BIO-ORGANIC CATALYST

THE POWER IN NATURE®

NONTOX®

TECHNICAL REPORT

OIL SPILLS • OIL TANKERS

MARITIME BILGES • COMMERCIAL BILGES

STORAGE TANKS • FUEL TANKS TRUCK TANKS

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Introduction

The cleaning and remediation of petroleum hydorcarbon contaminated water and soil is a fundamental environmental challenge that impacts most industries and transportation systems. NONTOX® is a specially formulated bio-organic catalytic composition which greatly accelerates remediation rates, at very low relative costs compared to traditional remediation technologies. NONTOX® provides a superior cleaning capability when used to wash petroleum hydorcarbon based contaminants, then allows for their subsequent biological breakdown, so that the cleaning/ remediation process is combined together into a synergistic and complementary procedure. NONTOX® is a highly concentrated bio-organic catalyst composition formulated to provide instant protection from accidental hydrocarbon ignition during cleanup of petroleum spills and wastes. NONTOX® contributes oxygen to encourage indigenous microbial colonies to quickly consume and neutralize these hazardous compounds in both water and soil applications.

Benefits

- Removes up to 90% to Total Petroleum Hydrocarbons (TPH) in 96 hours.
- Nontoxic formula does not require personal protective equipment to apply.
- Provides immediate and ongoing VOC odor suppression.
- Able to provide superior cleaning and breakdown of oil coatings and wastes
- Accelerates biodegradation rates of total petroleum hydrocarbons (TPH).
- Improves the effectiveness of most other remediation technologies.
- Treats all types of petroleum hydrocarbon contamination.
- Helps in the precipitation of metals in wastewater discharges.
- Can be used at extremely high dilution rates with no special equipment.
- Reduces costs associated with soil and water remediation and clean-up.
- Safe for Humans, Animals and Marine Life.

Petroleum Hydrocarbon Remediation and Cleaning

NONTOX® is a biocatalytic system in a liquid concentrate form that stimulates and accelerates natural biological reactions.

When combined with fresh or salt water and oxygen, the product will cause crude oil, jet fuel, diesel oil and other organic substances to rapidly decompose, eventually biodegrading them to carbon dioxide and water as end products.

- It is non-toxic and safe to humans, animals, marine life and plant life. It is 100% biodegradable.
- Works in concert with indigenous bacteria. No cultured or foreign bacteria are introduced into the ecosystem.
- Is nonflammable. It will reduce fire hazards by increasing flash points and auto ignition threshold points in substances such as gasoline or fuel oil.
- Eliminates obnoxious odors associated with crude oil, petroleum derivatives and other organic molecules that are proceeding through the natural decomposing process.
- Is fully compatible with most types of application equipment now in use. The product may be easily applied by hand or power sprayers, helicopter, airplane or floating equipment. Its application requires no special safety equipment.

Targeted Hydrocarbon Contaminants

In this case, the hydrocarbon compounds found in water, soil and air are the selected targets of NONTOX®. This would include such petroleum derived products as crude oil, drilling mud's, creosote, kerosene, coal tars, gasoline, diesel, bunker fuels, lubricating and hydraulic fluids. Other contaminant groups would include aliphatic and aromatic hydrocarbons, poly nuclear aromatic hydrocarbons, chlorinated aliphatic compounds, chlorinated aromatic compounds and chlorinated and non-chlorinated phenols.

It has been shown that the product has unique features in odor elimination of such gases as hydrogen sulfide, ammonia, mercaptan and other noxious odors emanating from anaerobic decomposition. The odor degradation activity happens in a very short period and effectively eliminates volatilization of light chain organic molecules, such as the BTEX group of petrochemicals, into the atmosphere.

Treatment Methodology

The product is fully compatible with most types of application equipment now in use. NONTOX® may be easily applied with hand or power sprayers, standard educator tubes, helicopters, airplane or floating equipment. No special nozzles or hoses are required.

Each treatment site may differ in its requirements and modality of treatment.

Factors that can influence the tactical use of NONTOX® are:

- Redo Potential Temperature
- Availability of Nutrients and Concentration of Contaminants
- pH or Heavy Metals

Should these variables complicate the application and treatment procedures, the NONTOX® works well with other commonly accepted treatment modalities such as venting, injection aeration, aeration lagoons and inoculants' for removal of heavy metals.

