



OPERATING MODE

Enzyme Catalysis in Fuels

FIELD OF ACTION OF THE XBEE ENZYMES

Combustion testing in all grades of diesel fuel, biodiesel, gasoline and E-10, as well as bunker fuel, demonstrate that **XBEE** improves combustion in all fuels. Several tests have shown that the enzymes in **XBEE** target aromatics. Additionally, **XBEE** appears to be breaking down the large, slow-burning Poly-nuclear Aromatic Hydrocarbons (PAH).

This accounts for the large reductions in soot observed in tests, as well as the reduction in PAH fraction in the exhaust. Aromatics, in particular the PAH molecules, are precursors to soot, and are also the source of carcinogens in diesel exhaust. **XBEE** has also been shown to reduce surface tension in fuel. The surface tension of the fuel has a large impact on the size of the droplets during fuel atomization. As **XBEE** reduces the surface tension, smaller fuel drops are formed, which allows for a faster and more complete vaporization of the fuel charge, and thus a more complete reaction with oxygen. This effect is corroborated by the reduction in CO in combustion testing, regardless of fuel type or aromatic content.

RESEARCH BACKGROUND

We now have over 20 years of field and laboratory experience with the enzyme formula used in **XBEE Enzyme Fuel Technology**.

We have conducted many laboratory studies on the effects of this unique product. Our independent laboratory testing has focused primarily on **XBEE**'s efficacy in purifying fuel and improving combustion, as well as engine and environmental safety. These tests, as well as field studies conducted by customers and OEM's, give us the empirical proof that **XBEE** is indeed an extraordinary fuel treatment.

A great deal of academic research on the mechanistic behaviour of enzymes on hydrocarbon substrates was conducted in the late 1970's and 1980's, primarily for oil-spill remediation and for bio-desulfurization of crude oil. In fact, **XBEE** can trace its roots to the industrial sludge treatment and water purification industries.



Mechanisms of enzyme catalysis

Mechanistic research and the development of the theoretical science about the molecular function of enzymes when suspended in organic solvents like fuels is more recent, and like all novel science, is still advancing...

There is a significant body of scientific theory on the mechanistic function of enzymes within biological cells, as well as many decades of research on the extracellular enzymes function in industrial applications such as enhancing petroleum recovery in wells and soil remediation. The mechanistic function of enzymes in non-polar, organic solvents such as fuel is less studied, but several research papers over the past ten years have described how enzymes suspended in organic solvents can catalyze novel reactions that are impossible in aqueous solutions.

Enzymes are catalysts which lower the activation energy of a reaction by providing a pathway(s) with lower activation energy than in a non- enzymatic reaction. For many years, a simple "lock and key" theory was used to describe enzyme-substrate specificity and function.

This simple model can still be used, but with a major stipulation: the substrate is not the "key". The key is the "Transition State". Generally, enzymes collide with substrates as they diffuse through the fuel, although some may be further attracted to substrates (target molecules) by electrostatic charges.

They contact their target molecules, and form Enzyme-Substrate complexes, called the "ES". Enzymes alter the electrical charges on substrates, temporarily binding to them. Between the starting point; a substrate binding to an ES and the release of the product, there is a temporary state called a transition state, which can be described as a high-energy structure somewhere between the reactant and product. After the ES is formed, the enzyme distorts the substrate, forcing it to the transition state. It is the transition state, rather than substrate, that is the key.

The substrate reacts only transiently with the enzyme. After catalysis, or "conversion", the product is released and the enzyme is free to perform more reactions. Some enzymes, under the right conditions, can perform catalysis as fast as the enzyme can diffuse



into the solution. Reaction rate acceleration can be as high as 1,017 faster than non-enzymatic reactions. One recent researcher has identified some enzymes could be as much as 1,021 faster. Enzymes can perform cleavage reactions, as previously described with PAH molecules. Hydrocarbons with covalent bonds (where pairs of electrons are shared by two atoms) are separated by the enzyme, with either one atom retaining both electrons, or both atoms each retaining just one electron. The substrate, once a large, difficult to burn molecule, is converted to two smaller molecules. To the engine, this means the fuel is more easily evaporated during injection, and can be ignited easier and react with more oxygen to burn more completely.

The enzyme's amazing speed at catalysis stems from binding modes. The binding forces behind ES complexes are charge-charge interactions, hydrogen bonds, hydrophobic interactions, and van der Waals forces. Charge-charge interactions are stronger in non-polar environments such as organic solvents than in water. It is believed that **XBEE's** extraordinary speed, efficacy, and longevity are derived from this phenomenon.

The substrate affinity and speed of catalysis are dependent upon the three-dimensional structure of the enzyme. Enzymes evolved to function in aqueous solutions and the position of individual water molecules within the enzyme's active sites are critical to both substrate sensitivity as well as the rate of catalysis. A monolayer, perhaps less than 500 water molecules, are always present, permanently attached to the surface of the enzyme. It is believed that when suspended in a non-polar, organic solvent, the hydrophobic force of the solvent pushes on the water molecules, in effect, forming a cage that holds the enzyme in its three-dimensional shape. This allows the enzyme to function over extraordinarily long periods of time, as well as improve its thermal stability.

