Dehalocounter™

～Volatile organic chlorine compound decomposing bacteria detection technology～

1 Background

Once soil or groundwater is contaminated with chlorinated volatile organic compounds (hereinafter referred to as "VOC") such as tetrachloroethylene or trichloroethylene, widespread contamination is likely. Since the conventional excavation and removal method as technology to decontaminate soil and groundwater involves huge costs, more economically rational methods that involve biostimulation have gained popularity in recent years.

Biostimulation is a method that involves injecting nutrients of decomposition microbes existing in soil or groundwater in order to activate their behavior, thereby rendering pollutants non-hazardous. Depending on the living condition of decomposing microbes in the ground, the pollutant decomposition is difficult or the decomposition process does not proceed to a state in which pollutants are detoxified in some cases. Still, confirming the applicability of a biostimulation method in advance can ascertain the cleanup effect.

As the applicability confirmation of biostimulation methods in advance, we conducted indoor cleanup tests and on-site demonstration cleanup tests for assessment using soil/groundwater samples taken from the contamination site. These purification tests, enabling highly accurate assessment for applicability, take about three months. Given this, we have started initiatives involving assessing the applicability of a biostimulation method more simply and in a shorter span of time by quantitatively understanding the living condition of VOC decomposition bacteria in the contaminated ground.

2 Advantages of Dehalocounter

Dehalococcoides spp. are the most common VOC decomposition bacteria. Dehalococcoides spp. use VOC for respiration, dechlorinating the VOC to render them harmless. (Refer to Fig. 1.) At present, they alone have been confirmed to decompose VOC to harmless ethylene.

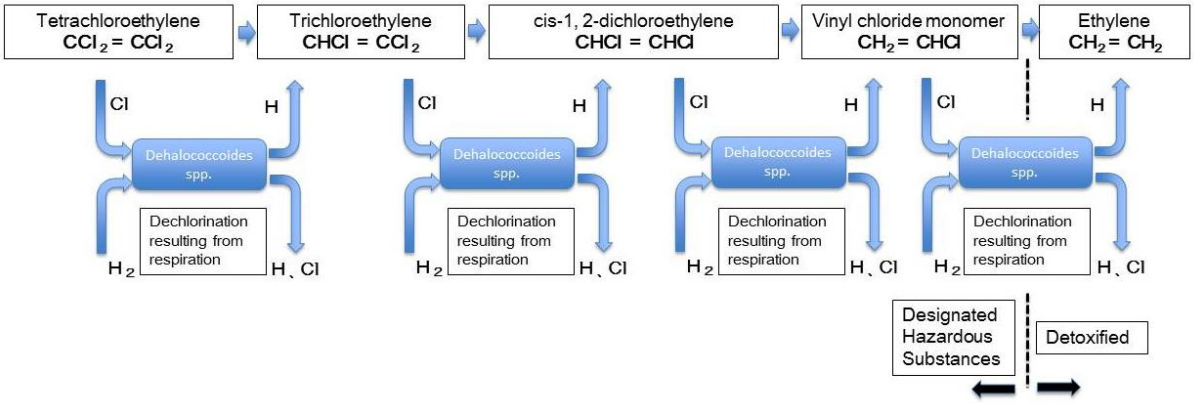


Fig. 1: VOC decomposition pathway and decomposition mechanism of Dehalococcoides spp.[1)]

Vinyl chloride monomer will be added to the Designated Hazardous Substance list on April 1, 2017.

Using a trace of Dehalococcoides spp. existing in the contaminated ground, our biostimulation method cultivates it to approx. 100 to 1,000 times. While the bacteria are increased, the decomposition of VOC proceeds, offering the prospect of a definite cleanup effect.

When it comes to quantifying environmental Dehalococcoides spp., samples taken from the contaminated soil/groundwater are analyzed using a real-time PCR method (increasing the gene arrangement specific to the target bacterium using a gene amplification method called the PCR method, and monitoring the process in real time). However, there were cases in the past in which some cleanup effect has been confirmed during a biostimulation test with no Dehalococcoides spp. detected. For this reason, we have continued our efforts to improve the quantitative accuracy.

Our Dehalocounter consists of technologies for detecting a trace of Dehalococcoides spp. without fail with high accuracy, characterized by the following advantages.

