a Variety of Conditions**

The key to consistent results is a target-specific blend of microbes in a quantity significant enough to overcome and consume the contaminant while benefiting and promoting the indigenous biological environment. Our microbiologist team has developed microbial packages that are specific to the contaminant being targeted while compatible with 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 remediated. 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. The proprietary fermentation process and the use of natural preservative packages maximizes the available microbial counts in every concentrate. Not only is the viability of every concentrate guaranteed, we also ensure exponential growth once the concentrate is diluted. The combination of bio-diversity, guaranteed quantities and viability ensure peak performance in a variety of conditions.

Key Differentiator: Ease of Use

Competitive microbial products often require the addition of special water and some type of carbohydrate to help the microbes grow. Many require preparation well in advance of use and then have a short time span when they are to be used before the solution deteriorates. Our microbial products are tolerant to many common water impurities. 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. The product has a flexible application window of up to three full days, after which the microbial viability will diminish if there are no contaminants to feed the exponentially growing colony.

Key Differentiator: Established Application Guidelines

Bioxy and its trained service partners 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.

Key Differentiator: Extended 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. The advanced formulation increases the performance ranges of our products and overcome these limiting factors.

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 to 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. This range extension can often help operators avoid the effort and expense of pre-treating for pH.

For 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.

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 produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation. 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.

Component #3: Enzymes

Soil microorganisms are surrounded by organic matter that is rich in carbon and nutrients 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 biochemical reactions by lowering the required activation energy and depolymerize organic compounds or contaminants into soluble oligomers and monomers. These less-complex entities 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 maintenance of the vast biodiversity of organisms in soils.

Key Differentiator: **Robust Amino Acid Makeup**

The enzymes found in Bioxy 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 sometimes referred to as “random coils”. Although the enzyme coils may contain hundreds of amino acids, only a few of the amino acids do the work. The polypeptide 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. We specially blend enzyme formulations to help catalyze 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.

Key Differentiator: **Thermal Stability**

As with microbes, enzyme activity is also temperature sensitive. Our enzymes possess greater thermal stability; i.e., they have the 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 Play Performance Summary

Bioxy’s unique product formulation introduces to the bio-remediation industry the first complete multi-action solution that supplies all the critical components to effectively manage multiple levels of contamination in a variety of environments. Bioxy products deliver three critical components needed for complete remediation while promoting the natural and indigenous environment: biopolymers, microbes and enzymes. The natural approach produces no harmful by-products, allowing the process to continue to work until the targeted contaminant food source is gone.

The process starts with the activation of our concentrated, target-specific microbial package complete with its own support system. Once the product is added to the contaminant zone, the biopolymer and enzyme components go to work to bind the



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contaminants and/or break them down into simpler forms more readily consumed by the microbes. 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 in an effective manner.

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

Whether you are dealing with soil or water contaminated with salts, heavy metals, PCBs, or hydrocarbons, we have the key to a successful remediation project. Adapting our Triple Play Technology and its application to almost any situation with proven scientific techniques, we will provide a positive result every time. Bioxy clients can enlist us to implement the solution or use their own employees and contractors. In any situation, we will always provide the product, services, training and support needed to ensure a successful remediation project.

Allow Bioxy to become a part of your team and we will close out the game on soil and water contamination with our Triple Play Technology.