A New Augmentation Method for Bioremediation of Pathogenic Bacteria-Contaminated Water and Sludge

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Dear Reader:

Concerns about the environment and health risks associated with pathogenic microorganisms from industrial, military, farming and ranching activities, and natural disasters stimulated the consideration of bioaugmentation processes for the reduction or removal of potential pathogens from contaminated water and soil. Some of these are known human pathogens (Escherichia coli, Salmonella species, Vibrio species, Enterococcus species and others), persist in the environment, and are a potential threat to human health and safety. Reducing or removing these potential pathogens from water and soil in an ecologically responsible, safe, and cost-effective way is a top concern for environmental management professionals. Bioaugmentation using non-pathogenic microorganisms is one way to do this. Through intensive study of specially treated non-pathogenic, heterotrophic bacteria (STNPHB), it has been determined that these microorganisms produce compounds that are bactericidal to potential pathogens. This makes them suitable for in-situ reduction or removal of many potentially pathogenic microorganisms from water and soil. STNPHB produce certain antimicrobial compounds that exert a bactericidal effect on potential human pathogens. This process and others like it have been extensively studied in the laboratory, showing great potential for the in-situ bioaugmentation and bioremediation capabilities of STNPHB.

Nature is able to clean up after itself, but it takes time. It's a slow, natural healing process – nature's own pollution control. The problem is that nature, and mankind's efforts to control nature, now can put far more microbial pollution into the environment than nature can remove in the time that man wants to allow. Science has devised ways of speeding nature up by adding certain microbial agents to contaminated water and soil.

We can speed up the rate of natural decontamination by the appropriate application of bioaugmentation agents such as NBT- PB1*. This agent is a unique microorganism suspension (Lactobacillus species) that has been specially processed to deliver non-toxic, bactericidal activity to pathogen-contaminated water and soil. These agents are new and effective Specially Treated Non-Pathogenic Heterotrophic Bacteria (STNPHB) that significantly reduces the number of pathogens in contaminated water and soil.

The consensus solution to controlling extensive pathogen-contamination in water and soil is encompassed in an effective STNPHB bioaugmentation program. Such a program helps fulfill the stated goal of environmental management professionals *to protect the environment and reduce the hazards associated with contamination events.*

I hope that by putting an effective bioaugmentation program into practice, environmental management professionals may be better able to protect human health and safety, rapidly reduce the number of microbial pathogens in biologically contaminated water and soil, and protect other critical infrastructure components of our society from water and soil that is contaminated with human pathogens.

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Historical Overview

The publication of Rachel Carson's Silent Spring in 1962 spoke to the American public about the direct link between the health of the ecological environment and the health of humanity. Since that time, all facets of American society have stepped up their efforts to prevent environmental degradation. Congress passed the Clean Air Act and the Clean Water Act and established the U.S. Environmental Protection Agency. Study results of the National Institute of Environmental Health Sciences have contributed to the increasing public awareness that human diseases often have preventable environmental components. Pollution prevention and environmental remediation are interwoven into all strategies proposed for sustaining human and environmental health. Remediation based on pollutant metabolism or absorption by normal, selected, and/or genetically engineered microbes is emerging as a distinctive and promising approach to cleaning up polluted environments. Harnessing microbial processes for good, rather than experiencing their harmful attributes as propagators of disease, is the goal of bioaugmentation. In this white paper is described an emerging measure of bioaugmentation based on biologically active microbes and focus on microbial processes. Microbes play an essential role in nature's cycles and they are the primary stimulant in bioaugmentation of contaminated environments. In the natural cycles of microbial pathogen reduction, for example, bacteria are essential elements of the reduction process. (4)

The need for a biological approach to improve environmental conditions directly relates to the increasing size of the human population on a planet of finite dimensions. The estimated population of the earth is expected to be twelve (12) billion people by the year 2100. Whereas the number of population doublings that might be sustained by advances in technology (without bringing unbearable pain and suffering) may be argued, no one believes that such an increase can go on indefinitely, and there are already great inequities in degree of pollution-related suffering among populations. As populations grow in size, increases in a variety of adverse human health and ecological effects (and associated costs such as health care expenses) are also expected. (2)