Several thousand enzymes have been identified in animals. While most textbooks discuss the highly specialized actions of enzymes, known as "substrate specificity", this is a derivative of enzyme research having been financed primarily for human or animal biological studies. Plant enzymology is less studied. Plants evolved roughly five hundred million years before animals, at a time when the prehistoric soil was relatively barren of organic matter.



Plants typically produce only a few dozen different enzymes, some of which evolved to catalyze multiple substrates in order to secure nutrients critical to their growth. This phenomenon is known as "enzyme promiscuity".

Over two dozen plant enzymes are used in **XBEE** which provides a wide range substrate reactivity.

As previously noted, **XBEE** has surfactant properties. Standardized ASTM testing has demonstrated this phenomenon in petroleum fuels. Recently, other researchers working with enzymes within the same class as those used in **XBEE** have demonstrated that enzymes can work in concert with each other, resulting in a surfactant affect that is collectively stronger than the individual enzymes. These enzymes break down sludge and disperse microscopic drops of water during fuel agitation. An enzymatic bio-surfactant differs from the chemical surfactants used in detergents in that they never become permanently bonded to the substrate. Enzyme breaks the electrical bonds holding the sludge to the fuel tank walls and helps dissolve the sludge into smaller complexes, as would a detergent. The enzyme then releases from the dissolved sludge, or "products", and is free to act over and over, almost indefinitely. Chemical surfactants act only once, and become bonded to the product. Therefore they have a finite reach and lifespan in fuel. Far fewer enzyme molecules are required to do the same amount of cleaning work as many times their amount of chemical surfactants. This eliminates the potential precipitation of chemicals that occasionally plagues conventional fuel additives if overdosed.



Effect on emissions

As mentioned previously, **XBEE**'s cleaving action reduces the size of the larger aromatic hydrocarbons that are precursors to soot. Aromatics have an opposite relationship to cetane index and number, and fuels that have high aromatics are typically lower in cetane ratings compared to fuels with low aromatics. **XBEE** can significantly increase cetane ratings in fuels by reducing these larger aromatics. This function helps improve cold-starts, as well as generally improves combustion, and reduces the most toxic exhaust fractions; the PAH molecules.

Additionally, **XBEE**'s effect on reducing surface tension results in smaller fuel droplets and faster evaporation. When combined, these two effects result in hyper-oxygenation of the fuel as it is injected into the combustion chamber. This leads to a better and more complete combustion. Emissions tests demonstrate this effect through a reduction in CO and HC, including the toxic PAH fractions. Less soot results in a reduced rate of soot contamination in the lube oil, as well as preventing hardened carbon deposits from forming in the combustion chambers.

As concussive forces in the engine slough off old carbon deposits, combined with **XBEE**'s action of preventing carbon deposits from forming, engines are cleaned over a period of time. Endoscopic studies of a commercial marine engines by OEM's confirm these benefits result from **XBEE**'s improved combustion.

Inside the combustion chamber, the nitrogen in the air is oxidized into NO_x . Higher combustion chamber temperatures result in higher NO_x . By improving the rate of vaporization and ignition characteristics, **XBEE** gives the fuel charge more time to fully burn. This reduces or eliminates the amount of still-burning fuel that contacts the cylinder walls or is pushed past the exhaust valves, super-heating them. This tends to reduce NO_x , even as the CO, HC and soot particles are reduced.



Reduction of sulphur emissions

During combustion testing, **XBEE** has exhibited an additional effect; the ability to reduce SO_x . Currently, there is no conclusive science explaining **XBEE**'s observed reduction in SO_x emissions other than the obvious reduction from improved combustion and reduced fuel consumption. Over a time-weighted study such as an emissions test, there is less SO_x because less sulphur-bearing fuel went into the engine over the course of the test.

In some tests, SO_x reductions are beyond the reduction in fuel consumption. This is not understood. There are two prevailing theories. In fuels with high levels of sulphur-bearing molecules, the enzyme cleaves some of these molecules, leaving a portion of the sulphur free. Atmospheric oxygen present in fuel tanks is believed to react with the elemental sulphur, forming sulphur oxides that are then reacting with water in the fuel.

The new molecules are denser and gravity separate. The water, containing trace amounts of sulphur, is collected in water traps and is thus prevented from being combusted.

Another theory is that after the enzymes have acted upon the sulphur-bearing molecules, after the combustion cycle of the engine, the small un-combusted portion tends to form sulphites or sulphate particles, rather than react with oxygen to form SO_x .

These particles would be discharged as a small amount of solid particles in the exhaust, and are not detected by standard SO_x testing.



