



System Description 111275954-3500

Ex-Situ Bioremediation System
Project number: Russo Development
Contract number:

Prepared By: Liberty Engineering

System Description Version Control

| Version | Date | Author | Change Description |
|----------|---------|---------------|--------------------|
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1 FUNCTION

Ex-Situ hydrocarbon bioremediation via microbial degradation in an oxygen rich environment.

2 DESIGN BASES

2.1 Safety Design Bases

ASME/ANSI B31.3, Process Piping, ASME Code for Pressure Piping, American Society of Mechanical Engineers, New York 1999.

ASME/ANSI Boiler and Pressure Vessel Code, ASME Code for Boilers and Pressure Vessels, Section VIII, Division I, *Rules for Construction of Pressure Vessels*, American Society of Mechanical Engineers, New York 2004.

CGA Pamphlet G-4, *Oxygen*, Compressed Gas Association, Arlington, VA.

CGA Pamphlet G-4.1, *Cleaning Equipment for Oxygen Service*, Compressed Gas Association, Arlington, VA.

NFPA 70 *National Electric Code* 2005 Edition. National Fire Protection Association. Quincy, Massachusetts.

U.S. Code of Federal Regulations Title 29 subpart 1910 Occupational Safety and Health Standards, GPO, 2007.

2.2 Contract Design Bases

CGA Publication P-8.1-2003. *Installation and operation of PSA, membrane oxygen and nitrogen generators*, Compressed Gas Association, Arlington, VA.

Eckenfelder, W.W. 1989. *Industrial Water Pollution Control*. 2d Ed. McGraw-Hill.

2.3 Codes and Standards

ASTM E 1739 2002, Standard Guide for Risk-Based Corrective Action Applied at Petroleum Release Sites, American Society of Testing and Materials, Philadelphia, PA.

3 DESCRIPTION

3.1 Microbial Degradation of Hydrocarbons

Microbial degradation of hydrocarbons is well documented in numerous publications and scientific literature. There are several commercially available processes where procaryotic organisms consume organic material under certain conditions to reduce them to carbon dioxide, water and biomass. By a substantial margin, bio-stimulation and augmentation are the fastest acting, most complete method for remediation of hydrocarbons and volatile organic compounds. Discussion here will be limited to this method.

Bio-stimulation (enhancement) involves adding specific nutrients for the indigenous bacteria in an oxygen rich environment, and bio-augmentation is addition of bacteria selected for affinity to the specific contaminants. These methods have proven to be valid under certain site conditions and have been utilized for the effective and successful cleanup of contaminated soil and water.

Bacteria, as all living organisms, have certain chemical and physical growth requirements. The proper application of these conditions is especially important in considering bioremediation.

3.2 Basic Mechanisms

An energy source is needed for biosynthetic reactions to make polymers such as proteins from amino acids and RNA and DNA from nucleotides.

Some Bacteria can utilize light energy, however the ones that we are concerned with oxidize chemical compounds to obtain their energy. The bacteria that are involved in bioremediation are chemoorganotrophs as they utilize organic compounds for their energy source.

A carbon source is required for all of the polymeric units in the cell such as DNA, RNA, and proteins. Some bacteria can utilize carbon dioxide as a sole carbon source ,however, the organisms concerned with bioremediation are heterotrophs as they require an organic source of carbon.

A nitrogen source such as ammonia, nitrite, nitrate, and organic nitrogen is required. Nitrogen is a component in the amino acids of proteins and in the purines and pyrimidines of RNA and DNA.

Phosphate is a component part of the nucleotides composing RNA and DNA these are required for energy transfer reactions.

Minerals such as magnesium, manganese, iron, etc., are required and are typically readily available in the soil. No additional augmentation is required.

Oxygen Supply

Utilization of aliphatic hydrocarbons by microorganisms is strictly an aerobic process. The initial oxidation step of aliphatic hydrocarbons involves molecular oxygen as a reactant and one of the oxygen molecules is actually incorporated into the oxidized product. The aromatic group of hydrocarbons can be viewed as derivatives of benzene. The breakdown of aromatic hydrocarbons involves the action of either oxygenases or mixed function oxygenases. These two reaction sequences both form catechol which can be degraded in a number of ways leading to either acetyl CoA or TCA cycle intermediates. Very importantly, the oxygen concentration is the rate limiting factor in the biodegradation of petroleum based products. Approximately, four pounds of oxygen is required for the biological oxidation of one pound of hydrocarbon. Microbial activity during bioremediation is often frequently limited by insufficient oxygen due to slow rates of diffusion into the interior of the soil layers or piles and into the center of soil aggregates. Excess water has been shown to severely limit the oxygen concentration and result in anaerobic conditions. Effective application can result in greatly accelerated cleanups as generally, the greater the mass of oxygen that can be distributed the more rapid and complete the cleanup.

3.3 Bacterial Growth Process

Bacteria are procaryotic organisms that reproduce primarily asexually by binary fission. One organism splits into two organisms and each one is capable of reproducing further.

The principle of bioremediation is based on the tremendous growth potential of bacteria with the binary fission process. For illustration purposes, consider the growth potential of one single bacterial cell, assuming a 20 minute generation time, for a few hours.