Two categories of bioaugmentation techniques have been identified. They involve distinct technologies for remediation *in situ* and for use of bioreactors. The first category, *in situ* land treatment - treatment of contaminated material in place - is a method for bioaugmentation of contaminated soil and, to some extent, of associated groundwater. The action may be as simple as nutrient enrichment or may involve further manipulation of site conditions, such as inoculation of the contaminated site with selected microorganisms, mixing and aeration of surface soils, or pH modification. *In situ* treatment involves a minimum amount of effort and cost since the contaminated material does not have to be contained or extensively handled. The expensive and potentially dangerous job of transporting waste is eliminated, and further disturbance of the site itself is minimized. The primary drawback of this approach is that degradation monitoring is difficult, and concerns about toxicant dispersal may rule out such methods altogether. A related bioaugmentation approach, referred to as land farming, is applicable to solids, sludges, liquids, or contaminated soil that is treated at a designated site. The wastes are sprayed onto, plowed into, or otherwise mixed with surface soils; aeration, water, nutrient,

and microbial enrichment is applied to achieve the best conditions for microbial growth. Land farming is identical in practice to *in situ* treatment of contaminated soil, except that treatment takes place at a different location from the site of contamination. Waste transport allows the use of a dedicated remediation facility where various considerations, such as contaminant, monitoring, accessibility, security, etc., can be optimized. In most cases, however, land farming as a final treatment for wastes is banned in the United States by the terms of the 1984 Hazardous and Solid Waste Amendments and the 1976 Resource Conservation and Recovery Act (RCRA). The so-called RCRA land ban prohibits any form of waste disposal on land unless the waste has been treated to prescribed standards. Thus, land farming can currently be used only as an intermediate step to be followed by further treatment or disposal. As with *in situ* land treatment, land farming requires large amounts of dedicated land and usually months or years before remediation is complete. (4)

Bioreactors are the second and most technologically sophisticated category of environmental bioaugmentation. Bioreactors offer a much faster means of waste biodegradation than land treatment and more control over reaction conditions and effluent quality than simple biofilters. In contrast to the months or years required for land treatment, bioreactors may require only days or weeks for effective degradation of specific pollutants. Slurry-phase bioreactors are suitable for remediation of high concentrations of microbes and soluble organic wastes in soil and sludges; these reactors are recognized as capable of handling up to at least 250 grams per kilogram of organic wastes. (10)

There are varied bioreactor designs, which allows for treatment of a wide variety of wastes with a varied water and organic content. In some designs bacterial growth is optimized in a well-mixed aqueous phase contained in a lagoon, tank, or other reactor vessel into which slurries of contaminated material are introduced. Other bioreactor designs specifically limit mixing. Control over mixing, aeration, temperature, nutrient levels, water content, etc., is increased dramatically over that which is possible with land treatment techniques. Degradation monitoring is made easier because the system is contained and output is regulated. Importantly, control over release of nonindigenous organisms to the environment is possible. (9)

Bioaugmentation is an emerging field, the full potential of which is as yet unknown. There is a tremendous need for further basic research and development, especially in the areas of environmental site and waste diagnostics, waste-technology matching, and integration of multiple remediation techniques. Bioaugmentation is a technological attempt to exploit the abilities of microbes and other members of the biosphere to restore and maintain environmental quality for all forms of life in the ecosystem, especially humans. Education is important in achieving the widespread practices of prevention, recycling, and remediation for the purpose of improving future environmental health and quality of life.

Perhaps the larger problem facing policy makers in the future is how to decide where available bioremedial dollars will benefit human and environmental health the most. For

instance, in the last 15 years, more than 40,000 hazardous waste sites have been identified in the United States alone. But remedial efforts have been undertaken at only a few hundred sites and are largely restricted to the approximately 1300 sites on National Priority List (Superfund sites). Target cleanup goals have been judged to be highly unrealistic in some cases. At most of these sites remedial efforts are incomplete, and some efforts have had little effect. In some cases, costs are astronomical, i.e., $1 billion for the Rocky Mountain Arsenal near Denver, Colorado. (5)(6)(8)(1)(4)

Although the issues involved are undeniably complex, a considerably improved and enlarged remedial campaign is necessary to deal with all identified toxic waste sites in an adequate, responsible, and expeditious fashion. In the meantime, ineffective sewage treatment plants, septic tanks, and improper methods for handling farm animal waste are also of significant practical concern; and these biologically generated contaminants are also adversely affecting the health of undetermined numbers of people. It is clear that new bioaugmentation technologies that can better monitor and control many types of societal wastes are emerging.

