

“BIOREMEDIATION OF HYDROCARBONS AND HEAVY METALS IN WATER”

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ABSTRACT

The purpose of this project was to examine the role of utilizing BioNutraTech's bioremediation technology to accelerate the break down process and reduction of containments in water and groundwater. The US Naval Surface Warfare Command Carderock Division, conducted tests on BioNutraTech's VB-591™'s breakdown ability of hydrocarbons in combination with dissolved oxygen on tens of thousands of gallons of oil filled bilge water. VB-591™ is not an enzyme nor is it a microbe, it is a green proprietary oleophilic bionutrient that arouses microbial activity by providing essential nutrients that stimulate and sustain the accelerated growth of naturally occurring microbes that are already exist in contaminated water, groundwater, and soil. VB-591™ has a unique ability to cling to organic contaminants while slowly releasing the nutrients necessary for enhancing microbial maturity. As a result, microbes increase in strength and concentration to convert organic wastes and contaminants into harmless products. When contamination in water occurs, oxygen is depleted thereby creating unhealthy environments for microbial life to mature effectively. By utilizing both EPA registered VB-591™ and dissolved oxygen, NSWCC's test findings confirm the accelerated remediation process to within hours. Tests performed in Florida and Japan's bilge water; reveal significant reduction levels of containments that include BTEX (Benzene, Toluene, Ethylbenzene and Xylene) and MTBE in less than just seven days. Within seven days Ethylbenzene, 1-Methylnaphthalen, 2-Methylnaphthalene, Naphthalene and Xylene levels were reduced over ninety-four percent, along with over a seventy-five percent decrease in Fluorene, and Phenanthrene. Employing both VB-591™ and dissolved oxygen to contaminated sites will effectively expedite the reduction of harmful levels of containments in water and groundwater.

1.0 INTRODUCTION

Tests were carried out to evaluate the effect of Accelerated Bioremediation Nutriation Technology (ABNT) for the treatment of bilge wastewater containing hydrocarbons generated by naval vessels. The testing was performed to evaluate BioNutraTech's VB-591™ bionutrients and dissolved oxygen delivery (DO). BioNutraTech VB-591™ nutrient is a proprietary bionutrient designed to enhance bioremediation of hydrocarbon contamination. The bionutrient replenishes key nutrients consumed by the accelerated population growth of microbial communities. The oxygen delivery system delivers large amounts of oxygen into waste streams without loss through bubbles; the oxygen rich water remains stable, odor-free and biologically available with virtually no off gassing. The use of this technology intermixed is said to cut down significant bioremediation timeframes. The US Naval Surface Warfare Command Carderock Division conducted two tests in April 2008 at the Mayport Navel Station and in October 2010 at the Commander Fleet Activities Yokosuka Japan. The purpose of these reports is to illustrate the success of these results.

2.0 WHAT IS BIOREMEDIATION

Biodegradation is the natural process whereby microorganisms alter and break down organic molecules. Microbes produce and emit enzymes that are catalysts that either begin or speed up chemical reactions. These enzymes break apart the bonds that hold together various contaminants. When a potential food source (the contaminant) is introduced to a microbial environment, the organisms adapts to enzymes to optimize the breakdown of that particular food source. Often many cycles of regeneration are required for microbes to adapt to the contaminant, and in the process, life-sustaining nutrients are depleted. The entire conversation process can be slowed or stopped altogether due to the fact that microbes gorge on the carbon sources, thereby multiplying quickly and depleting vital nutrients.

Bioremediation is the process of using naturally occurring microorganisms to enhance normal biological breakdown. Microorganisms will break down complex wastes, fats, oils and hydrocarbons into simple, non-toxic by-products. It is one of the oldest and yet newest pollution fighting tools.

3.0 HOW DO YOU ACCELERATE BIODEGRADTION

One can substantially accelerate biodegradation by providing the necessary support and stimulation for native microbes to quickly multiply and produce enzymes to speed up the metabolic process of biodegradation. How do you provide the necessary support to stimulate already existing microbes, through employing BioNutraTech's ABNT. BioNutraTech's nutriation technology provides key life-source nutrients for the beneficial microbial family. This particular form of technology ensures microbial accelerated reproduction and activity through both an enriched nutrient and the oleophilic coating that surrounds it.