Effect on biomass

Organic contaminants such as bacteria and fungi are present throughout the environment. Many species can utilize the carbon in fuel for energy. All fuels, but particularly low-sulphur fuels and biofuels, are an excellent food source for microbial growth. During refining, a process called hydro-treating breaks down the sulphur-bearing hydrocarbon molecules. This allows the refiner to remove the sulphur as it lowers soot-forming aromatics, and together, this process allows the refiner to obtain a higher yield of cleaner burning fuel from each barrel of crude oil. Unfortunately, the elimination of sulphur has two negative results. First, the sulphur-bearing hydrocarbons provided diesel fuel with natural lubricity. To restore lubricity, the refinery may add a chemical lubricity agent. These additives are polar, meaning they will attract moisture.

For economic and environmental reasons, Fatty Acid Methyl Ester (FAME, also known as biodiesel) is mandated in much of the diesel fuel around the world and as FAME is an excellent lubricity agent, this is often the lubricity agent of choice. FAME is also polar and, it too, attracts moisture into the fuel. Water is necessary to support infestations.

Second, sulphur is a natural biocide. Its removal makes the fuel more prone to microbial infestation. With no natural biocide, and fuel now being hygroscopic, the incidence of microbial infestations and microbial influenced corrosion (MIC) in diesel has skyrocketed. An additional development in parts of the world has recently been uncovered. Ethanol, which is used as an additive in E-10 gasoline (petrol) or as an alternative fuel (E-22 and E-85) is often cross-contaminating diesel fuel during switch-loading. This is when diesel fuel is delivered in the same tankers as ethanol fuels, and the hoses are not thoroughly cleansed before fuel is switched. Further, highly volatile ethanol passes from its compartment through the venting system of the tanker to the non-volatile diesel, where it is absorbed. In 2016, in an extensive US EPA study, ethanol was found in 90% of the fuel tanks. Ethanol is broken down by bacteria of the acetobacter species and converted to acetic acid. This is the acid in vinegar. The volatile acid vapours rise to the fuel tank's ullage (vapour space) and rapidly corrode the metal components. The EPA study found 83% of the fuel tanks had moderate to severe rust, with the most severe damage being in the vapour space.



Although distillate fuels are sterile at their completion, with every transfer of fuel from a bulk tank to a pipeline or tanker, then the transfer to a service station or fuel wholesaler, then another transfer to the customer's fuel tank, the fuel must be vented to the air to prevent a vacuum. As air enters the fuel system, so does moisture and microbes, as well as dust and dirt. Even fuel in back-up tanks is exposed, as during the day, the fuel heats up and expands, driving out air. At night as the fuel cools, it contracts, sucking in air, along with contaminants. This was not a serious problem until the implementation of ultra-low sulphur diesel and biofuels like FAME and ethanol.

Biocides have been used for 50 years to control fungal growth. They are toxic to both the environment and the mechanic that gets diesel fuel on his hands in the shop. These can be harsh pesticides. No engine is perfect in burning all its fuel, and pesticide is spewed into the air we breath and the water we drink. The better solution is to use **XBEE** enzymes for breaking water emulsions, dismantling fungal growth and the dissolving of petroleum based sludge.

In effect, the enzyme is really nothing more than an organically obtained surfactant (detergent) which neutralizes charged atoms and keeps them from clinging to each other or to other surfaces. One benefit of using **XBEE** will be to disperse a small quantity of water on a microscopic level during fuel movement and eliminating it during combustion. As the water is removed from the fuel, microbial spores will not thrive, thus preventing filter plugging and MIC.

As corrosion in the vapour space of diesel tanks was not common until the implementation of ULSD and ethanol, there is no ASTM standardized test for additives to prevent vapour space corrosion. A 2013 test designed in-house by a petroleum engineer for a marine consumer report (Practical Sailor, August 2013) found that out of the largest national marine fuel additive brands, our enzyme technology was the best at preventing corrosion in contaminated diesel and the only additive that protected in the vapour space.

Along with Ultra low sulphur diesel and biofuels, an additional change in diesel engines, the implementation of High Pressure Common Rail fuel systems, has changed fuel housekeeping necessities.



With fuel pump pressures now commonly over 30,000 PSI, most OEM's have added fuel filters with pores as small as 4 to 5 microns. Some have adopted filters as low as 2 microns and are considering the possibility of 1 micron in the future.

Today, fuel must be nearly free of particulates. Warranties insist on fuel meeting ISO 4406, a rating standard for 4, 6 and 14-micron particulates. The intent of this specification, and the smaller filters, is to protect small fuel injector orifices from hard particles like rust, silicate and dirt. However, soft organic particulates like gums and biomass can coat the filters, preventing them from passing clean fuel to the engine.

Testing has demonstrated that the enzymes in **XBEE** dissolve organic contamination and can significantly reduce the organic particulates as measured under ISO 4406, even in old, oxidized fuel. This enzymatic action is the same action that provides **XBEE** with its stabilization function. Not being self-limiting like a chemical additive, **XBEE's** effects on preventing or reducing gums and particulate can last for months, or even years in fuel stored at ambient temperatures.