Water Or Beach Spills

Reducing the danger of oil reaching beaches and shore structures is best achieved by spraying the oil slick perimeter with a diluted solution of 10 gallons of NONTOX® mixed with 150 gallons of sea water or fresh water for each 40,000 square feet of surface area to be treated. If the slick has a heavy consistency, it is recommended that a 1:15 diluted NONTOX® solution is applied over a three-day period, using one-third of the mixed solution each day. For best results, the product should be applied at a high pressure - generally above 500 psi.

Product Actions

Bacterial Proliferation

The successful biodegradation of petroleum is dependent on two factors: 1) having the bio-organic catalyst reduce the petroleum to a form, which can be readily assimilated, by bacteria and 2) stimulating the proliferation of naturally occurring nonpathogenic heterotrophic bacteria. NONTOX® to significantly increase beneficial bacterial activity in bay water by 12,857% and ocean water by 14,333%.

Accelerated Bioremediation

Independent laboratory studies from specialists in petroleum technology have quantified the ability of NONTOX® to dramatically reduce petroleum contaminants. Showing a 90% reduction in Jet-A, Diesel-2 and Heavy Duty Lube Oil within 96 hours. While treatment time required may vary dependent on conditions previously noted, the mode of action is the same. NONTOX® is a unique biocatalytic system that accelerates natural biological reactions with hydrocarbon products in water.

Metal Contamination Precipitation

Another benefit of NONTOX® use is its ability to break the matrix that suspends metals.

Flammability Reduction

Open cup flash points and auto ignition temperature tests quantify the ability of NONTOX® to render petroleum products nonflammable and dramatically increase their auto ignition temperatures. NONTOX® alters the molecular structure that dramatically reduces flammability and the elimination of volatile organic compounds (VOCs) and their odors. The importance of this feature cannot be overstated in terms of shipboard safety and survivability. In addition, the use of other ecologically incompatible materials, such as AFFF Foam, may be significantly reduced.

Safety Profile

Extensive independent laboratory testing utilizing accepted standards for dermal and ocular effects on animal and human subjects have been performed. Phytotoxicity, bacteria community and internal aquatic organism safety studies are well documented.

Other Possible Applications

- Initial Actions for Fire Fighting, i.e. cover the fire hazard with a layer of AFFF and flash point reducing product.
- Fuel or Oil Tank Cleaning
- Engine / Generator Wipe down
- Galley Drain Line Unclogging
- CHT Tank Cleaning / Degreasing
- Flight Deck Cleaning (should be able to hose it over the side).
- Trough Cleaning

Ecological Restoration of Petroleum Hydrocarbon (TPH) Pollutants

Oil spills in a marine environment presents massive challenges to the entire ecological chain of life. The toxicity of petroleum hydrocarbons can wreck damage to marine life and significantly compromise the invisible microscopic living organisms that support the entire food chain.

The blockage with booms, and the absorption of the oil spill through a wide variety of equipment and systems, has evolved over the years with oil spill response services. What has remained the greatest challenge has been how to mitigate the resulting toxicity and vast contamination of seawater and shoreline habitats impacted by widely dispersed petroleum pollutants.

Scientific studies of previous oil spills, such as Exxon Valdez, have shown that the most effective approaches have been those that enhanced the natural microbiological ecologies that consume these pollutants. Unleashing nature's own mechanisms through supplemental nutrients, such as fertilizers and organic carbon sources, allow much higher populations of indigenous microbiology to work in digesting the pollutants, and reducing them into harmless bio-products.

Bacterial/enzymatic cultures exhibiting hydrocarbon preferences can be useful in depleted soils and some water bodies, but in oceans and seawater bodies there is abundance of complex and interconnected microbiological ecologies that overwhelm any additional seeding of bacterial/ enzymatic cultures. In fact, they could be counterproductive in the ecology's own vitality being activated. However, nutrient, or metabolic stimulation, additives can be very useful in supportive in establishing an ecological restoration of these marine environments.

