

# Enviro-BAC

## Green Remediation Technology

### About Enviro-BAC

In conjunction with a leading microbial manufacturing expert, we have developed an innovative and unique blend of non-pathogenic, non-genetically modified microbial organisms (Enviro-BAC) to facilitate the bioremediation of petroleum spills in both soil and groundwater.

Enviro-BAC has undergone extensive testing and product application under various conditions and environments including laboratory bench top trials as well as actual field applications.

Enviro-BAC consists of a proprietary blend of Bacillus bacteria spores. The particular strains in Enviro-BAC are all naturally occurring facultative anaerobes and are blended at a high concentration for optimum performance.

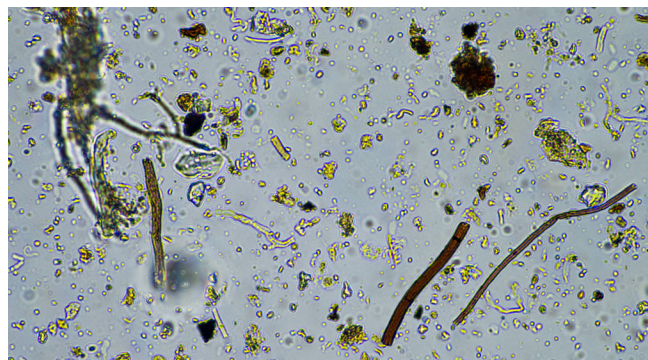
Facultative anaerobes are capable of thriving in the presence or absence of oxygen, making them ideal candidates for bioremediation applications. Utilizing the natural spore form of the bacteria ensures long term stability and flexibility for storage and applications. Enviro-BAC is manufactured as a spray dried powder and is easily dispersed in soil and water for a variety of applications.

The strains formulated into Enviro-BAC have been produced for more than 20 years under controlled fermentation conditions in order to maintain a reliable, consistent supply.

Enviro-BAC has been listed with the North Carolina Department of Environmental Quality (NCDEQ) Division of Water Resources (DWR) as an approved injectant. The product has been approved without use restrictions. Additional states within the southeastern United States have also provided acceptance of Enviro-BAC for use as an injection/remedial product.

**Subsequent sampling of the soil in the source area indicated a decrease in Total Petroleum Hydrocarbons as Diesel (TPH-D) of nearly 50% within four weeks of application.**

The introduction of Enviro-BAC proved to be a cost effective and efficient methodology for remediation, and, more importantly, reduced the time required to achieve regulatory closure by more than two years. With Enviro-BAC, HRP has the solution to remediate soil and groundwater impacted by a release of petroleum products efficiently.



## Case Study #1

### Laboratory Treatment and Analysis of Petroleum Contaminated Groundwater

An initial laboratory study evaluated the potential impact of Enviro-BAC on petroleum-contaminated groundwater. The groundwater samples were collected in the field from a petroleum impacted site and immediately delivered to an independent laboratory for analysis and treatment.

The laboratory decanted the groundwater sample into 1,000 ml beakers and divided the sample into untreated (control sample) and treated samples. Laboratory tests indicated that Enviro-BAC significantly degraded (i.e., > 92% decrease in contaminant levels) petroleum constituents in the groundwater sample under laboratory conditions. Laboratory data summarizing contaminant reduction of the groundwater sample is provided in Table 1 below.



Table 1

Sample ID	Groundwater Day 1	Untreated Groundwater Day 30	Treated Groundwater Day 30	% Improvement Treated (Untreated v. Treated)
<i>Volatile Organic Compounds (ug/l):</i>				
Benzene	6,090	1,600	17.7	99.7%
Ethylbenzene	1,900	BQL	BQL	100%
Toluene	2,960	31.0	BQL	100%
Total Xylenes	5,500	1,300	151	96.66%
Total BTEX	16,450	2,931	168.7	98.99%
Naphthalene	61.0	35.0	BQL	100%
1,2,4-Trimethylbenzene	1,610	58.5	82.1	92.03%

BQL = Below Quantitation Limits  
Results in ug/L

## Case Study #2

### On-Site Treatment of Soil Contaminated with Fuel Oil

In an effort to evaluate the effectiveness on petroleum contaminated soil, Enviro-BAC was applied to a surface spill of fuel oil that occurred within a residence basement. Using hand tools, two areas approximately 24" x 24" x 20" deep of contaminated soil were agitated within the basement around the perimeter of the slab foundation. The areas were sampled and analyzed for Total Petroleum Hydrocarbons as Diesel (TPH-D) and Gasoline (TPH-G). Following sampling, approximately 140g of Enviro-BAC was mixed with approximately 2.5 gallons of water and applied to one of the contaminated areas.



Note that an untreated, control area was also disturbed and sampled per above. Confirmatory sampling and analyses conducted approximately six weeks later indicated that the TPH-G in the treated and control areas had decreased at similar rates. The TPH-D concentrations remained essentially unchanged for the treated and control areas. However, a perceptible odor change was noted in the treated area and the laboratory indicated that there had been a significant change in the associated chromatogram which included peak reduction and retention time changes. An additional soil sample from the treated area was collected approximately 12 weeks following application of the product and indicated a 72% decrease from the initial concentration. As a result, soil was collected from the contaminated area for additional ex-situ treatment and analysis.

An additional 30 grams of Enviro-BAC, 5 grams of sucrose, and one liter of water were added to the contaminated soil, as a second dosage. A soil sample was collected and tested for TPH-D approximately nine weeks later. The sample exhibited an approximately 81.9% decrease in TPH-D since the August 14, 2015, sampling event, with an approximately 35.1% decrease since the October 9, 2015, sampling event. Summary of soil analytical results are in Table 2 below.

**Table 2**

Sample ID	Sample Collection Date	TPH-D (mg/Kg)	% Reduction
Contaminated Source Area	07/02/15	4,140	N/A
Contaminated Source Area	08/14/15	4,150	-1.0
Contaminated Source Area	10/9/15	1,160	72.0
Contaminated Source Area	12/18/15	753	81.9

## Case Study #3

### On-Site Treatment of Petroleum Contaminated Groundwater Monitoring Well

In 2017, State of North Carolina regulatory personnel approved pilot testing of Enviro-BAC at a petroleum contaminated groundwater site. The site is a gasoline station located within a coastal plain area with sandy soils and relatively shallow groundwater (i.e., approximately 5-6 feet below ground surface). An on-site monitoring well located within the source area had exhibited persistent dissolved-phase petroleum groundwater contamination. One kilogram of Enviro-BAC was introduced into the monitoring well via suspension within a five-foot, one-inch Schedule 40 PVC pipe with 0.10 slotted screen. A small amount of sucrose (approximately 1-2% by weight) was added in order to stimulate microbial activity.

The goal was to introduce microbes into the contaminant plume to facilitate petroleum degradation. Groundwater samples were collected from the monitoring well prior to and, at approximate 30-day intervals, following injection activities in order to evaluate the effectiveness of Enviro-BAC on dissolved-phase petroleum constituents and influence on bacteriological populations under field conditions. The groundwater samples have been analyzed for volatile organic compounds (VOCs) by Standard Method 6200B including ethanol, methyl tert-butyl ether (MTBE), and isopropyl ether (IPE). In addition, groundwater samples were collected for analysis via heterotrophic plate count (HPC). This procedure is used for estimating the number of live culturable heterotrophic bacteria in water. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term “colony-forming units” (CFU).

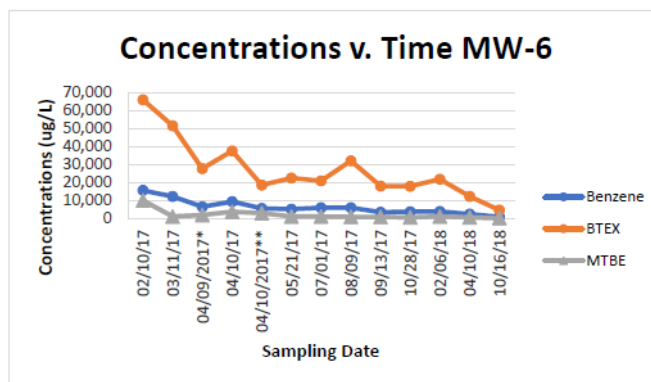
Subsequent sampling and analysis of the Enviro-BAC treated monitoring well indicated that, after 60 days following the initial Enviro-BAC injection:

- Benzene decreased 64%;
- Benzene, toluene, ethylbenzene, and total xylenes (BTEX) decreased 72%; and,
- Methyl tert butyl ether (MTBE) decreased 70%.

In addition, CFUs have increased from 130, prior to treatment with Enviro-BAC, to as high as 186,700,000. For the purposes of the pilot study only two, one-kilogram passive injection devices were introduced into the contaminated monitoring well at an approximately five-month interval. One year following the second injection event, groundwater samples collected from the monitoring well have not indicated evidence of rebound. The most recent data collected from the monitoring well indicates:

- Benzene decreased 94%;
- BTEX decreased 93%; and,
- MTBE decreased 98%.