1--2--4--8--16--32--64--128--256--512--1024--2048--4096--8192--16384--3268--65536--131472--262944--525888--1,051,776—in a little more that 6 hours the population has increased over 1 million fold. This tremendous growth rate is the secret to successful remediation.

In the case of bioremediation, the hydrocarbon is used both as a carbon source and an energy source to produce the Million fold increase in cell mass.

Large amounts of dissolved oxygen to stimulate the process and proper choice of bacteria is key to rapid remediation.

3.4 Ex-Situ Bioremediation System

Liberty Engineering's ex-situ bio remediation system will completely remediate a nominal 270,000 cubic feet of hydrocarbon contaminated soil in 90 days of treatment with proper application and supervision. Significant reduction in contaminates will be obtained in the first 30 days.

3.5 Operational concept

Molecular sieves are used to produce high purity oxygen from air employing the pressure-swing adsorption process. Pressurized oxygen is used to activate the bacteria and is continuously supplied via water sparge to meet the biological oxygen demand as the contaminates are broken down.

Chloride/hydrocarbon free water and supplemental nutrients are supplied from an external source. Water and bacteria slurries are pumped to the oxygen sparger and to the soil in batch operations.

When hydrocarbon contaminates are depleted, the bacteria are deprived of food and self-remediate. The process can be terminated on short notice if required, by sterilization.

3.6 Environmental Requirements

3.6.1 Temperature

Temperature must remain above freezing to prevent killing off the bacteria. Microbes go dormant below 65°F and require additional nutrients if ambient temperature dips for an extended period during the process

3.6.2 Moisture

The soil should be kept between 15-20% moisture. Not wet.

3.6.3 pH

pH should be between 6.5 and 8.0. A pH of 7.2 is optimum

3.7 Description of Principle Components

3.7.1 PSA Oxygen Generator

The oxygen generator system will supply oxygen of no less than 96% oxygen at 50 +/- 5 psig.

Low air and oxygen pressure protection are standard equipment.

In the event of power failure, the oxygen generator fails-safe.

The system is designed for in-door operation. The system components are designed not to pose any danger to existing equipment or create a hazardous environment.

Ambient operating temperature range is 32°F to 120°F. Storage conditions are -20°F to 140°F.

The adsorber skid requires a 240 VAC, 60 Hz, 20 A feeder breaker. Full load amperage of the oxygen and auxiliary equipment is 17 A.

The system is designed to operate at an elevation of 577 feet above sea level (Hamburg, NY).

The oxygen generator and associated equipment operate without causing or suffering any unacceptable performance degradation due to electromagnetic interference to or from other equipment in the same environment. Harsh environmental conditions such as industrial environments, blowing sand, snow, sleet, dust, rain, and salt fog are protected against by the enclosure.

If necessary to dispose of spent adsorbent, the molecular sieve will not pose a threat to the environment. To dispose of molecular sieve, it must be wetted with water to deactivate it. Adsorption of water generates heat and care must be taken to prevent property damage or personal injury. Instructions are provided for disposal.

The oxygen generator skid does not present a safety hazard to personnel, facilities or the environment in the event of fire or catastrophic failure while operational or non-operational in all planned environments.

3.7.2 Sparger

Dissolved oxygen at the required concentration for bio-augmentation is introduced to the microbial slurry and then continuously distributed to the soil to keep up with biological demand. The sparger has an integral recirculation and oxygen injection system and controls to meter the required oxygen/water/microbe mixture.

3.7.3 Water Storage and Transport

Approximately 78,000 gallons of water is required for a 90 day treatment. Water must be free of chlorine, grease, oil, pesticides, high sodium content, and other components deleterious to life of the bacteria.

3.8 Computer Resources

The complete system is designed to operate automatically with little or no human interface beyond initial start-up. A Supervisory Control and Data Acquisition (SCADA) system is incorporated that allows for PC based control, data logging and alarm functions either on a LAN based or wireless platform. The primary function of the SCADA is to control oxygen purity and sparge functions.

3.9 Maintenance

The system skid requires no periodic maintenance. Consumable materials are disposed of as non-hazardous waste.

4 OPERATION

4.1 Start-up

Start-up takes approximately 2-1/2 days to activate bacteria and establish the initial 30 day campaign.

4.2 Remediation Time

Substantial results will be obtained immediately. An approximate 50% reduction occurs in 21 days, 80% in 60 days and full remediation in 90 days.

4.3 Full-Cost Utility Costs

4.3.1 Adsorber System Operating Cost

| | | Northeast |
|-------------|---------|-----------|
| Electricity | \$/kWhr | 0.08-0.12 |

Average load of operating system = 3.391 kW. Cost for 30 days of operation at \$0.10 per kW-hr is \$244.15.

4.3.2 Water Cost

Supplier's labor cost is 6.5 hrs X \$110 hr = \$715.00

Trailer Rental = \$75.00 per month.

Water is supplied to specification at no additional cost.

5 REFERENCES

| | |
|---------------------|---------------|
| Design Criteria | 111275954-300 |
| Design Calculations | 111275954-400 |
| P&ID | 111275954-700 |
| SWD | 111275954-800 |