4.0 WHAT IS NUTRIATION TECHNOLOGY

The technology behind BioNutraTech's bionutrient product formulations is the unique proprietary oleophilic coating and its nutrients. The green oleophilic (oil loving) coating is made up of a dry, prilled, texture, that physically clings to the organic contaminants (hydrocarbons, fats, oils and greases) while slowly releasing the nutrients necessary for enhancing microbial growth. BioNutraTech's bionutrients cling to organic wastes without harmful residual or being able to be washed away; this assures that bionutrients are always available in useful concentrations for microbes. As a result, proper nutrients are made available on a time-release basis and lower dosing levels are possible than those required with common nutrient supplements; due to the fact that there is less of a need for secondary applications. BioNutraTech's products employ enriched bionutrients that include: carbon, nitrogen, sulfur, phosphorus, and trace minerals help microbes speed non-toxic mineralization. These nutrients provide vital nutrients for the growth and health of native microbes present at the contamination site with no threat to the extant ecological balance. As a

result, contaminants are broken down to carbon dioxide, water and traces of fatty acids. By utilizing these bionutrients, local microbes can colonize the organic contaminants and use them as a source of food.

4.1 Nutriation Technology versus the Krebs Cycle

Microbes are suited for consuming dissolved and undissolved particles of organic materials (food source). These single celled organisms grow in wastewater by utilizing the Krebs Cycle. This cycle constitutes the major terminal pathway of biological oxidation in most animal, plant and bacterial cells, a series of chemical reactions by microorganisms, by which two-carbon fragments produced by the metabolism of carbohydrates, fats, and proteins are converted to carbon dioxide and water, liberating energy in the process.

The Krebs Cycle begins when a form of acetic acid combines with oxaloacetic acid. This combination forms citric acid, molecules of which are rearranged to produce isocitric acid. The molecules then pass through a series of stages during which six intermediate compounds are formed. But, unfortunately the continued loss of intermediates, by not having the right mix of carbs, fats and protein in the receiving stream will ultimately shut down the Krebs Cycle altogether and the bacteria will starve. However, many bacteria possess an alternative metabolic pathway that allows them to cope with this situation. Bacteria have the appropriate enzymes to bypass several steps in the Krebs Cycle. It is called the glyoxylate shunt. The 'shunt process' by-passes steps in the process so that the cycle can proceed. It has been suggested that fatty acids lead bacteria to utilize the 'shunt process'. Accordingly, the shunt process is more carbon efficient than the Krebs Cycle. A particular advantage to this process is glyoxylate's shunt which prevents the loss of two carbon dioxide molecules and utilizes four additional carbon atoms for production of intermediates.

The result is that fatty acids can efficiently replenish Krebs Cycle intermediates and support increases of biomass across a variety of biomolecules. It is even suggested that the intermediates unique to the glyoxylate shunt may be utilized to support the synthesis of other biomolecules. VB591's oleophilic coating is made up of a combination of fatty acids to lead the bacteria to utilize the 'shunt process

5.0 VB-591™ WATER NUTRIATION TECHNOLOGY

BioNutraTech's formulation, EPA registered VB-591™, IS NOT a microbe but a green bionutrient that is attracted to oil globules. This particular attraction overthrows molecular dissimilarity and mutual bonding forces between like molecules that causes oil and water to be immiscible. In conjunction with oxygen, the ABNT process has demonstrated the ability to reduce the time required to effectively remove oil from rocks, sand, and vegetation. VB-591™ has been tested for efficacy and toxicity; VB-591™ can be used without negative effects or causing injury or discomfort to the marine life.

The use of VB-591™ supports the growth of all non-fastidious natural microbial populations; it contains no toxic chemicals, surfactants or hazardous substances. VB-591™ has shown no indication of significant adverse impacts, algae bloom formation and / or overcome primary limitation of natural biodegradation. This product remains stable, since it is not affected by the pH, salinity, normal temperature fluctuations or alkalinity.