A summary of the monitoring data and groundwater analytical results is provided in Table 3 on the following page.



## Case Study #3 - continued

Table 3

### Passive Injection at Gasoline Contamination Site – Coastal Plain

Contaminant of Concern			Benzene	Ethylbenzene	i-Propylbenzene	MTBE	Naphthalene	n-Propylbenzene	Toluene	1,2,4-Trimethylbenzene	1,3,5-Trimethylbenzene	Total Xylenes	Total BTEX
Well ID	Date Collected (mm/dd/yy)	Depth to Water (Ft. Below TOC)											
MW-6	02/10/17*	5.54	15,640	5,560	1,340	10,080	6,240	3,920	26,390	15,600	9,060	18,340	65,930
	2/10/17	--	1 Kilogram of <i>Enviro-BAC</i> passively injected										
	03/11/17	6.44	12,300	1,900	148	1,198	614	210	26,760	1,900	556	10,550	51,510
	04/09/17	5.69	6,480	1,310	168	1,890	896	332	12,900	1,800	505	7,070	27,760
	04/10/17	5.60	9,430	1,800	164	3,770	840	272	16,900	2,130	628	9,440	37,570
	04/10/17	5.60	5,580	932	188	2,980	904	436	7,560	1,160	352	4,450	18,522
	05/21/17	5.61	5,220	1,180	BQL	1,170	605	BQL	9,500	1,520	440	6,530	22,430
	07/01/17	5.72	6,040	1,050	112	1,200	618	218	9,230	1,190	330	4,560	20,880
	07/01/17**	--	1 Kilogram of <i>Enviro-BAC</i> passively injected										
	08/09/17	NM	5,970	1,290	BQL	910	400	BQL	17,840	1,340	290	6,930	32,030
	09/13/17	4.62	3,440	1,100	108	869	528	BQL	7,150	1,430	BQL	6,370	18,060
	10/28/17	5.40	3,760	1,060	BQL	625	530	BQL	7,850	1,360	370	5,220	17,890
	02/06/18	5.51	3,930	1,090	BQL	1,090	595	208	10,100	1,540	420	6,680	21,800
	04/10/18	4.82	2,410	739	115	660	533	252	6,600	904	222	2,480	12,229
	10/16/18	4.32	1,000	439	58.6	215	313	BQL	1,660	730	BQL	1,594	4,693
	% Reduction	-		94%	92%	96%	98%	95%	100%	94%	95%	100%	91%

\*Pre-Treatment Sample Followed by *Enviro-BAC* Treatment

\*\* Second *Enviro-BAC* Treatment Conducted

BQL = Below Quantitation Limits

Exceeds North Carolina 2L Groundwater Quality Standards (NC2LGWQS)  
 Exceeds North Carolina Gross Contamination Levels

Based on the results of the October 2018 sampling event, the site was granted a No Further Action Letter by the North Carolina Department of Environmental Quality (NCDEQ). The use of *Enviro-BAC* significantly shortened the duration of the natural attenuation processes at the site. Receiving regulatory closure optimizes the use of the property, maximizes the property value, and allows for an unencumbered property transaction.





## Case Study #4

### On-Site Treatment of Petroleum Contaminated Groundwater Plume

Enviro-BAC has been used at multiple leaking UST sites resulting in similar, immediate results. Enviro-BAC was employed at a dormant gasoline release site at which only natural attenuation monitoring had been occurring for the past several years. Enviro-BAC was actively injected into the groundwater plume via 17 injection points. Table 4 documents the pre-existing concentrations and the two post-application sampling events.