Over the past decade, extensive work has been underway in using innovative biocatalytic compositions to enhance and accelerate the inherent biological processes that are essential in all waste pollution reduction. Rather than attempting to supplement the indigenous microbiological with cultured stains of foreign colonies of organisms, a new appreciation of releasing the natural biocatalysts' mechanisms within the existing microbiological ecology has been developed. Understanding Nature's own inherent dynamic mechanism for regeneration has illuminated the synergistic confluence of air and water.

The beauty of utilizing a bio-catalytic agent in triggering accelerated bioremediation of petroleum hydrocarbon (TPH) pollutants within the marine environment lies in unleashing the natural ecological processes that ultimately must be called upon to bring about the health and vitality of the ecosystem to support the viability of marine and shoreline species. Their stimulation of the proliferation of microbiological communities, and promotion of higher oxygen transfer and dissolved oxygen penetration, within the seawater and shoreline soils, supports the entire shellfish and fish mortality.

The most toxic and insidious of petroleum hydrocarbon pollutants (TPH) are the generally invisible and rapidly diffused light volatile components, as they are absorbed within the metabolisms of living species and either kill, or mutate, the populations at levels they are unable to tolerate. Oil spill capture approaches are unfortunately limited in their ability to stop these components from escaping into the environment. However, these pollutants have been shown to be particularly responsive to bio- catalytic reduction, with instantaneous effectiveness, and substantial bioremediation accomplished within hours.

Oil spills are very illustrative by their adhesion characteristic of coating surfaces and living creatures with a slick, oily, sheen that can form globs of tar-like clusters. The emulsification of these clusters into smaller sizes is the objective of the dispersants used in oil spills to minimize these elements, but they have the disadvantage of both diffusing the petroleum hydrocarbons over larger areas, plus they have their own biodegradation problems. Bio-catalytic reductions of the petroleum hydrocarbons will both initiate an acceleration of the bioremediation of the diffused dispersed oil spill components, as well as accelerate the bioremediation of the dispersants themselves.

In an oil spill incident, there is the challenge of reducing the immediate toxicity of the petroleum hydrocarbon pollutants, especially the light volatile components that travel quickly, poisoning the ecologies that are immersed within the water borne environment. There is also the simultaneous requirement to stimulate the indigenous microbiological systems that will need to be called upon to as rapidly as possible to consume these pollutants and render them harmless byproducts of their digestive work. Bio-catalytic reactions can offer an invaluable tool to supporting these ecological restoration aspects.

The extensive expanse of oil spills across vast stretches of open ocean and coastal waters requires approaches that are not limited to enclosed containment strategies, but allow broad and readily delivered coverage with high dilutions of bioremediation agents. Biocatalytic solutions are by design highly water soluble and are able to be used effectively at extremely high dilution through any water spraying system. The safety and economy of using bio-catalytic compositions surpasses any alternative chemistry, or biological additive, in addressing the primary challenges and underlying ecological restoration mechanisms that are required in oil spills in a marine environment.

CSU Chico: Bench Scale Testing of BOC NONTOX® for Degrading Petroleum Hydrocarbons in Soil

Information in this report was provided by a Californian Environmental Engineering Company. It is the follow study to the first USAF base case study and was performed in collaboration with the manufacturer and it's representatives. The first study revealed that biocatalytic soil additives can preferentially degrade soluble fractions of total petroleum hydrocarbons (S-TPH) (Gaudette et al, 1996).

Soluble fractions of TPH are the most mobile in the environment and as such, tend to be of highest concern to regulatory agencies. The experimental program described herein focuses on confirming NONTOX®'s tendency to preferentially degrade S-TPH so that soluble fractions are degraded at highly accelerated rates. Oxygen, temperature and soil moisture will be held constant for the purposes of this work.

The experiment consists of monitoring TPH and S-TPH degradation over time as a result of NONTOX® treatment as compared to controls. The work was carried out in a controlled laboratory environment with constant temperature and humidity. The data in this study summarizes the first four weeks of an ongoing study which was anticipated to last for up to twelve weeks. This document also provides the testing procedures and quality control measures to be followed during implementation of the experimental program.