Bioaugmentation

Bioaugmentation is the controlled addition of specially formulated microbial cultures to assist those found naturally in the soil. It is done in conjunction with the development and monitoring of a growth environment in which selected bacteria can live and work.

In most cases, the targeted contaminants either serve as the food source or are co-metabolized. Essential elements are added to the “food source” to provide the required nutrient levels, and water provides the media in which the bacteria function.

The simple addition of bacteria will not, in itself, solve the problem. Studies conducted in 1979 by Dibble and Bartha demonstrated that sewage sludge actually inhibited hydrocarbon degradation in soil, and the use of yeast extract had no effects. The selected microorganisms must be carefully matched to the waste contamination present in the soil, as well as the metabolites formed. They must favorably compete with the ubiquitous organisms found in the environmental conditions.

Bioaugmentation allows for the control of the nature of the biomass. It provides a certain element of predictability. Bioaugmentation ensures that the proper complement of microorganisms is present in the soil in sufficient type, number, and comparability to effectively and efficiently attack the harmful waste constituents and reduce them to harmless substances.

Bioaugmentation has the advantage over other bioremediation processes in that it allows for the control of the biomass of the contaminated site. The additional control of the biomass enables one to increase the kinetic rate of removal from the contaminated site by selecting more efficient consortia of microorganisms than might be naturally present at the site. By increasing the kinetic rates, it has been possible to remediate sites in a shorter time frame using the addition of selected consortia of microorganisms. (3)

By selecting the bioaugmentation process beforehand, it is possible to select for organisms that will not produce nuisance odors such as hydrogen sulfide.

Laboratory Study 1 – Environmental Sludge

Laboratory Study 1 shows how Bioaugmentation Product NBT-PB1* was used to reduce the number of total coliform bacteria in environmental sludge.

Issue: Total coliform-contaminated environmental sludge collected in New Orleans, Louisiana following Hurricane Katrina.

Goal: Determine the feasibility for the laboratory bioaugmentation of sludge contaminated with total coliforms.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB1* as a treatment alternative for environmental sludge contaminated with total coliforms. Microbial enumeration and growth potential tests indicated the sludge contained high levels of total coliform bacteria. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB1* on total coliform reduction. Differential plate counts for the total coliform contaminants conclusively demonstrated greater than 96 per cent reduction after 60 minutes of exposure to the Bioaugmentation Product NBT-PB1*. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB1* on Total Coliform Bacteria Present in Environmental Sludge

Exposure Time (minutes)	**Total Coliforms Actual Count (cfu/gram)**	**Percent Reduction**	**Log_{10} Reduction**
5	**1.0×10^4**	**96.875**	**1.51**
10	**1.0×10^4**	**96.875**	**1.51**
15	**1.0×10^4**	**96.875**	**1.51**
30	**2.0×10^4**	**93.750**	**1.20**
60	**$<1.0 \times 10^4$**	**>96.875**	**>1.51**

Laboratory Study 2 – Environmental Flood Water

Laboratory Study 2 shows how Bioaugmentation Product NBT-PB1* was used to reduce the number of total coliform bacteria in flood waters.

Issue: Total coliform-contaminated flood water collected in New Orleans, Louisiana following Hurricane Katrina.