Table 4

#### Active Injection at Gasoline Contamination Site – Piedmont

Analytical Method		Standard Method 6200B											
Well ID	Date Collected (mm/dd/yy)	Benzene	Ethylbenzene	I-Propylbenzene	MTBE	Naphthalene	n-Butylbenzene	n-Propylbenzene	sec-Butylbenzene	Toluene	1,2,4-Trimethylbenzene	1,3,5-Trimethylbenzene	Total Xylenes
		MW-1 (Source Area)	11/09/16	31,60	30,10	1,630	< 0.5	18,700	< 0.5	4,980	< 0.5	111,00	35,700
08/27/18	7,320		13,40	1,630	2,000	4,160	< 0.5	< 0.5	< 0.5	47,500	7,760	2,000	44,700
10/16/18	Enviro-BAC Injection (Pilot Test)												
11/12/18	842		2,330	92.5	< 25	677	< 25	286	< 25	2,830	868	464	3,880
12/18/18	519		1,930	91.0	< 25	496	< 25	267	< 25	304	465	282	1,870
04/23/19	Enviro-BAC Injection												
06/28/19	566		2,250	145	< 40	815	152	416	< 40	173	722	212	1,990
09/23/19	656		1,890	98.8	< 1.0	594	31.0	226	16.2	259	731	249	2,470
% Improvement from		91.0%	85.9%	93.9%	NA	85.7%	NA	NA	NA	99.5%	90.6%	87.6%	94.5%
MW-3	11/09/16	10,50	1,770	55.0	< 0.5	265	< 0.5	157	< 0.5	561	279	201	2,170
	08/27/18	13,90	8,980	< 0.5	< 0.5	3,730	< 0.5	770	< 0.5	39,100	5,370	1,340	36,400
	10/16/18	Enviro-BAC Injection (Pilot Test)											
	11/12/18	6,240	1,110	< 50	< 50	155	< 50	93	< 50	305	253	301	1,270
	12/18/18	7,790	1,070	< 50	< 50	154	< 50	< 0.5	< 50	410	224	226	1,280
	04/23/19	Enviro-BAC Injection											
	06/28/19	1,710	255	16.6	< 10	46.4	10.6	26.6	10.2	122	55.0	21.2	363
	09/23/19	727	135	18.0	< 1.0	20.7	6.9	25.6	9.4	25.8	24.8	15.2	102
% Improvement from		94.8%	98.5%	NA	NA	99.4%	NA	96.7%	NA	99.9%	99.5%	98.9%	99.7%
NC2LGWQS (µg/L)		1	600	70	20	6	70	70	70	600	400	400	500
GCL (µg/L)		5,000	84,500	25,000	20,000	6,000	6,900	30,000	8,500	260,00	28,500	25,000	85,500

Exceeds North Carolina 2L Groundwater Quality Standards (NC2LGWQS)  
 Exceeds North Carolina Gross Contamination Levels

## Case Study #5

### On-Site Treatment of Surface Spill of Mineral Oil

Following a lightning strike that resulted in the release of approximately 300 gallons of mineral oil at an electrical substation, approximately 750 gallons of an Enviro-BAC slurry were applied to the affected area. Note that site conditions, including energized electrical equipment and the need for uninterrupted facility operations, precluded the use of more traditional remedial methods such as over-excavation of contaminated soils. The site measured approximately 750 square feet and was characterized by an approximately four-inch gravel layer underlain by clayey silts and silty sands soils.

The application procedure included the on-site mixing of 16 kg of Enviro-BAC (and sucrose) in three approximate 250-gallon batches. The Enviro-BAC was mixed with water using a high shear mixer and added to an on-site 275-gallon tote. The slurry was applied directly to the contaminated area (three applications) via a gravity fed two-inch hose. Subsequent sampling of the soil in the source area indicated a decrease in Total Petroleum Hydrocarbons as Diesel (TPH-D) of nearly 50% within four weeks of application. At twelve weeks following the application of Enviro-BAC, the soil samples generally exhibited an average decrease of approximately 76% in TPH-D concentrations, with the soil sample collected in the immediate source area of the release exhibiting an 87% reduction in TPH-D concentrations.



Applications for Enviro-BAC include: open hole excavations; surficial spills, soil borings; and, passive and active groundwater injections. Enviro-BAC is available to environmental consultants, contractors, responsible parties, state agencies, and other environmental stakeholders that realize the environmental and cost benefits of using a naturally occurring product to remediate soil and groundwater from petroleum releases. Benefits of utilizing an in situ bioremedial product include: minimizes disturbance to facility and its operations; decreases remedial duration; provides an efficient remedial alternative to traditional methods; is landfill conscious; is effective on soil and groundwater; and, is environmentally responsible.



Enviro-BAC has been approved for injection in North Carolina and Florida by the Underground Injection Control (UIC) Programs for those states and has also been injected in the District of Columbia. Technical information has been presented in South Carolina and Georgia; however, there are no formal review processes in those States. Remedial proposals are reviewed and approved on a case-by-case basis. Similarly, Virginia does not have a formal review process, a UIC permit would be required for injection (site specific process). Approval is pending in Tennessee.