Experimental Procedure Description

Start-up procedure

Latex gloves and laboratory gloves were during all procedures Twenty one 5 gal buckets of JP-7 contaminated soil were obtained from the USAF base from AOC-34. These buckets were labeled A-U, and stored in the California State University, Chico laboratory. One bucket of soil from the JP-7 fuel site was obtained that has no evidence of contamination. This bucket was labeled "V".

Six liters of neat JP-7 fuel were obtained from the USAF base and stored in CSUC laboratory Five 37.85 liter glass test reactors (61cm x 25.4 cm x 25.4 cm) were used as testing containers, and eighteen 1 liter capacity aluminum scoops will be used to mix the soil during the course of the experiment.

Testing containers and scoops were washed with a mild detergent and rinsed thoroughly, followed by a final rinse with distilled water.

Testing containers and scoops were labeled 1-5. Label assignments will be summarized in the key below:

LABELING KEY		
Scoop/Test Cell ID	Surfactant Concentration Ratio (Water Surfactant)	Biocatalyst Concentration Ratio (Water : Biocatalyst)
1	None	None
2	None	None
3	NONTOX® Standard	NONTOX® Standard
4	None	NONTOX® biocatalyst only
5	NONTOX® surfactant only	None
(a)	Test cell no 2 was autoclaved to remove microbes prior to initiating testing.	

In order to ensure that the testing soil is relatively homogenous in both texture and JP-7 contamination, an equal amount of soil was taken from each of the 21 field sample buckets, labeled A-U, and placed in the labeled testing containers (glass aquariums). One scoop of soil was taken from each bucket consecutively (A-U). The total amount of soil to be placed in each aquarium was approximately 1 liter x 21 scoops = 21 liters = 5.5 gallons = 0.74cu.ft x 100lbs/cu.ft = 74 lbs = 33.6kgs. Each scoop was used with the corresponding testing container throughout the experiment to avoid any cross contamination. Soil from bucket V was used for test cell No. 2.

- Test cell No. 2 was sterilized to remove existing natural micro-organisms. Soil for test cell No. 2 was autoclaved at 15psi and 1210C for a period of 30 minutes. A sample of the soil was then tested for microbes in accordance with procedure outlined below. Autoclaving was repeated until all existing microorganisms has been eliminated.
- A sample of JP-7 fuel was sent to a California state certified laboratory and a gas chromatograph was run to determine the JP-7 fingerprint.
- Each of the testing containers was spiked with 200 ml of JP-7 fuel to ensure an initial soil concentration of at least 5,000 mg/kg JP-7 fuel, as demonstrated by the following calculation:
    - @ 1 gm/ml, 200 ml JP-7 weighs 200 gms = 200,000 mg;
      we have 33.6 kg of soil
      therefore concentration is 200,000/33.6 = 5950mg/kg
- Each sample was mixed thoroughly to achieve a uniform composition for each of the initial soil samples. Mixing was performed under a fume hood provided by CSUC. The samples were then stored in the environator and turned once per day for a period of one week. This provided "set up" time for the added JP-7 fuel. The air quality in the environator was checked daily with a Photo Ionization Detector (PID) to monitor potential build up of volatile organic compounds. If the monitoring revealed air concentrations in excess of 100 ppm the equipment was to be delayed until special ventilation equipment could be installed.
- Appropriate treatment dilutions were made to each container according to the procedure described below and each test cell was mixed thoroughly.

NONTOX® Addition for Test Cell No. 3

Seven ml of neat NONTOX® product was mixed into 28ml of distilled water creating a total volume of 35ml. This volume shall be added to the test cell No.3 and mixed into the soil.

The moisture content of the soil in each container was tested by time domain reflectometery testing using an Environmental Sensors moisture probe as follows:

- Insert probe 10cm into soil
- Percent moisture was read from digital display
- Instrument was calibrated weekly using soil with known water content; or if soil is not at 50% saturation, distilled water was added in 100ml increments until this level was obtained. The sample was mixed thoroughly after each water application. Fifty percent soil saturation was maintained throughout the experiment. Fifty percent soil saturation was approximately 10-15 percent on a mass basis (i.e. 10- 15 grams water per 100 grams dry soil).

Containers were placed in the temperature controlled environment chamber.

Chamber temperature was maintained at 20°C throughout the experiment.