(1) Certainty in collecting decomposition bacteria

- Dehalococcoides spp. are unique anaerobic bacteria that require careful handling when taking samples. We have optimized the sampling equipment and sampling techniques based on the accumulated experience, enabling the collection of the bacteria with certainty.

(2) Established highly accurate analysis method

At the element technology level, we have taken various measures to improve the accuracy of the analysis using the real-time PCR method.

1) Pretreatment techniques

- For the pretreatment of an analysis, chemicals that contain no contaminating DNA are used, eliminating the influence of any impurities on the analysis results.

- If any substance that inhibits the PCR reaction is contained in the sample, the gene amplification efficiency is significantly reduced, and a lower quantitative value will be estimated. Since we use a kit with better ability to remove PCR inhibitors (compared to various commercially available kits), highly accurate analyses can be performed.

2) Real time monitoring using the QP method[2)]

- For the analysis, a method called QP is used. The QP method uses fluorescent-labeled probes, called QProbes, that bind to growths amplified under the PCR method. Binding to a growth quenches the fluorescence of a QProbe. The amplification statuses can be monitored in real time by checking the percentage of QProbes with quenched fluorescence. Since this enables analyzing the sample while confirming the smooth amplification progress under the PCR reaction, accurate quantification results can be obtained (Fig. 2).

- Conventionally, there are detection methods including those with an accuracy problem caused by labeling both specific and non-specific growths or involving a significant cost resulting from the use of two types of fluorescent dyes. In contrast, QProbes used for the QP method bind to specific growths alone, and only one type of fluorescent dye is used, which reduces costs.

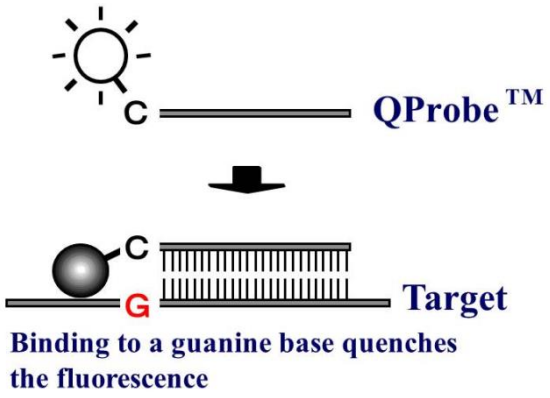
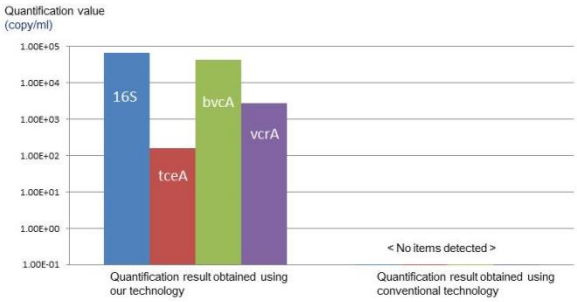


Fig. 2: Schematic of quenching of the fluorescence possessed by a QProbe[2)]

3 Example of Assessment Results

Figure 3 shows the quantification results obtained using our technology described above to take a sample from a contamination site before performing any cleanup treatment and conduct an analysis as an example of the applicability assessment of a biostimulation method, together with the results obtained using conventional technology to take samples and analyze the same site for comparison. Using our technology, an abundance of Dehalococcoides spp. were detected. During a follow-up biostimulation test as well, a very favorable cleanup effect was confirmed, supporting the high accuracy of the technology.



16S: Genes that Dehalococcoides spp. have in common

tceA, bvcA, and vcrA: VOC degradative enzyme genes possessed by Dehalococcoides spp.

Fig. 3: Example of detection results

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4 Conclusion

Detecting VOC decomposition bacteria with high accuracy enables labor-saving and shortening the time involved in the applicability assessment of a biostimulation method to a VOC pollution site. We hope that this technology assists in the further spread of biostimulation methods.

References

1) Masahiro Mizumoto et al., Cleanup Technologies of Soil/Groundwater Contamination, Tatemasa Hirata, et al., Cleanup and Restoration Technologies of Contaminated Soil/Groundwater, NTS, Inc., PP. 60–61 (2008),

2) Shinya Kurata, Complex Microbial System Analysis Technology Using Fluorescence Quenching Phenomenon, supervised by Ryuichiro Kurane, New Applications & Developments of Biotechnology for Industrial Pollutant-remediations and Wastewater-treatments, CMC Publishing, PP. 56–64 (2012)