

**Probiotics in the remediation of soil  
contaminated with 1,1,2-trichloroethene (TCE)  
Our experience at Loni Kalbhor, India**

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*Abstract*

1,1,2-trichloroethene (TCE) contaminated soil was treated *in-situ* with Probiotics to decontaminate the soil and the ground water for over 18 months; results of analysis of TCE in soil (gas analysis) and ground water after six months and twelve months of initiating the Probiotics treatment do indicate effective decontamination of the soil and the ground water by Probiotics. The methodology used is simple, needing no electricity or elaborate infrastructure or qualified manpower. The cost of decontamination of TCE using Probiotics is less than 5 % of the estimated cost of decontamination using conventional methods.

## INTRODUCTION

1,1,2-trichloroethene (tri, trichloroethylene, TCE) has been traditionally used as a solvent for cleaning punched/formed metal parts to remove lubricants (oil) from their surface before taking them up for processes like electroplating. Vapour degreasing equipments have been conventionally used for this process in the industry. Improper handling of TCE, TCE waste and exhausting TCE (being heavier than air), invariably lead to contamination of soil and ground water in and around the area where such activities are carried out.

Conventional methods of decontamination (remediation) of the soil and ground water contaminated by TCE include Pump and Treat (P & T), Permeable Reactive Barriers (PRB) and *in-situ* Chemical Oxidation (ISCO). These methods take many years (three to thirteen years and beyond depending on the extent of contamination) for decontamination of the soil and ground water and the results are difficult to predict<sup>(1)</sup>.

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In the recent past there has been an interest in using microbes to carry out the remediation of TCE (and other halogenated hydrocarbons) contaminated soil and ground water and the results have been encouraging both in terms of time frame for the treatment and the cost involved <sup>(2)</sup>.

In this paper we describe our experience in using the Probiotics<sup>(3)</sup> solution to effectively decontaminate soil and ground water contamination by TCE in one of our industrial sites at Loni Kalbhor, Near Pune, India.

## BACKGROUND INFORMATION

The Plastics and Metalware Factory (PMF) of Philips Electronics India, started in 1958 at Loni Kalbhor, near Pune, India, had been involved in the manufacture of metal and plastics parts for the electronic industry. Metal parts were punched and formed in the manufacturing process, using lubricants as process aids. Before the metal parts were taken up for further processing, such as galvanic plating, these parts were cleaned of oil/grease with TCE using a vapour degreasing equipment. In the vapour degreasing equipment liquid TCE was heated to give TCE vapour and the vapours were allowed to condense on the metal parts and clean them; the condensate went through many such vapourization and condensation cycles. Excess vapour in the system was regularly removed, to avoid excess pressure build up, by the exhaust system attached to the equipment. As TCE is heavier than air, most of the exhausted TCE settled down around the exhaust.

When Royal Philips Electronics decided to divest its interest in the Plastics and Metalware Factories, Loni Kalbhor, