Remediation of Subsurface Contamination Using Bacteria

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Abstract

Contamination of subsurface environments (soil, groundwater) is a world wide environmental issue. There are many bacteria in subsurface environments and some of them help us to maintain clean environments. I would like to introduce various abilities of bacteria and a case study at the contaminated site in Japan.

Remediation of subsurface contamination using bacteria

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National Institute of Advanced Industrial Science and Technology (AIST)
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Today's talk

1 Subsurface contamination in Japan
2 What is "bacteria", a key player in bioremediation?
3 Various functions of bacteria
4 How to investigate subsurface contamination
5 Bioremediation utilizing bacteria

1. subsurface contamination in Japan

(geo-pollution, soil&groundwater contamination)

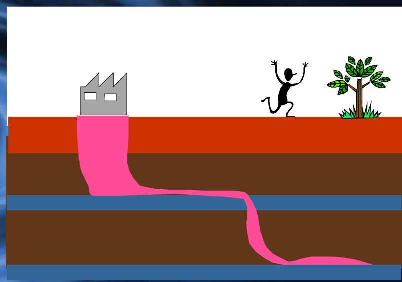
When contaminated,

Direct inhalation of soil

Direct intake of groundwater

⇓

Risk for human health



Regulated by "Soil Contamination Countermeasures Act"

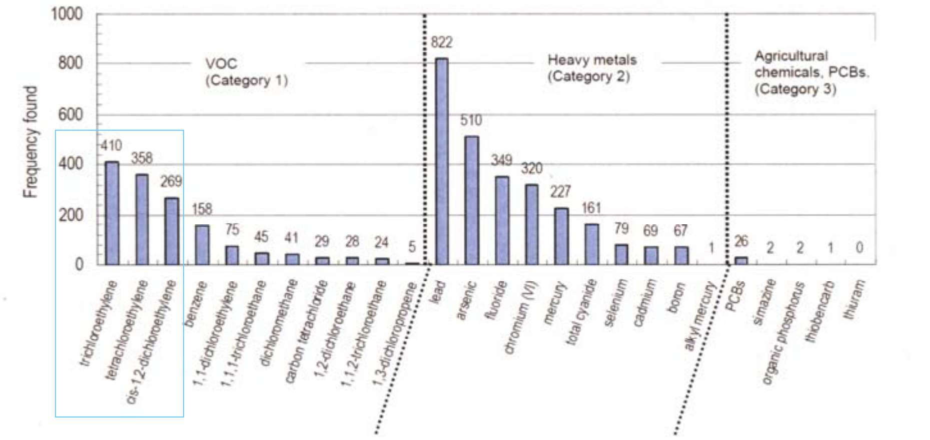
Environmental Quality Standards are set for...

Inorganic substances

Cd, Pb, Cr(VI), As, Hg, Cu, Se, F, B

Organic substances

total cyanide, organic phosphorus, PCBs, dichloromethane, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethylene, cis-1,2-dichloroethylene, 1,1,1-trichloroethane, 1,1,2-trichloroethane, trichloroethylene, tetrachloroethylene, 1,3-dichloropropene, thiuram, simazine, thiobencarb, benzene



Frequency of designated hazardous substances exceeding standards (Ministry of Environments, 2006)

Tetrachloroethylene and its degradation products are major VOCs (volatile organic compounds)

Remedial actions adopted in the past (Ministry of Environments, 2006)

	# of countermeasures		VOCs Category 1		Heavy metals Categories 2 & 3		complex contaminations	
	FY2004	total	FY2004	total	FY2004	total	FY2004	total
Monitoring of groundwater quality	9	(315)	7	(166)	1	(101)	1	(48)
Removal of Soil Contamination	382	(1,860)	81	(622)	211	(898)	90	(340)
Excavations	296	(1,246)	32	(209)	205	(844)	59	(193)
Insitu Cleanup	86	(614)	49	(413)	6	(54)	31	(147)
Bioremediation	18	(44)	10	(27)	0	(3)	8	(14)
Chemical Decomposition	16	(54)	7	(25)	2	(7)	7	(22)
Soil Vapor Extraction	20	(199)	13	(160)	1	(4)	6	(35)
Pump and Treat	24	(282)	13	(189)	2	(31)	9	(62)
Soil Washing	5	(15)	4	(5)	0	(6)	1	(4)
Others	3	(20)	2	(7)	1	(3)	0	(10)
Insitu Containment	11	(85)	0	(7)	7	(54)	4	(24)
Sheetpiles	8	(37)	0	(3)	5	(21)	3	(13)
Soil/Cement Mixing Walls	0	(20)	0	(2)	0	(13)	0	(5)
Others	3	(28)	0	(2)	2	(20)	1	(6)
Offsite Containment	3	(8)	0	(0)	2	(5)	1	(3)
Stabilization (insitu)	3	(62)	0	(2)	2	(51)	1	(9)
Stabilization (exsitu)	2	(51)	0	(2)	2	(43)	0	(6)
Concrete Vault Containment	0	(31)	0	(2)	0	(23)	0	(6)
Topsoil shuffling	13	(25)	3	(4)	7	(15)	3	(6)
w/ on-site clean soils	3	(4)	0	(0)	2	(3)	1	(1)
w/ off-site clean soils	10	(21)	3	(4)	5	(12)	2	(5)
Soil Caps	10	(72)	0	(2)	10	(61)	0	(9)
Pavements	24	(167)	0	(8)	21	(129)	3	(30)
w/ concrete	12	(81)	0	(4)	11	(66)	1	(11)
w/ asphalt	12	(86)	0	(4)	10	(63)	2	(19)
Signs and fence	1	(58)	0	(11)	1	(37)	0	(10)
Others	6	(249)	1	(112)	4	(109)	1	(28)
total	362	(1,681)	66	(431)	232	(1,018)	64	(232)

Bioremediation has been used for VOCs (volatile organic carbons) contamination

Today's talk focuses on...

Bioremediation of VCOCs

(Volatile Chlorinated Organic Compounds)

1,1-dichloroethylene (1,1-DCE)
cis-1,2-dichloroethylene (c-DCE)
trichloroethylene (TCE)
tetra(per)chloroethylene (PCE)

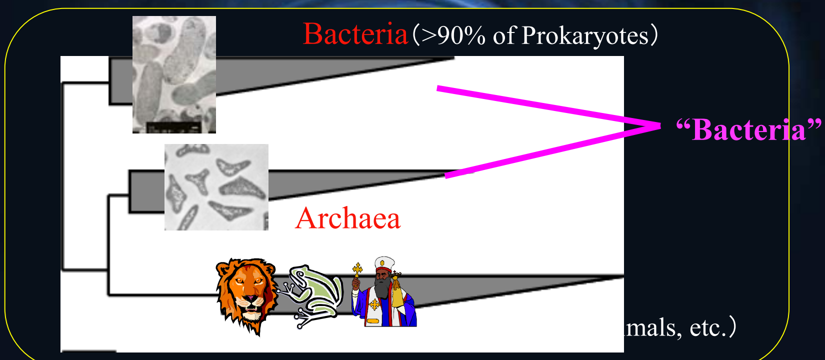
Bioremediation:

Remediation of contaminated environment utilizing organisms such as plants, fungi, and bacteria.

2. What is " bacteria"

Microorganisms： organisms
with small size（bacteria，fungi，algae）

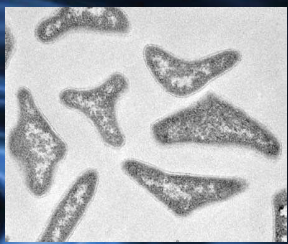
Phylogenetic tree of life

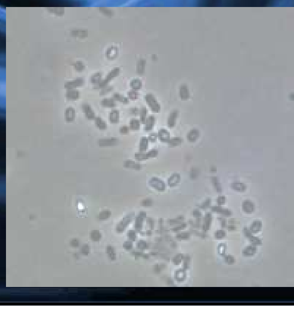


" Bacteria" : important microorganisms in subsurface environment

Size: around 1 μm

Shape: variable





What do they need?

(1) Carbon source

- CO_2 autotrophs
- organic chemicals heterotrophs

(2) Energy source

- light phototrophs
- chemical compounds chemotrophs

(3) Minerals

N, P, Mg, K, Ca, Fe…..

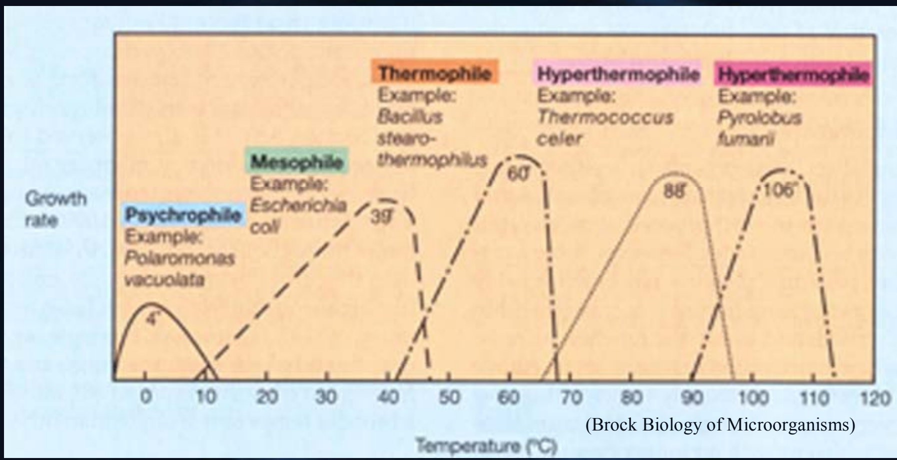
(4) Oxygen

aerobes use oxygen as terminal electron acceptor

anaerobes use chemical compounds as terminal electron acceptor

(5) water

(6) temperature



(Brock Biology of Microorganisms)

(7) pH

Acidophile, alcaliphile....

3. Various functions of bacteria

With organic contaminants: degradation

1. **Hydrocarbons** (aromatic, alkanes, alkenes..)

   effectively degraded under aerobic condition

   (*Pseudomonas*, *Acinetobacter etc.*)

2. **VCOCs (PCE, TCE, DCE…)**

   Low-Cl VCOCs are effectively degraded by aerobic bacteria

   High-Cl VCOCs are effectively degraded by anaerobic bacteria

With metals

1. **Leaching (direct or indirect)**

   leaching with *Thiobacillus* in the copper ore is famous

   $Cu_2S + O_2 \rightarrow CuS + Cu_2+ + H_2O$

2. **Absorption/accumulation**

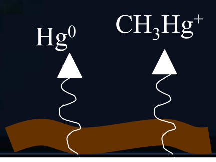
   utilization as a bioreactor (Cd, Cu, Ni, As, Pb, Fe, U..)

3. **Oxidation/Reduction**

   Cr(VI), U(VI) can be insoluble and less toxic when reduced to Cr(III), U(IV)

4. **Volatilization**

   Hg^{2+} is volatilized when reduced to Hg or methylated



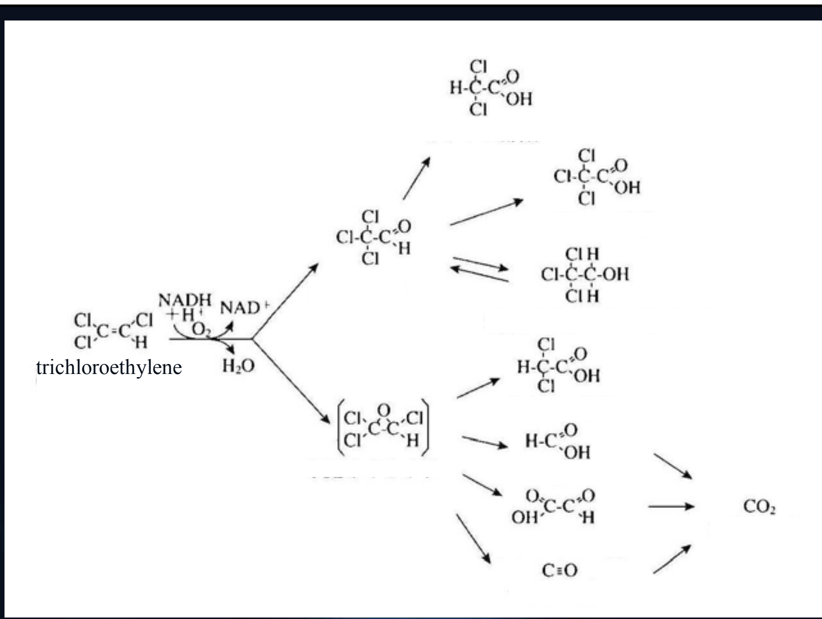
Degradation of VCOCs by aerobic bacteria

- **Toluen/phenol degrading bacteria**
  - *Pseudomonas cepacia* G4
  - *Psedomonas putida* F1
  - *Pseudomonas mendocina* KR1
  - *Pseudomonas stutzeri* OX1
- **methanotrophic bacteria**
  - *Methylosinus trichosporium* OB3b
  - *Methylocystis* sp. M
  - *Methylomonas methanica* 68-1
- **propane degrading bacteria**
  - *Mycobacterium vaccae* JOB5
- **ammonium oxidizing bacteria**
  - *Nitrosomonas europaea*

Degradation by co-metabolisms by oxygenase

↓

They can't use VCOCs as carbon source nor as energy source



Degradation pathway of trichloroethylene by aerobic bacteria

（「バイオレメディエーション実用化への手引き」）

Characteristics of aerobic degradation

- VCOCs are degraded to non-toxic CO_2 and Cl^-
- Degradation is caused by co-metabolism,
  they need other substance
- High-Cl compounds are less degradable

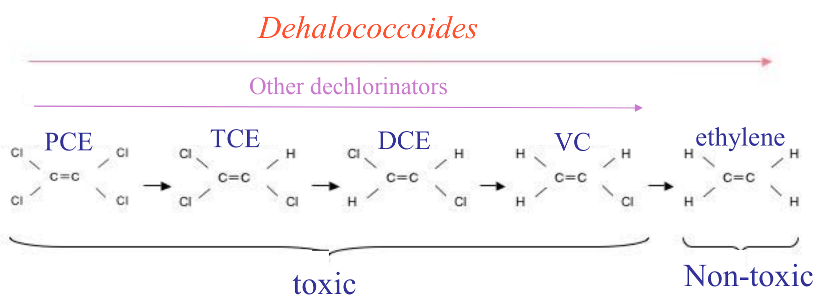
Degradation of VCOCs by anaerobic bacteria

Methanogens, sulfate reducing bacteria, *Dehalococcoides*

Enterobacter agglomerans biogroup 5
Methanosarcina sp. DCM
Sporomusa ovata
Dehalococcoides ethenogenes 195
Dehalobacter restrictus
Dehalospirillum multivorans
Desulfitobacterium sp. Viet1
Desulfuromonas michiganensis BB1

Halorespiration : obtain energy by VCOCs degradation

Utilize H_2, acetate as electron donor, utilize VCOCs as electron acceptor

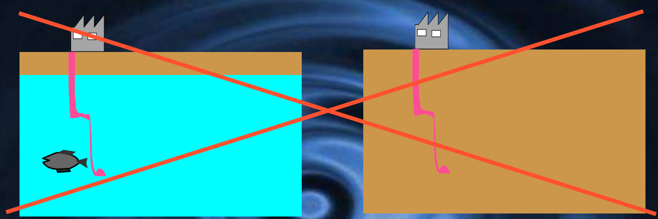


Degradation pathway of tetrachloroethylene by anaerobic bacteria

Characteristics of anaerobic degradation

- In many cases, degradation stops at vinyl-chloride (still toxic)
- Halorespiration, obtain energy through the degradation process
- High-Cl compounds are more degradable

4. How to investigate subsurface contamination

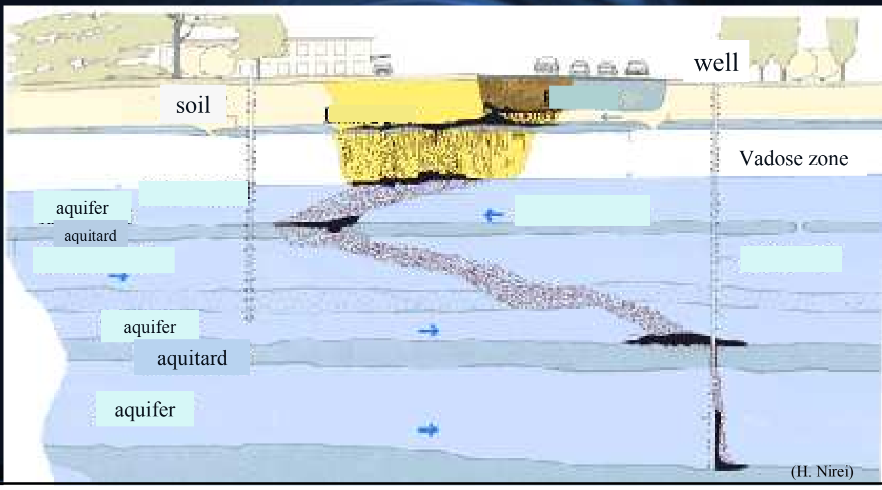


It' s not a simple world

It' s not a black box

Importance of Geology & Hydrology

It is regulated by geological structure, groundwater flow, and structure of monitoring well



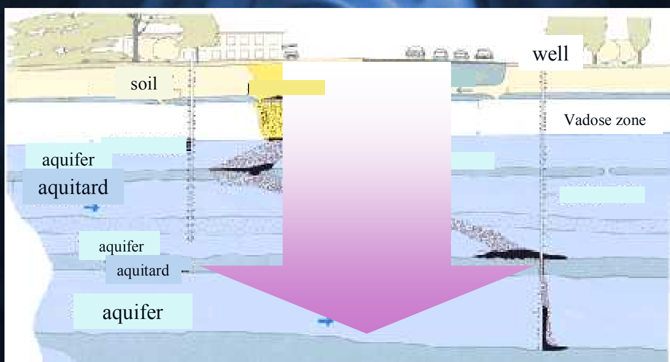
Example of VCOCs contamination

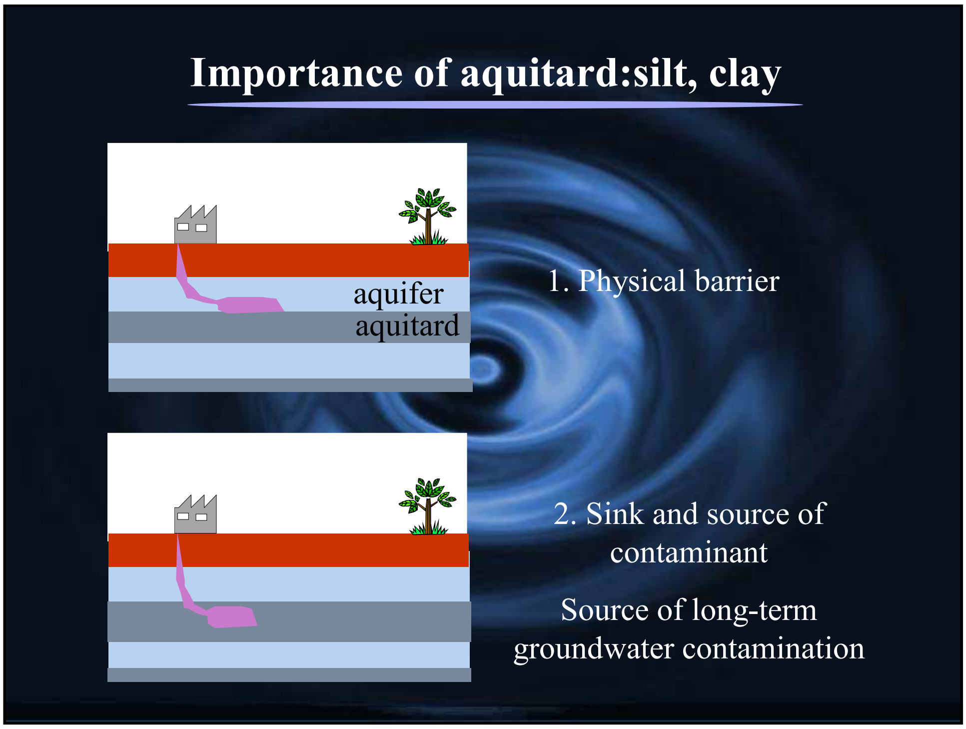
High specific gravity

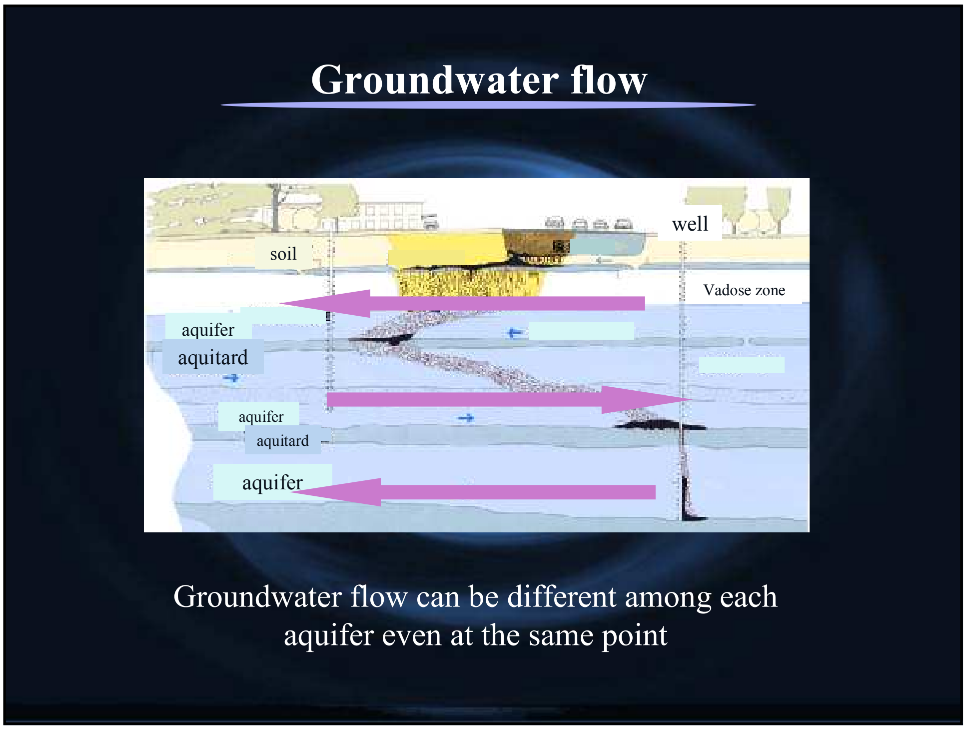
Generally migrate to downward

Hydrauric conductivity varies from

clay: 10^{-7}cm/sec to sand: 10^{-1} cm/sec



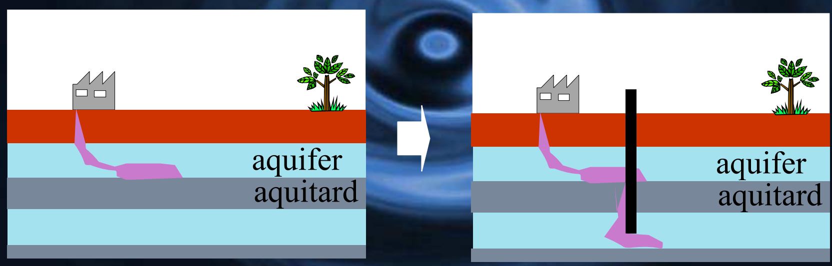

Importance of aquitard:silt, clay
aquifer
aquitard
1. Physical barrier
2. Sink and source of contaminant
Source of long-term groundwater contamination


Groundwater flow
well
soil
Vadose zone
aquifer
aquitard
aquifer
aquitard
aquifer
Groundwater flow can be different among each aquifer even at the same point

Important drilling strategy

1. Check sedimentary facies and contamination at each 1m to find aquitard

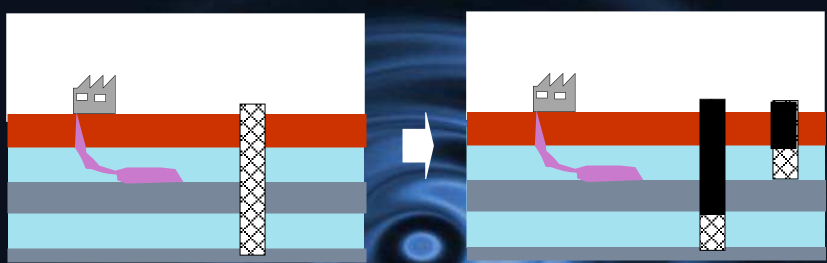
Penetration of aquitard without care can lead to unnecessary expansion of the contaminant



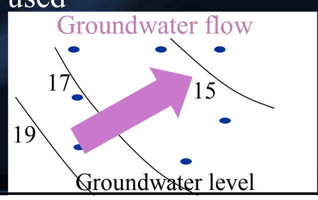
If VCOCs are detected in above the aquitard, casing is necessary

(NPO, The Geo-pollution control agency, Japan)

2. Install monitoring well which has a screen to each one aquifer



- Water from each aquifer, and even surface water can mix inside all-screened well
- In a proper well, groundwater level can be used to know the groundwater flow



(NPO, The Geo-pollution control agency, Japan)

5. Bioremediation utilizing bacteria

1. Biostimulation
   Inject nutrients to contaminated subsurface to stimulate the bacterial activity to degrade contaminants

2. Bioaugmentation
   Incubate bacteria with ability to degrade contaminants in the lab, and inject the cells into the contaminated subsurface

3. Monitored Natural Attenuation (MNA)
   When natural attenuation by intrinsic bacteria are occurring, the risk for the human health can be reduced by monitoring the contamination and controlling it not to further expand (US EPA,1999).

Bioremediation studies on VCOCs contaminated site utilizing methanotrophs

Site	Type of bioremediation	depths(m)	contaminants	Conc.(ppb)	Injected substrate	effect
Moffest[1]	stimulation	4.3-5.8	TCE*	100	CH_4, O_2	23%（2ｍ downgradient）
Kururi[2]	stimulation	8-17	TCE	200	CH_4, O_2, N, P	10%（ 0.75-1.5m downgradient）
Savanna River[3]	stimulation	27-45	TCE	<14,000	CH_4, O_2, N, P	<95%（monitoring wells）
Chikura[4]	stimulation	12-20	DCE*	35	CH_4 rich groundwater	50&（2ｍdowngradient）
Chico[5]	augmentation	26-28	TCE	425	*M. trichosporium*	<98%（injection well）
Abiko[6]	augmentation	3-5	TCE	128	CH_4 rich groundwater	>78%（2ｍdowngradient）

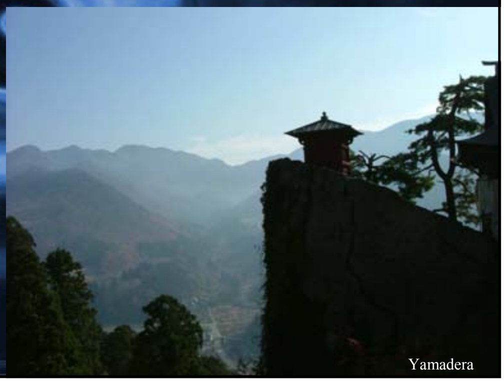
References

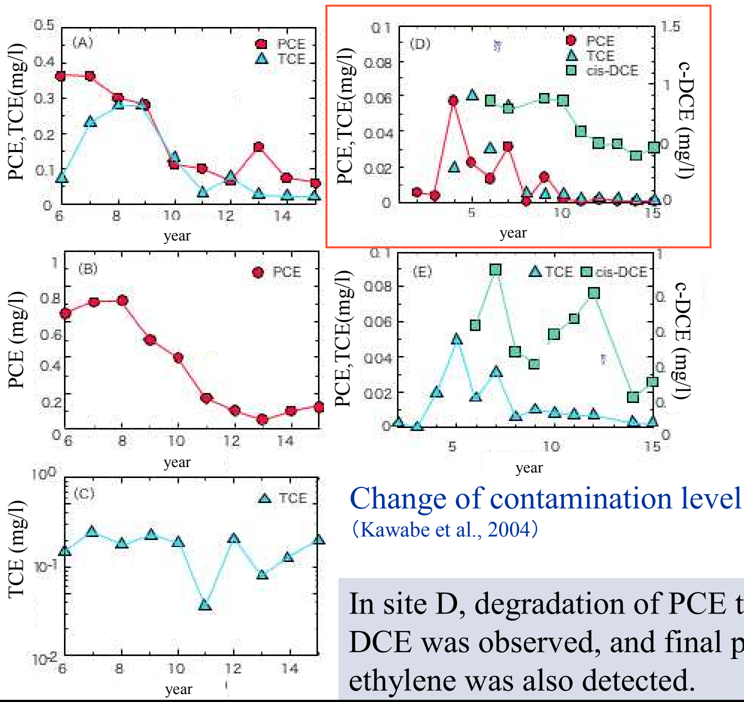
1: Roberts et al., 1990; Semprini et al., 1990; Semprini et al., 1991, 2: Eguchi et al., 2001, 3: Pfiffner et al., 1997, 4: Takeuchi et al., 2005, 5: Duba et al., 1996, 6: Takeuchi et al., 2004

Case study-MNA site in Japan

For MNA, precise evaluation of the contamination (source of the contaminant, movement of the contaminant based on geology and hydorology) and microbiology are important.

In Yamagata Prefecture, Japan, we have been monitoring groundwater contamination with VCOCs at 5 sites for almost 10 years.



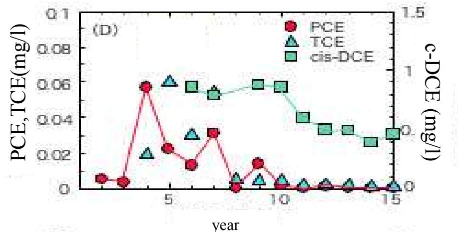


Change of contamination level
(Kawabe et al., 2004)

In site D, degradation of PCE to TCE and DCE was observed, and final product, ethylene was also detected.

PCE is degraded to c-DCE, and gradually decreasing

PCE→TCE→DCE→VC→ethylene (non-toxic)

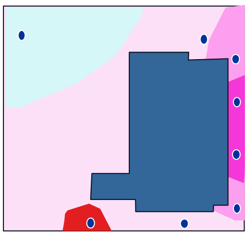


Highest concentration in one well in each year

Site D was designated as MNA candidate, and further study was conducted in this site.

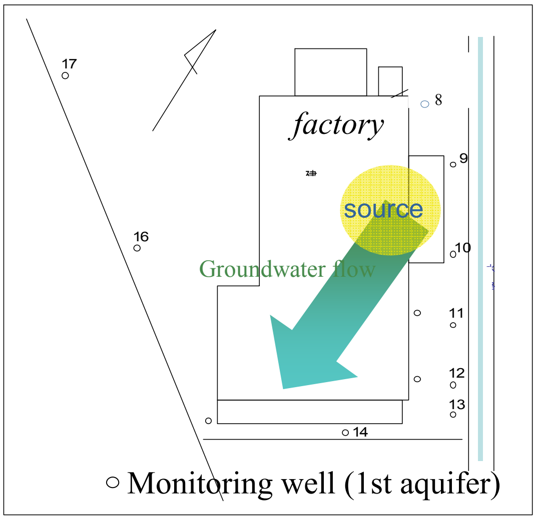
Are there bacteria with PCE degrading ability?

If so, why are they living there?



Contour of groundwater contamination

PCE was considered to be spilled at inside the building of laundry, and spread into the 1st aquifer.



Location of the monitoring wells

- Groundwater was collected from the monitoring wells and served for chemical and microbiological analyses
- Bacteria in the groundwater was collected by filtering 150 ml of groundwater.
- DNA was extracted from the cells, and the copy number of 16S rRNA gene of *Dehalococcoides* (biomass) was determined by Real-time PCR.

Chemical characteristics and the number of *Dehalococcoides* in GW

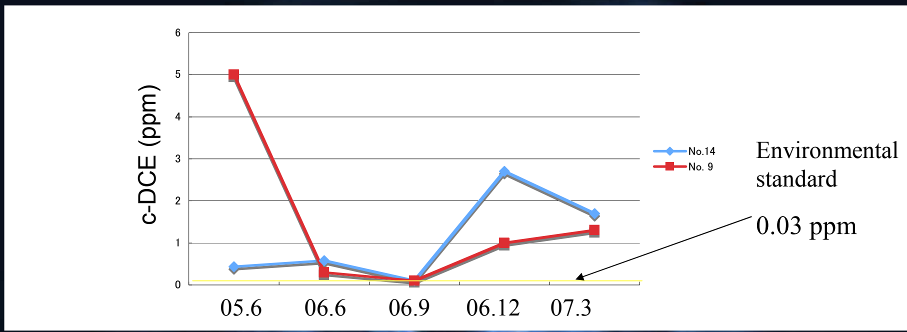
Deha/Eub (%)	TOC (mg/l)	CH4 (mg/l)	Fe (mg/l)	ORP (mV)	NH4 (mg/l)
0.01-3.7	1.4-16	0.2-4.6	21-181	-42 - +100	0.5-3.5

Deha/Eub: Percentage of *Dehalococcoides* to total bacteria

- *Dehalococcoides* existed in every well
- Percentage of *Dehalococcoides* was highest in the well with lowest ORP (highly reduced condition)
- Incubation of groundwater added with PCE exhibited degradation activity.

Natural attenuation must be occurring. Contamination was expected to disappear.

However....



High concentration of c-DCE were still detected in some wells.

Something is wrong.
There must be a source of contamination

When we joined the monitoring program, monitoring wells already existed there.

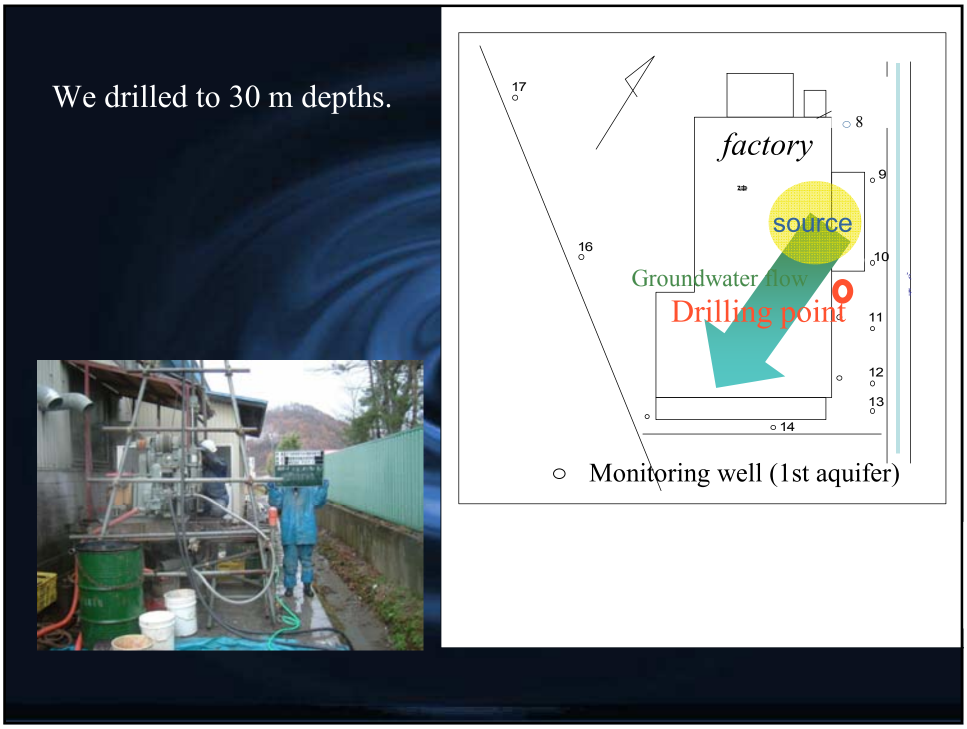
Monitoring wells were all-screened well installed by a private company.

Mechanism of contamination was not very clear.

Investigation should be done to get fundamental information on the contamination, geology and hydrology.

We decided to get a core sediment and install a new monitoring wells to reveal,

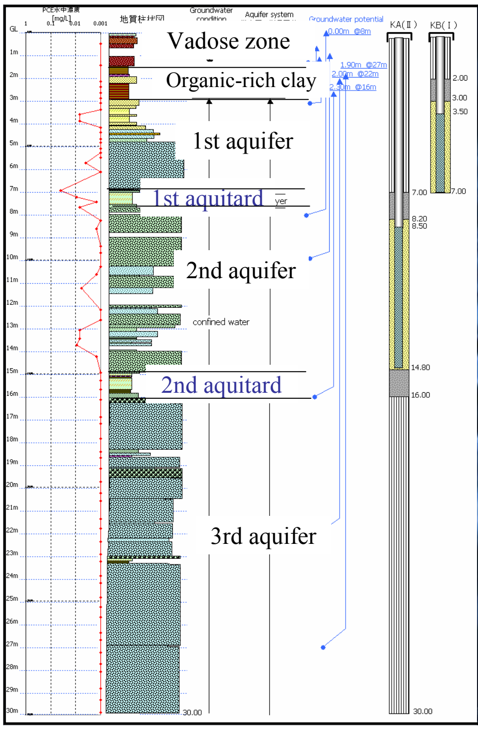
1. Mechanism of contamination, geology, and hydrology
2. Detailed ecology of bacteria which degrade VCOCs


We drilled to 30 m depths.
17
8
factory
9
source
16
10
Groundwater flow
Drilling point
11
12
13
14
Monitoring well (1st aquifer)


Observe sediment facies to reveal geological structure
Take samples from each layer.
Above and below the interface are important zones.

Analyse the VCOCs in the sediment by a detection tube on site.

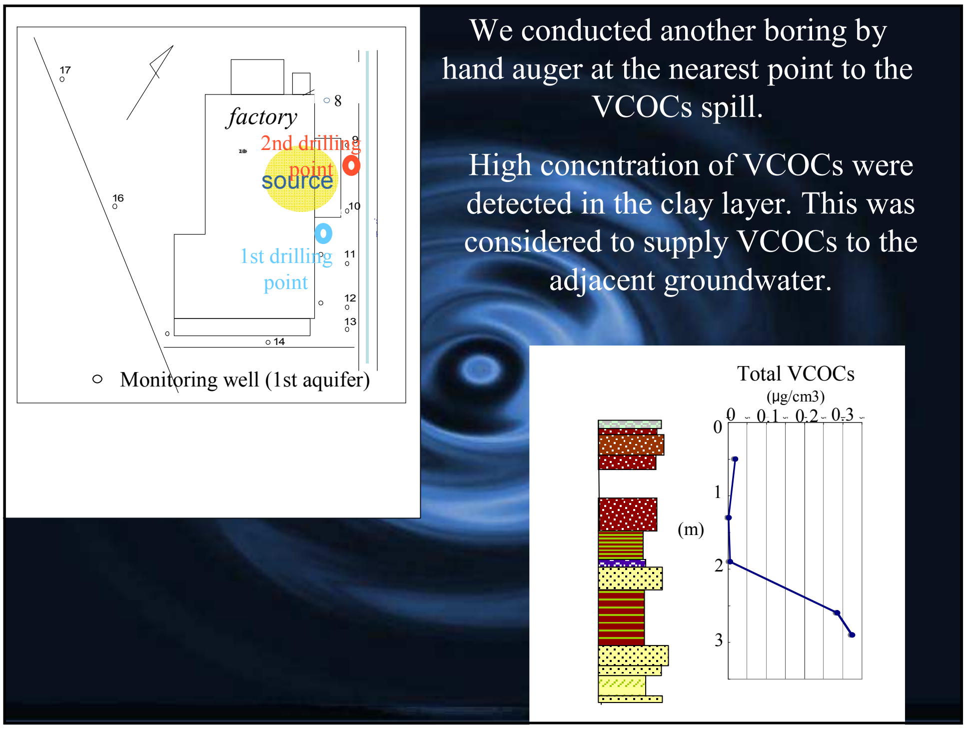
Subsamples for microbiological analyses were taken from each 1m of core sediment inside the portable clean bench brought to the field.



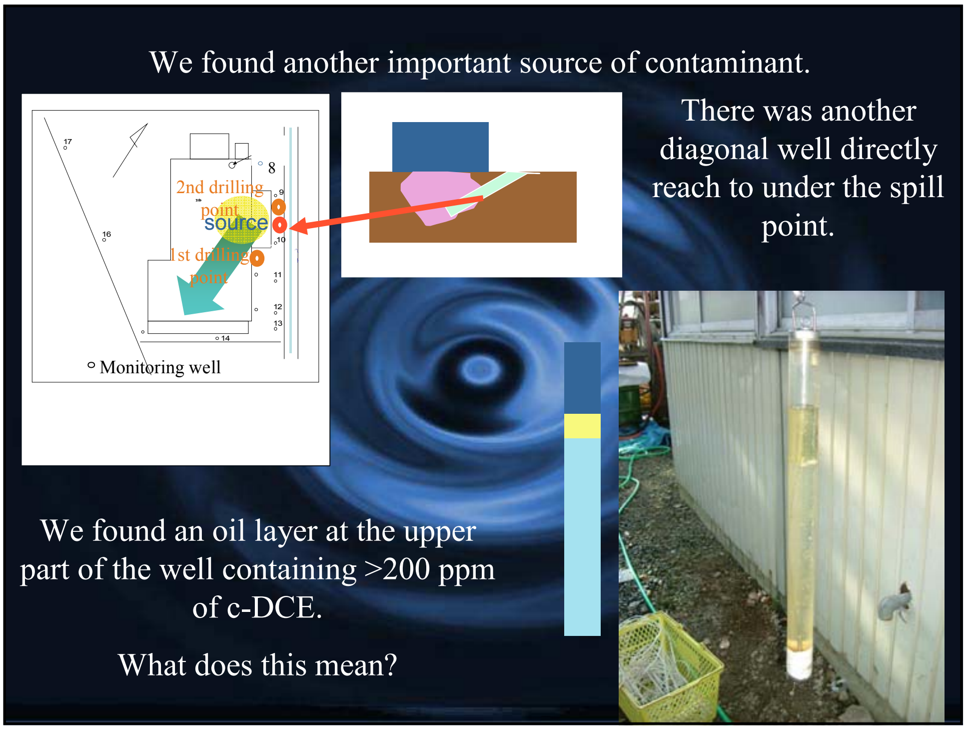
Now geological structure of the site is clear.

Higher concentrations were detected in silt layers and above the silt layer.

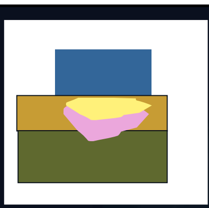
One of the source of the contamination was considered to be VCOCs trapped in the organic-rich clay layer above the 1st aquifer.



factory
2nd drilling point
source
1st drilling point
Monitoring well (1st aquifer)
We conducted another boring by hand auger at the nearest point to the VCOCs spill.
High concntration of VCOCs were detected in the clay layer. This was considered to supply VCOCs to the adjacent groundwater.
Total VCOCs
(μg/cm3)
0 0.1 0.2 0.3
(m)
0
1
2
3



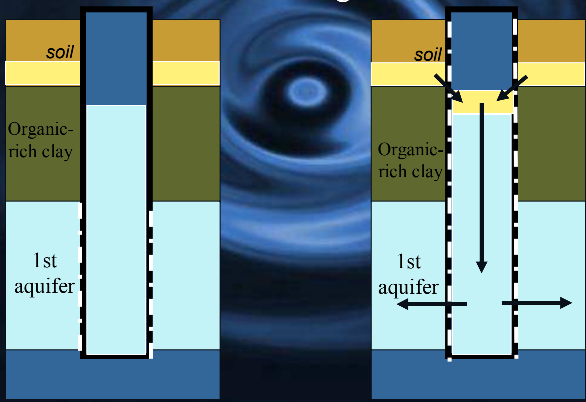
We found another important source of contaminant.
2nd drilling point
source
1st drilling point
Monitoring well
There was another diagonal well directly reach to under the spill point.
We found an oil layer at the upper part of the well containing >200 ppm of c-DCE.
What does this mean?



After they spilled VCOCs, they spilled oil.

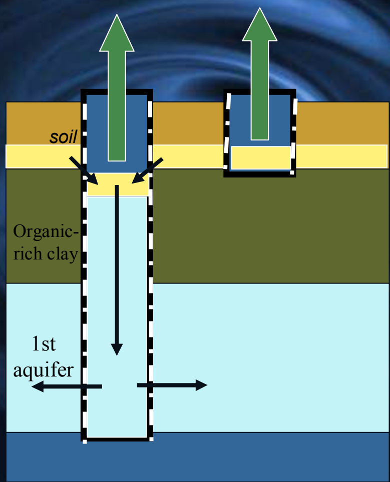
Oil dissolved VCOCs at high concentration and stayed above the clay layer.

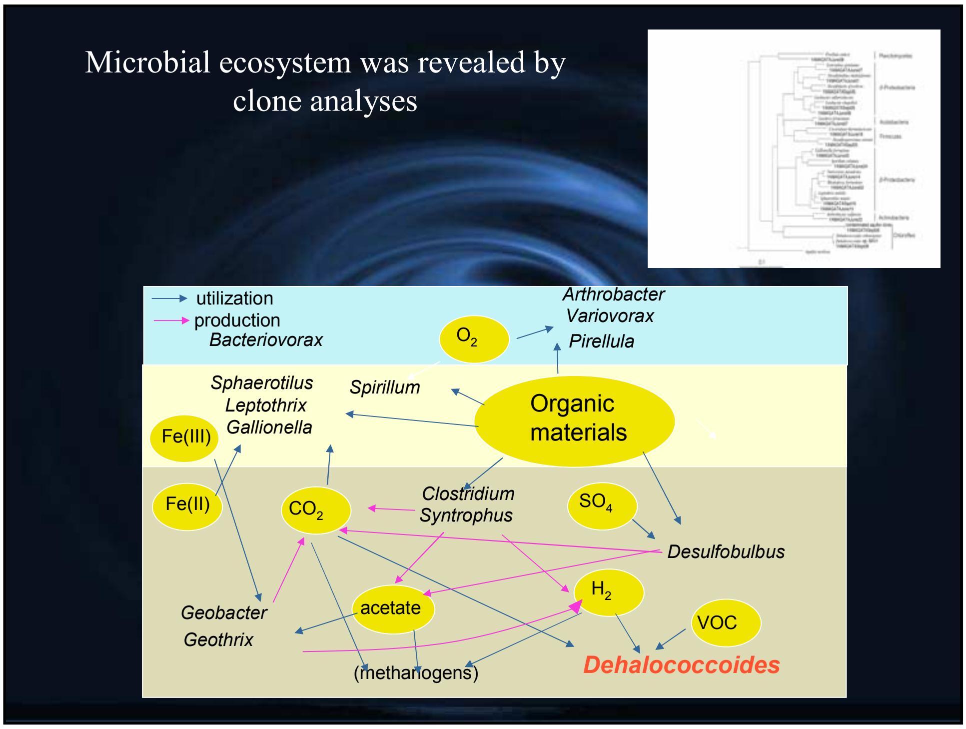
Because it was an all-screened well, the oil moved into the well, may have supplied VCOCs into the groundwater.

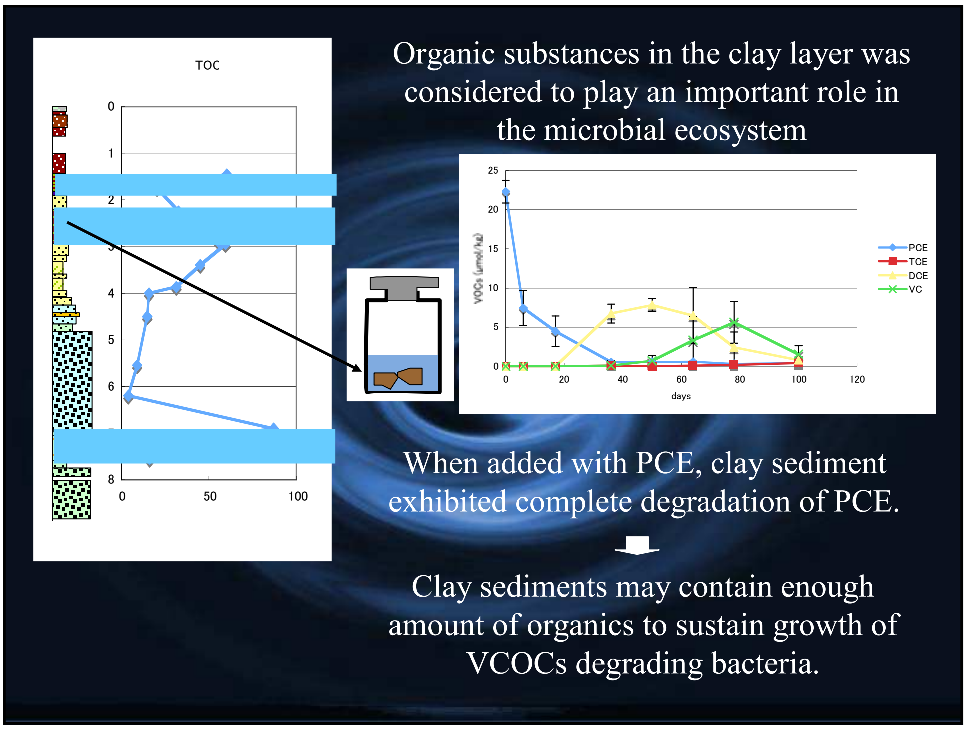


Well for the vadose zone was also installed.

Oil layer with contaminants was extracted.




Microbial ecosystem was revealed by clone analyses
utilization
production
Bacteriovorax
O2
Arthrobacter
Variovorax
Pirellula
Sphaerotilus
Leptothrix
Gallionella
Spirillum
Organic materials
Fe(III)
Fe(II)
CO2
Clostridium
Syntrophus
SO4
Desulfobulbus
H2
acetate
Geobacter
Geothrix
VOC
(methanogens)
Dehalococcoides


TOC
0
1
2
4
5
6
8
0
50
100
Organic substances in the clay layer was considered to play an important role in the microbial ecosystem
25
20
15
10
5
0
VOCs (μmol/kg)
0
20
40
60
80
100
120
days
PCE
TCE
DCE
VC
When added with PCE, clay sediment exhibited complete degradation of PCE.
Clay sediments may contain enough amount of organics to sustain growth of VCOCs degrading bacteria.

Natural attenuation is considered to be occurring in the clay layer and adjacent aquifer using H_2 produced by a degradation of organics in the clay layer.

Monitoring is still on-going after removing the oil layer with high concentration of c-DCE.

By using a proper methodology, we can know what's going on in subsurface.

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