Goal: Determine the feasibility for the laboratory bioaugmentation of flood water contaminated with total coliforms.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB1* as a treatment alternative for flood water contaminated with total coliforms. Microbial enumeration and growth potential tests indicated the flood water contained high levels of total coliform bacteria. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB1* on total coliform reduction. Differential plate counts for the total coliform contaminants conclusively demonstrated greater than 99 per cent reduction after 5 minutes of exposure to the Bioaugmentation Product NBT-PB1*. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB1* on Total Coliform Bacteria Present in Flood Water

Exposure Time (minutes)	Total Coliforms Actual Count (MPN)	Percent Reduction	Log_{10} Reduction
5	1.04×10^7	99.320	2.17
15	5.79×10^6	99.622	2.42
30	5.29×10^6	99.654	2.46
60	4.22×10^6	99.724	2.56

Laboratory Study 3 – Environmental Flood Water

Laboratory Study 3 shows how Bioaugmentation Product NBT-PB1* was used to reduce the number of total coliform bacteria in flood waters.

Issue: Total coliform-contaminated flood water collected in New Orleans, Louisiana following Hurricane Katrina.

Goal: Determine the feasibility for the laboratory bioaugmentation of flood water contaminated with total coliforms.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB 1* as a treatment alternative for flood water contaminated with total coliforms. Microbial enumeration and growth potential tests indicated the flood water contained high levels of total coliform bacteria. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB1* on total coliform reduction. Differential plate counts for the total coliform contaminants conclusively demonstrated greater than 99 per cent reduction after 5 minutes of exposure to the Bioaugmentation Product NBT-PB1*. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB1* on Total Coliform Bacteria Present in New Orleans Flood Water

Exposure Time (minutes)	Total Coliforms Actual Count (MPN)	Percent Reduction	Log_{10} Reduction
5	1.1×10^6	99.928	3.14
10	3.1×10^5	99.980	3.69
15	8.6×10^5	99.944	3.25
60	1.0×10^5	99.993	4.18

Laboratory Study 4 – Escherichia coli ATCC 11229

Laboratory Study 4 shows how Bioaugmentation Product NBT-PB1* was used to reduce the number of Escherichia coli ATCC 11229 bacteria contained in an aqueous suspension.

Issue: Aqueous suspension of Escherichia coli ATCC 11229 prepared in the laboratory.

Goal: Determine the feasibility for the laboratory bioaugmentation of an Escherichia coli ATCC 11229 suspension prepared in the laboratory.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB 1*as a treatment alternative for an Escherichia coli ATCC 11229 suspension prepared in the laboratory. Microbial enumeration and growth potential tests indicated the suspension contained high levels of Escherichia coli ATCC 11229. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB 1*on Escherichia coli ATCC 11229 reduction. Differential plate counts for the Escherichia coli ATCC 11229 contaminants conclusively demonstrated greater than 98 per cent reduction after 60 minutes of exposure to the Bioaugmentation Product NBT-PB1*. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB1* on E. coli ATCC 11229

Exposure Time (minutes)	E. coli Actual Count (cfu/ml)	Percent Reduction	Log_{10} Reduction
5	2.6×10^8	96.533	1.46
10	2.7×10^8	96.400	1.44
15	2.0×10^8	97.333	1.57
30	1.53×10^8	97.960	1.69
60	1.25×10^8	98.333	1.78

Laboratory Study 5 – Aspergillus niger ATCC 16404

Laboratory Study 5 shows how Bioaugmentation Product NBT-PB1* was used to reduce the number of Aspergillus niger ATCC 16404 fungus/mold contained in an aqueous suspension.

Issue: Aqueous suspension of Aspergillus niger ATCC 16404 prepared in the laboratory.

Goal: Determine the feasibility for the laboratory bioaugmentation of an Aspergillus niger ATCC 16404 suspension prepared in the laboratory.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB 1* as a treatment alternative for an Aspergillus niger ATCC 16404 suspension prepared in the laboratory. Microbial enumeration and growth potential tests indicated the suspension contained high levels of Aspergillus niger ATCC 16404. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB 1* on Aspergillus niger ATCC 16404 reduction. Differential plate counts for the Aspergillus niger ATCC 16404 contaminants conclusively demonstrated greater than 98 per cent reduction after 60 minutes of exposure to the Bioaugmentation Product NBT-PB 1* . Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB 1* on Aspergillus niger ATCC 16404

Exposure Time (minutes)	A. niger Actual Count (cfu/ml)	Percent Reduction	Log_{10} Reduction
5	1.34×10^7	81.259	0.73
10	1.8×10^6	97.483	1.60
15	1.15×10^6	98.392	1.79
30	1.3×10^6	98.182	1.74
60	1.1×10^6	98.462	1.81

Laboratory Study 6 – Environmental Flood Waters

Laboratory Study 6 shows how Bioaugmentation Product NBT-PB 1* was used to reduce the number of heterotrophic bacteria contained in flood waters.