Start up soil pH readings were obtained using the following procedure:

- A soil distilled water slurry was made using 1.0 gallons of soil vortexes in 9.0 ml deionized water for 10 seconds.
- This was measured using either Cole Palmer hand held pH tester or a Beckman Zeromatic SS-3 pH meter. The meters were standardized using standards of pH 4, 7 and 10 daily before use.

Obtain start up TPH samples for total TPH and S-TPH analysis using the following procedures:

- Latex gloves and laboratory gloves were worn during all sampling procedures
- Eight oz. glass jars with Teflon lid were provided by a California state certified laboratory.
- Each jar was packed tightly with soil. There were no air spaces within the jar.
- Each jar was labeled with the date and time of sampling; sample ID number (testing container number), and the requested tests. Samples were immediately packed on ice to maintain temperature at or below 4°C.
- Chain of custody forms were completed and samples were shipped to a California state certified laboratory for analysis. Samples were analyses for total petroleum hydrocarbons in the diesel range (TPH-D) using EPA Method 8015, total petroleum hydrocarbons in the gasoline range (TPH-G) using EPA Method GCFID/5030, and BTEX compounds using EPA Method 8020. Soluble TPH (S-TPH) was measured by performing a waste extraction test with Deionized water (DI-WET) and analyses for the same compounds as for total TPH.

Start up measurements of petroleum hydrocarbon degrading bacteria were obtained using the following procedures:

- Two soil samples from each sample cell were taken on day 0,1,3,7 and every 7 days thereafter.
- Gallon of soil was vortexed in 9.0 ml of Deionized water for 10 seconds.
- Appropriate dilutions were made to yield 30-300 CFU using the spread plate technique.
- 1:10 serial dilutions were prepared out 10-6.
- 0.1ml of each dilution were plated onto media for enumeration of total heterotrophs, psudomonads, and petroleum degraders (see media below).
- Samples were incubated for 7 days at 28°C.
- Plates were made with thick, 22ml media/disposable plate.

Media preparation techniques for identification of petroleum hydrocarbon degrading bacteria:

Total Heterotrophies

- Basal mineral salts media (M-9)
- Na2HPO4(6.0g), KH2PO4(3.0g),NaCL (0.5g), NH4CL (1.0g), Bacto- Agar (20.0g)
- Add deionised water to 979ml
- Autoclave and cool to 500C.
- Add 1 M MgSO47H2) (1.0ml) and 0.01 M CaCL2 (10ml) to above media.
- Autoclave separately and cool to 500C.
- Add sterile 20 percent glucose (10ml).
- Add Amphotericin B (fungicide) to 10ug/ml and pour plates.

Pseudomonades

- Pseudomonades Isolation Agar (PIA)(Difco).
- Suspend 45g dehydrated PIA in 980 ml Deionized water.
- Add glycerol (20ml) and boil to dissolve completely.
- Autoclave and cool to 500C.
- Add Amphotericin B to 10ug/ml and pour plates.
- Petroleum Degraders
- Bacto Bushnell-Haas Broth (Difco).
- Dissolve 3.27g dehydrated broth in 990ml Deionized water.
- Add Bacto-Agar (20g)
- Autoclave and cool to 500C.
- JP-7 was added to the media (10g/l). the JP-7 was prepared by adding silica gel (3.35g) to filter sterilized JP-7 (6.35g)
- Add Amphotericin B to 10ug/ml and pour plates.

Optional Procedures

Each testing container was aerated daily by mixing thoroughly with the designated scoop.

The environmental chamber temperature was checked and recorded daily. Humidity was measured using a GECNO Sling Psychrometer using the following procedures:

- The sock was wetted with Deionized distilled water.
- The psychrometer was swung rapidly at waist level for 60 seconds.
- Temperatures on the wet and dry thermometers was read
- Relative humidity was determined from a standard chart based on the dry temperature and the difference between the wet and dry temperatures.
- Soil moisture content was checked daily using the procedure specified in item 12 of the Start Up Procedures. Any adjustments that were made were recorded in the daily log book, including date of adjustment, initial moisture content, final moisture content, and required amount of distilled water to reach 50% saturation.
- Soil removal for sampling purposes (date, amount), changes in protocol, or deviation from the specified parameters (temperature, sampling procedures, etc) during the course of the experiment must be recorded in the daily log book.
- Soil samples were taken for pH measurement as specified in item 16 of the Start Up Procedures. In addition to the pre-treatment testing, soil pH testing were performed on day 1,3,7 and weekly thereafter for the duration of the experiment.
- Soil samples were taken for total TPH and TPH-DI Wet analysis as specified in item 17 of the Start Up Procedures. In addition to the pretreatment testing, TPH testing occurred on day 1,3,7 and weekly thereafter for the duration of the experiment.
- Soil samples were taken for bacterial analysis as specified in item 18 of the Start Up Procedures. In addition to the pre-treatment testing, bacteriological testing was done on day 1,3,7 and weekly thereafter for the duration of the experiment.

Results And Discussion

Reductions in total TPH in soil could not be evaluated in this study because rapid reductions in untreated control soil clearly indicated that volatilization, rather than degradation, was responsible for most of the TPH lost in all treatments. Therefore, the role of NONTOX® in the breakdown of TPH could not be discerned in these data. However, the data do suggest that the presence of NONTOX® or the NONTOX® surfactant actually reduced the rate of volatilization.

The primary mechanism by which surfactants alone could reduce the rates of volatilization of TPH from soil involves the formation of a hydrophobic environment around individual soil particles. Surfactant molecules are amphiphilic, having both hydrophobic and hydrophilic regions. As a surfactant monolayer is formed around individual particles, the hydrophobic region orients towards the TPH impacted particle and the hydrophilic region orients outwards towards the aqueous environment. The association of TPH with the hydrophobic region of surfactant molecules would result in a reduced tendency for loss by volatilization.

The disappearance of soluble TPH is summarized in the table below. Degradative mechanisms appear to be responsible for the disappearance of soluble TPH. Soil treated with NONTOX® showed greater reductions in soluble TPH than untreated controls or soil tested with surfactant alone or biocatalyst alone. Those results confirm that surfactants and biocatalysts combined behave as a bioorganic catalyst (BOC) which is responsible for soluble TPH reduction. It is likely that the tendency of NONTOX® components to organize into clusters, aggregates, or gas filled bubbles provides a platform for reactions to occur. The resulting localized increase in reactant concentrations and reduction in transition energy for reactions significantly increase the rate of catalysis. These aggregates of BOC and partially degraded TPH may also facilitate the presentation of the TPH to micro-organisms for complete mineralization.

Plate count data indicate that specific hydrocarbon degrading populations of bacteria emerge much more quickly in NONTOX® treated soil and remained at least 10 fold higher during the study interval.

14 SOLUBLE TPH { JP7 RANGE} DATA SUMMARY {MG/L}					
Cell Identification Number					
Days	1 Untreated	2 Sterile Untreated	3 NONTOX®	4 Biocatalyst Alone	5 Surfactant Alone
0	70	30	88	100	63
1	52	18	11	25	35
3	52	24	5.1	11	39
7	61	29	13	37	50
14	49	33	14	44	21
21	45	34	11	60	21
28	51	27	8.4	34	14.2
Total Reduction	27%	10%	90%	66%	77%

In conclusion, optimization of treatment parameters resulted in a fourfold decrease in the time required for treatment and a 50% reduction in the cost of NONTOX® to meet clean up criteria as compared to the original study using NONTOX®. The results of the current optimization study confirms that the cost of NONTOX® treatment is comparable to the cost of fertilizer in the removal of soluble TPH from oil. Based on these results, the environmental Engineer anticipates USAF and regulatory agency approval for the use of remediating impacted soil at the USAF base in the summer of 1997.

Orange County, CA Sanitation District
Dissolved Oxygen Increase from Open Air

In April of 2004, in conjunction with other laboratory tests conducted by project coordinator, Yu-Li Tsai, Ph.D., of the Environmental Science Laboratory, Inorganic Chemistry Section, Orange County Sanitation District, Costa Mesa, California. The laboratory found that by adding Bio-Organic Catalyst, there was substantial increases in dissolved oxygen (DO) in both non-purged (no air added) and in purged (air added) waters that contained approximately 250 PPM of BOD. The water was stabilized to eliminate a biological reaction.

