REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-018

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1995	

4. TITLE AND SUBTITLE

Using Stable Isotopes of Carbon to Monitor Biodegradation of Pollutant Compounds

5. FUNDING NUMBERS

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESSE(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

TDS-2013-ENV

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESSES

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The rate at which microorganisms breakdown petroleum contaminants in soil and water can be measured by analyzing their waste product, carbon dioxide (CO_2). The ability to measure the actual biological breakdown of contaminats can be quantified by analyzing the differences in the ratio between the different carbon molecules isotopes ^{12}C and ^{13}C, which make up petroleum contaminants and released carbon dioxide.

14. SUBJECT TERMS

Biodegradation of pollutant compounds, microorganisms, petroleum contaminants, stable isotopes

15. NUMBER OF PAGES

2

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

TechData Sheet

TDS-2013-ENV **April 1995**

Naval Facilities Engineering Service Center
Port Hueneme, California 93043-4328

SERDP
Strategic Environmental Research and Development Program
A Partnership to Improve the Environment

Using Stable Isotopes of Carbon to Monitor Biodegradation of Pollutant Compounds

INTRODUCTION

The rate at which microorganisms breakdown petroleum contaminants in soil and water can be measured by analyzing their waste product, carbon dioxide (CO_2). The ability to measure the actual biological breakdown of contaminants can be quantified by analyzing the differences in the ratio between the different carbon molecules isotopes ^{12}C and ^{13}C, which make up petroleum contaminants and released carbon dioxide.

PURPOSE OF CARBON ISOTOPE MONITORING AT PORT HUENEME

Carbon isotope monitoring is being conducted on contaminated petroleum soil and groundwater and in remediation demonstrations at the Construction Battalion Center (CBC) in Port Hueneme to indicate if bioremediation of petroleum compounds is occurring.

ADVANTAGES

By comparing the carbon isotope ratios of the different carbon containing compounds, this will indicate that the bacteria are indeed consuming the pollutant compounds and are converting them into harmless $^{12}CO_2$ and $^{13}CO_2$.

TECHNICAL DESCRIPTION

Many elements exist as two or more isotopes. Isotopes of an element have the same number of protons in their nuclei but different numbers of neutrons. For example, ^{12}C has six protons and six neutrons, and ^{13}C has six protons and seven neutrons. Both ^{12}C and ^{13}C are stable, i.e., they are not radioactive like their ^{14}C counterpart. The ^{12}C isotope is the most abundant isotope, making up about 98.89% of all carbon, whereas ^{13}C constitutes approximately 1.11% of all carbon (^{14}C makes up less than 0.00001% of all naturally occurring carbon).

The additional neutron in ^{13}C element makes it slightly heavier than ^{12}C. Because of this weight difference, the two isotopes tend to react at slightly different rates in biological and chemical reactions. The most significant reaction where this occurs is during photosynthesis, in which CO_2 in the air is incorporated into plant tissue. Once the carbon isotope ratio has been established in the plant, further transformations of carbon cpmpounds do not change the isotope ratio to a large degree. Throughout millions of years, animal, plant, and tiny marine organism residues have been subjected to geological conditions, which have converted them into a complex mixture of organic substances called petroleum. There are hundreds of individual carbon containing compounds in every crude oil. The composition of ^{12}C and ^{13}C elements in each crude oil varies with its origin. Crude oil is refined by fractional distillation into commercially usable petroleum products. Petroleum products are a complicated mixture composed primarily of molecule chains containing 5 to 10 carbon atoms for gasoline, 11 to 18 carbon atoms for jet fuel, and 15 to 40 carbon atoms for lubricating oils.

Biological processes are varied and depend on the fuel hydrocarbon contaminant chemistry and soil and water conditions. The microorganisms act to transform contaminants from relatively toxic forms to relatively nontoxic forms (CO_2, methane) of carbon. Stable carbon isotope ratios are being measured as indicators of the bioremediation of petroleum products, such as diesel and gasoline, as shown in Figure 1.

Figure 1. Measuring stable carbon isotope ratios.

Since the development of an instrument called a gas chromatograph/ isotope ratio mass spectrometer, it has been possible to separate some 20 different chemical components of diesel and gasoline and to measure their individual $^{13}C/^{12}C$ ratios as shown in Figure 2. It has been discovered that the compounds in diesel and gasoline have $^{13}C/^{12}C$ ratios that are similar to those of the organic matter that formed the petroleum million years ago, however, they are distinct enough to allow the biodegradation of 20 some compounds to be traced with respect to their $^{13}C/^{12}C$ ratio.

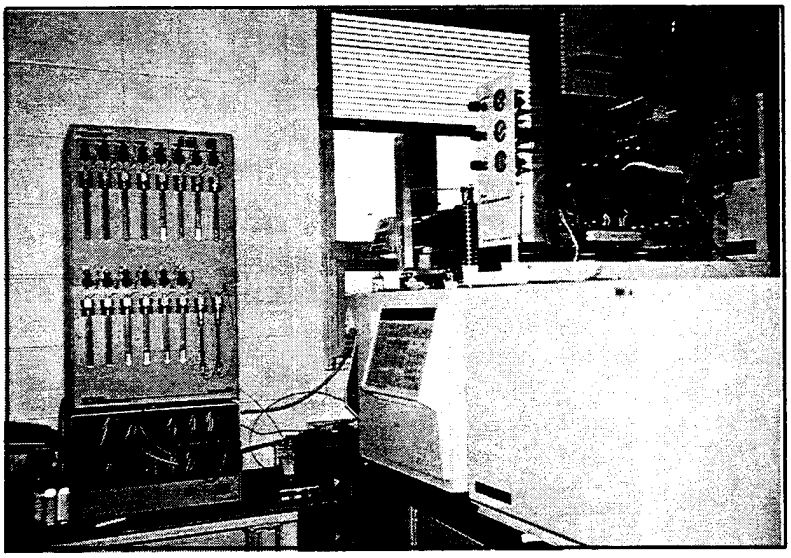

Figure 2. Gas chromatograph/isotope ratio mass spectrometer.

It has been found that bacteria take on the carbon isotope ratios of their food; furthermore, the CO_2 that they release during the breakdown of the diesel and gasoline has the same carbon isotope ratio as their food. The implication of this is that when bacteria consume pollutant compounds in diesel and gasoline, their tissues and the carbon dioxide waste product that they respire have $^{13}C/^{12}C$ ratios that are the same as the pollutants that they are degrading.

At contaminated sites, scientists hope to use carbon isotope ratios to indicate whether or not bioremediation of pollutant compounds is occurring. They will measure the carbon isotope ratios for the pollutant compounds (in Port Hueneme this will consist of gasoline and diesel) as well as non-pollutant organic matter. Scientists will then measure the carbon isotope ratios of bacteria collected from the polluted ground waters and soils, and will measure the isotope ratios of CO_2 collected from soil gas samples.

For more information about this monitoring system, contact Mr. Jeff Heath, Manager, Technology Application Branch, Code ESC414, at (805) 982-1657 or DSN: 551-1657, or call our 24-hour number: (805) 982-4070 or DSN: 982-4070.