VB-591™ stimulates the metabolic rate of the native non-pathogenic bacteria thereby increasing the rate of conversion of simple and complex hydrocarbons. The granular nutrient is a formulation of essential nutrients required for effective bacterial, microbial development and stimulated metabolic bioremediation. The nutrient and hydrocarbon become a concentrated food source easily accessed and metabolized by native bacteria, converting it to soluble natural fatty acid that in turn becomes food for marine life. This elevated metabolic activity increases the demand for available oxygen to support the process. This biological process eliminates the need for dispersants, surfactants, exogenous microbes and enzymes.

BioNutraTech's VB-591™ is a proven nutrient formula that works in fresh, brackish, and salt water. VB-591™ is formulated to address oil spills in open water, coastal waterways, sensitive environments, refineries, bilge water, tank cleaning, waste treatment facilities and petroleum contaminants.

6.0 BIOREMEDIATION NUTRIATION TECHNOLOGY TEST HISTORY

A series of subjective and viability field tests have been performed that have demonstrated the ability of the ABNT process to rapidly reduce a broad range of hydrocarbons under varying conditions. Tests have proven that BioNutraTech's products provide vital bionutrients that accelerate and maintain microbial growth. Our latest test was performed by the US Naval Surface Warfare Command Carderock Division (NSWCCD) in Yokosuka, Japan. These tests demonstrated BioNutraTech's VB-591™'s ability to breakdown hydrocarbons in combination with dissolved oxygen on tens of thousands of gallons of oil filled bilge water utilizing the proprietary oleophilic bionutrient, VB-591™. VB-591™ has demonstrated a significant reduction in time and hydrocarbon levels under a variety of conditions which has been witnessed in several other industrial site tests as well. Head-to-lab and field tests utilizing the ABNT process has validated the capability of the system to meet the increased nutrition demand during enhanced degradation activity. This biological process lowers Total Petroleum Hydrocarbons (TPH), Diesel Range Organic (DRO), Gasoline Range Organic (GRO), organics, some inorganics and fat, oil and grease (FOG) levels.

7.0 RESULTS

Before the test application of VB-591™, test samples were found to be dark black in color with a heavy odor of hydrocarbons. Test samples contained concentrations of Biological Oxygen Demand (BOD, 220 mg/L), Total Suspended Solids (TSS, 1,200 mg/L), mineral oil (630 mg/L), and animal and vegetable fat (200 mg/L) exceeded the Japanese Environmental Governing Standards for Wastewater (JEGS). Tables summarizing the analytical detections are provided as Table 1 through Table 3. The full analytical results are provided as Attachment 1.

After the application treatment of VB-591™, test samples were found to be light tan in color with a reduced odor of hydrocarbons. The NSWCCD's test results showed a reduction of BOD by 94%, TSS by 97%, mineral oil by 98%, and animal and vegetable fat by 97%. In detail, the treated samples also revealed significant reduction levels of containments that include BTEX (Benzene, Toluene, Ethylbenzene and Xylene) and MTBE in less than just seven days. Within seven days the BOD levels of Ethylbenzene, 1-Methylnaphthalen, 2-Methylnaphthalene, Naphthalene and Xylene levels were reduced over ninety-four percent; along with over a seventy-five percent decrease in Chemical Oxygen Demand (COD) levels of Fluorene, and Phenanthrene. Tables summarizing the analytical detections are provided as Table 1 through Table 3. The full analytical results are provided as Attachment 1.

Table 1

Pre and Post Treatment Test Results in Comparison to JEGS

	JEGS	JEGS Pretreatment (10/25/2010)	Post-treatment (11/5/2010)
Copper (mg/L)	3	2.2	0.3
Zinc (mg/L)	5	18	6.1
Coliform Colony (colonies/cm3)	3,000	170	4,100

Notes:

1. Samples were analyzed via Japanese Industrial Standard (JIS K0102)
2. Coliform Colony was analyzed via Ordinance 1, Ministry of Welfare
3. mg/L – Milligrams per Liter
4. Colonies/cm3 – colonies per cubic centimeter
5. JEGS – Japanese Environmental Governing Standards
6. This table includes all compounds detected on the “Fixed Monitoring Requirement Standards for All Direct and Indirect Discharge Waste Water Flows” JEGS (Table 1)
7. Shading and Bold – Concentrations above the JEGS (Table 1)

Table 2

Pre and Post Treatment Test Results in Comparison to JEGS

	JEGS	Pretreatment (10/25/2010)	Post-treatment (11/5/2010)	% Reduction
pH	5.0 – 9.0	6.5	6.6	NA
BOD (mg/L)	160	220	16	94%
COD (mg/L)	160	120	30	75%
TSS (mg/L)	200	1,200	29	97%
Mineral Oil (mg/L)	5	630	8.2	98%
Animal & Vegetable Fat (mg/L)	30	200	5.5	97%
Nitrogen (mg/L)	120	43	110	+
Phosphorous (mg/L)	16	1.8	1.5	17%

Notes:

1. BOD – Biological Oxygen Demand
2. TSS – Total Suspended Solids
3. COD – Chemical Oxygen Demand
4. NA – Not applicable
5. This table includes all compounds detected on the “Effluent National Standards for Waste Water Flows”
JEGS (Table 2)
6. Shading and Bold – Concentrations above the JEGS (Table 2)

Table 3

Pre and Post Treatment Test Results in Comparison to JEGS

	JEGS	Pretreatment (10/25/2010)	Post-treatment (11/5/2010)
Mercury (mg/L)	0.005	0.0012	<0.0005
Lead and its Compounds (mg/L)	0.1	0.02	0.01
Boron and its Compounds (mg/L)	230	3	2
Ammonia (mg/L)	100	4.4	34
Nickel (mg/L)	1	0.67	0.45
Fluoride and its Compounds (mg/L)	15	1	1

Notes:

This table includes all compounds detected on the “Monitoring Requirement Effluent Standards (Hazardous Substances) for Discharge from Non-Domestic Sources Including Industrial Waste Water Treatment Plants” JEGS (Table 3)

Attachment 1

OKA TANK#1 AT BOWT's FACILITY, WASTE WATER FROM CG-67

Sample ID					S-11025	S-11025
Sampling Date					25-Oct-10	5-Nov-10
Sampling Time					13:40	13:40
Water Temperature on-site					20.3	18.5
pH					7.05	6.64
Analytical Items	Test Method	Unit	RDL	JEGS'08 Allowable Limit		
FIXED MONITORING REQUIREMENT STANDARDS FOR ALL DIRECT AND INDIRECT DISCHARGE WASTE WATER FLOWS (J.E.G.S Table 4-1)						
1 Phenol	JIS K 0102 28.1.2	mg/L	0.5	5	<0.5	<0.5
2 Copper	JIS K 0102 52.4	mg/L	0.3	3	2.2	<0.3
3 Zinc	JIS K 0102 53.3	mg/L	0.1	2	18	6.1
4 Iron (soluble)	JIS K 0102 57.4	mg/L	1	10	<1	<1
5 Manganese (soluble)	JIS K 0102 56.4	mg/L	1	10	<1	<1
6 Chromium	JIS K 0102 65.1.4	mg/L	0.05	2	<0.05	<0.05
7 Coliform Colony	Ordinance 1, Ministry of Welfare	total colonies / cm ³	1	(3000)	170	4,100
EFFLUENT NATIONAL STANDARDS FOR WASTE WATER FLOWS (J.E.G.S Table 4-2)						
1 pH	JIS K 0102 12.1	-	-	5.0 - 9.0 (Sea Areas)	6.5	6.6
		-	-	5.8 - 8.6 (Public Water Areas)	-	-
2 BODs	JIS K 0102 21.32.3	mg/L	1	N/A (Sea Areas)	220	16
		mg/L	1	160 (Public Water Areas)	-	-
3 COD	JIS K 0102 17	mg/L	1	160 (Sea & Lakes)	120	30
		mg/L	1	N/A (Public Water Areas)	-	-
4 TSS	JEA Notices 59 Table 8	mg/L	1	200	1200	29
Normal Hexane Extracts ^(*)	JEA Notice 64 Table 4	mg/L	0.5		830	13
5 Mineral Oil	JIS K 0102 Ref. II	mg/L	1.0	5	630	8.2
Animal & Vegetable Fat	JIS K 0102 Ref. II	mg/L	0.5	30	200	5.5
6 Nitrogen	JIS K 0102 45.2	mg/L	0.2	120	43	110
7 Phosphorus	JIS K 0102 46.3.1/46.1.1	mg/L	0.1	16	1.8	1.5
MONITORING REQUIREMENT EFFLUENT STANDARDS (HAZARDOUS SUBSTANCES) FOR DISCHARGE FROM NON-DOMESTIC SOURCES INCLUDING INDUSTRIAL WASTE WATER TREATMENT PLANTS (J.E.G.S Table 4-4)						
1 Cadmium and its compounds	JIS K 0102 55.3	mg/L	0.01	0.1	0.01	<0.01
2 Cyanide	JIS K 0102 38.1.2, 38.3	mg/L	0.1	1	<0.1	<0.1
3 Organic Phosphorus Compounds	JEA Notices 64 Table 1	mg/L	0.1	1	<0.1	<0.1
4 Lead and its compounds	JIS K 0102 54.3	mg/L	0.01	0.1	0.02	0.01
5 Hexavalent Chromium	JIS K 0102 65.2.1	mg/L	0.05	0.5	<0.05	<0.05
6 Arsenic and its compounds	JIS K 0102 61.4	mg/L	0.01	0.1	<0.01	<0.01
7 Mercury, Alkyl mercury, and its compounds	JEA Notices 59 Table 1	mg/L	0.0005	0.005	0.0012	<0.0005
8 Alkyl Mercury Compounds	JEA Notices 59 Table 2	mg/L	0.0005	ND	ND	ND
9 PCB	JEA Notices 59 Table 3	mg/L	0.0005	0.003	<0.0005	<0.0005
10 Trichloroethylene	JIS K 0125 5.2	mg/L	0.01	0.3	<0.01	<0.01
11 Tetrachloroethylene	JIS K 0125 5.2	mg/L	0.001	0.1	<0.001	<0.001
12 Dichloromethane	JIS K 0125 5.2	mg/L	0.001	0.2	<0.001	<0.001
13 Carbon Tetrachloride	JIS K 0125 5.2	mg/L	0.001	0.02	<0.001	<0.001
14 1, 2-dichloroethane	JIS K 0125 5.2	mg/L	0.001	0.04	<0.001	<0.001
15 1,1-dichloroethylene	JIS K 0125 5.2	mg/L	0.001	0.2	<0.001	<0.001
16 cis-1, 2-dichloroethylene	JIS K 0125 5.2	mg/L	0.001	0.4	<0.001	<0.001
17 1, 1, 1-trichloroethane	JIS K 0125 5.2	mg/L	0.01	3	<0.01	<0.01
18 1, 1, 2-trichloroethane	JIS K 0125 5.2	mg/L	0.001	0.06	<0.001	<0.001
19 1, 3-dichloropropene	JIS K 0125 5.2	mg/L	0.001	0.02	<0.001	<0.001
20 Thiuram	JEA Notices 59 Table 4	mg/L	0.001	0.06	<0.001	<0.001
21 Simazine	JEA Notices 59 Table 5	mg/L	0.001	0.03	<0.001	<0.001
22 Thiobencarb	JEA Notices 59 Table 5	mg/L	0.001	0.2	<0.001	<0.001
23 Benzene	JIS K 0125 5.2	mg/L	0.001	0.1	<0.001	<0.001
24 Selenium and its compounds	JIS K 0102 67.4	mg/L	0.05	0.1	<0.05	<0.05
25 Boron and its compounds	JIS K 0102 47.3	mg/L	1	230 (Marine Areas)	3	2
		mg/L	1	10 (Non-Marine Areas)	-	-
26 Fluoride and its compounds	JIS K 0102 34.2	mg/L	1	15 (Marine Areas)	1	1
		mg/L	1	8 (Non-Marine Areas)	-	-
27 Ammonia, Ammonium Compounds, Nitrate Compounds & Nitrite Compounds	Calculation Method	mg/L	0.1	100	4.4	34
28 Silver	ICP-AES	mg/L	0.05	1.2	<0.05	<0.05
29 Nickel	JIS K 0102 59.4	mg/L	0.004	1	0.67	0.45
30 Toluene	JIS K 0125 5.2	mg/L	0.06	-	<0.06	<0.06
31 Xylene	JIS K 0125 5.2	mg/L	0.04	-	<0.04	<0.04
32 Ethylbenzene	JIS K 0125 5.2	mg/L	0.0005	-	<0.0005	<0.0005
33 MTBE	HS-GC/MS	mg/L	0.001	-	<0.001	<0.001

ND = Not Detected

RDL = Reporting Detection Limit

☐ Exceed JEGS Allowable Limit

- : Item Not Analyzed

() : Allowable Limit for Coliform Colony only applies to Direct Discharge

*1) If Exceed 5 mg/L, Analyze both "Mineral Oil" and "Animal & Vegetable Fat" Contents.

8.0 CONCLUSION

The product formulation of VB-591™ has a proprietary oleophilic coating that clings to oily organic contaminants while slowly releasing the nutrients necessary for enhancing microbial growth. VB-591™ formulation takes advantage over nature's existing waste disposal system. Oxidation-reduction reactions are the primary reactions created during bioremediation that yield free energy that microorganisms can use for cell growth and reproduction. These beneficial microorganisms can then grow in large quantities due to the formulation and will breakdown complex wastes, fats, oils, and hydrocarbons into simple, nontoxic by-products of CO₂, water and traces of fatty acids.

As the bionutrient, VB-591™ solution comes in contact with the oil on these surfaces, it attracts the oil present, reduces surface tension, enables the oil residuals now carried by the solution for a greater surface interface for more native bacteria to access the oils and perform the breakdown and conversion of the hydrocarbons. The ABNT process, demonstrates that it can effectively remediate a wide range of hydrocarbons without the use of dispersants, surfactants, exogenous microbes and enzymes.

The NSWCCD witnessed how VB-591™ effectively remediated 40,000 gallons of oil filled bilge water within hours. The enhanced bionutrient VB-591™ and oxygen reduced contaminant concentrations in the waste water in as few as five days. Utilizing the ABNT process to contaminated sites will successfully speed up the reduction of harmful levels of contaminants in water and groundwater.

9.0 AUTHOR(S) BIOGRAPHICAL DETAILS

Sandra L. Hruza President and CEO, has 20+ successful years experience, in the corporate world. She has built the complete corporate infrastructure of BioNutraTech from the ground up. She took one concept and invented seven (7) patents products with ten (10) applications. She is the Winner of WE300, top 300 women owned companies under \$1,000,000.00. She has worked in conjunction with the Texas Bioremediation Council to further educate the private, business, and regulatory sectors, by advocating professional performance standards to help monitor, and influence regulatory standards. She was invited by Hitachi Plant to speak on U.S. standards regulating hydrocarbons in groundwater, to improve legislation to regulate not only VOC's but also hydrocarbons in Japan. She travels extensively to educate and inform government officials on the consequences of excessive nitrogen in our waters.

Cassandra Hruza graduated in Business from Concordia University in 2002. She obtained a Master degree in Operations Management at the University of Colorado in 2011. In 2006, she opened her own business, specializing in the selling and distribution of Together; a small animal litter box product of BioNutraTech. From 2008 to 2009, she worked with different aspects of company operations within the medical industry. Since 2009, Cassandra has been contracted with BioNutraTech, specializing in developing and implementing business process and marketing campaigns to increase efficiency and increase revenue for the company.

10.0 CERTIFICATE

Sandra L. Hruza and Cassandra Hruza certify and submit this paper titled, "Bioremediation of Hydrocarbons and Heavy Metals in Water" for consideration for the Conference on "India Water Week" to be held in New Delhi from April 10-14, 2012 is original and has not been published or presented at any other forum.

Sandra L. Hruza

Cassandra Hruza

Place: Houston, Texas

Date: March 13, 2012