TABLE 1 P1 INFLUENT NON-PURGED			
Time (Min.)	0 PPM Control	250 PPM of Bio-Organic Catalyst	Increase
3	0.33	0.51	54
6	0.69	1.41	104
12	0.48	1.69	252
30	1.20	2.78	132
60	2.90	3.04	5

TABLE 2 P1 INFLUENT PURGED			
Time (Min.)	0 PPM Control	250 PPM of Bio-Organic Catalyst	Increase
3	1.50	1.89	26
6	2.77	3.84	39
12	4.38	5.08	16
30	6.33	6.39	0
60	5.81	6.15	6

These data show the substantial increase of oxygen transfer to dissolved oxygen, from the open air, which is important in gravity mains, rivers or open bodies of water.

UC Irvine: Indigenous Bacterial Growth Study

Executive Summary
University of California, Irvine
For Bio-Organic Catalyst

BOC's bio-organic catalyst based products cause naturally occurring nonpathogenic (harmless) bacteria to grow rapidly, while not affecting the growth of pathogenic (harmful) bacteria.

The following studies were performed at the University of California, Irvine. Figure 1: Demonstrates that Bio-Organic Catalyst does not contribute to the growth of pathogenic (harmful) bacteria. In the researcher's conclusion, BOC did not have a significant impact on E. Coli cell growth.

The following studies were performed at the University of California, Irvine.

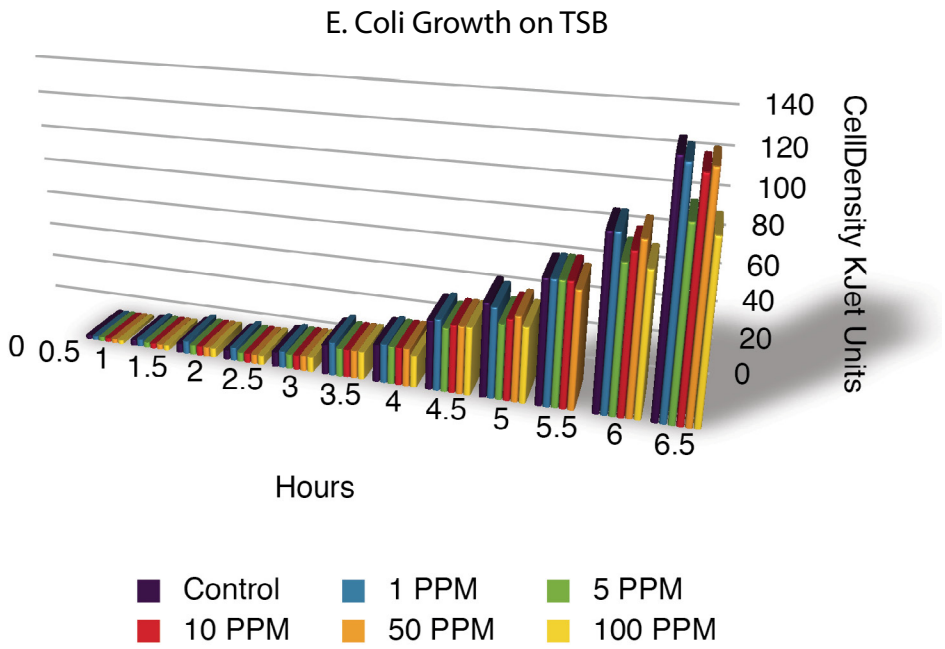

Figure 1: Demonstrates that Bio-Organic Catalyst does not contribute to the growth of pathogenic (harmful) bacteria. In the researcher's conclusion, Bio-Organic Catalyst did not have a significant impact on E. Coli cell growth.