Issue: Heterotrophic bacteria present in flood water collected in New Orleans, Louisiana following Hurricane Katrina.

Goal: Determine the feasibility for the laboratory bioaugmentation of heterotrophic bacteria present in flood waters.

Process: Laboratory studies were conducted to evaluate Bioaugmentation Product NBT-PB 1* as a treatment alternative for flood water containing high numbers of heterotrophic bacteria. Microbial enumeration and growth potential tests indicated the flood water contained high levels of heterotrophic bacteria.

Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB1* on total heterotroph reduction. Heterotrophic plate counts for total heterotrophy conclusively demonstrated greater than 87 per cent reduction after 6 hours of exposure to the Bioaugmentation Product NBT-PB 1 *. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bactericidal Activity of NBT-PB 1* on Bacteria contained in Flood Water

Exposure Time (hours)	Total Bacteria Actual Count (cfu/ml)	Percent Reduction	Log_{10} Reduction
2	2.09×10^6	71.370	0.54
4	1.09×10^6	85.068	0.83
6	9.4×10^5	87.123	0.89

Laboratory Study 7 – Bacteria Contained in New Orleans Environmental Sludge – Organism Identification

Laboratory Study 7 shows how Bioaugmentation Product NBT-PB 1* reduced the number of various heterotrophic bacteria contained in sludge.

Issue: Heterotrophic bacteria present in sludge collected in New Orleans, Louisiana following Hurricane Katrina.

Goal: Determine the identity of heterotrophic bacteria present in sludge following bioaugmentation with NBT-PB 1*.

Process: Laboratory studies were conducted to identify the heterotrophic bacteria present in sludge following treatment with Bioaugmentation Product NBT-PB 1*. Microbial enumeration and growth potential tests indicated the sludge contained high levels of heterotrophic bacteria. Additional studies conducted in closed slurry reactors evaluated the impact of Bioaugmentation Product NBT-PB1* total heterotroph reduction. Laboratory studies were conducted according to Official Methods of Analysis, 15th Edition, Association of Official Analytical Chemists (AOAC), Method 960.09C.

Bacteria Identified in Sludge Following Treatment with NBT-PB 1*

Sample Description	Bacteria Identified
Direct Sludge Sample:	Bacillus cereus Bacillus megaterium Acinetobacter lwoffi Stenotrophomonas maltophilia Escherichia coli Aeromonas hydrophilia Pseudomonas stutzeri
Sludge and NBT-PB 1* after 2 hours exposure:	Bacillus cereus Pseudomonas species
Sludge and NBT-PB 1* after 4 hours exposure:	Bacillus species Probiotic product* Pseudomonas species
Sludge and NBT-PB 1* after 6 hours exposure:	Bacillus species= Probiotic product*

Conclusion

Reducing the numbers of pathogenic bacteria and other heterotrophic bacteria in flood water and sludge can be significantly accelerated by the appropriate application of bioaugmentation agents such as NBT-PB1*. Each of these agents is a unique microorganism suspension (Lactobacillus species) that has been specially processed to deliver non-toxic, bactericidal activity to pathogen-contaminated water and sludge. These agents are new and effective Specially Treated Non-Pathogenic Heterotrophic Bacteria (STNPHB) that significantly reduces the number of pathogens in contaminated water and sludge.

The consensus solution to controlling extensive pathogen-contamination in water and sludge is encompassed in an effective STNPHB bioaugmentation program. Such a program helps fulfill the stated goal of environmental management professionals *to protect the environment and reduce the hazards associated with contamination events.*

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*NBT-PB1 = Living Streams Pro-Biotic Solutions co.