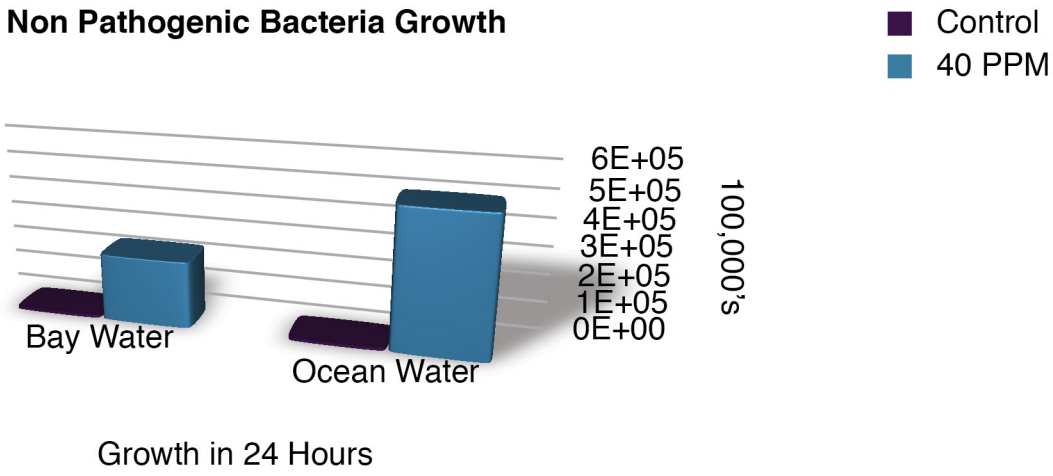

Figure 2: Demonstrates Bio-Organic Catalyst's ability to proliferate non-pathogenic (harmless) bacteria in bay water and ocean water in a 24 hour period of time. These bacteria are the same bacteria required by Nature to biodegrade organic petroleum products back to their individual parts before they formed to become petroleum.

Bacterial Growth Study Summary

Clearly these graphic displays of BOC's ability to clean, along with the catalytic reactions caused by BOC's bio-organic catalyst based product, clearly lead on to the conclusion as its effectiveness as a surface cleaning agent. It also does not cause harmful bacteria to grow, and enhances Nature's harmless bacteria to begin biodegrading petroleum.

E.coli Growth Curve On TSB

This experiment was done to show the toxicity of BOC to bacteria. Several replicate flasks were prepared with sterile growth medium. The medium used was Tripticase-Soy broth (TSB), this is a rich medium ideal for the cultivation of E.coli. When the broth is inoculated with E.coli, the number of E.coli cells in the broth increases. If there is a substance toxic to E.coli present in the broth, the number of cells will not increase as rapidly or not increase at all.

The growth of E.coli in TSB amended with different concentrations of BOC was measured. The BOC concentrations used were: 0, 1, 5, 10, 50, and 100 ppm (v/v). The density of cells in the broth was measured periodically with a Klet meter.

Cell density as Klet Units (KU) is proportional to the number of cells present in the broth.

The attached chart shows the result of the experiment. The plots are typical pf the early stage of logarithmetic cell growth in all cases. Also, none of the plots are significantly different from one another. None of the concentrations of BOC had a significant impact on E.coli cell growth.

Growth Response Of Natural Marine Bacteria To Bio-Organic Catalyst

This experiment was done to show any toxicity that BOC might have to seawater bacteria. The seawater sample was tested for total heterotrophic plate count (HPC) after it was collected. The sample was then split into several flasks with BOC concentrations of 0, 50, 100, 500, 1000 ppm v/v. The flasks were incubated for 24 hours on a shaker at room temperature. After the incubation period the water samples were tested for heterotrophic plate count (HPC). The results are represented on the bar graph. Adding any amount of BOC up to 1000 ppm v/v results in a large increase (approximately 2 orders of magnitude) in HPC over that if no BOC is added.

Each sample was divided into four 500 ml flasks, 250 ml per flask. One flask of each water sample was amended with BOC @ 40 ppm. After 24 hours incubation shaking at room temperature, each flask was plated on sea water medium to determine the number of bacteria present in each flask.

	Bay Water	Ocean Water
No Bio-Organic Catalyst	2.1 x 106 CFU/ml	3 x 103 CFU/ml
40 ppm Bio-Organic Catalyst	2.7 x 105 CFU/ml	4.3 x 105 CFU/ml

It is apparent that at 40 ppm, Bio-Organic Catalyst is not toxic to marine bacteria and in fact promotes growth. This data shows that 40 ppm of Bio-Organic Catalyst in natural marine water causes over a 100 fold increase in the number of heterotrophic bacteria.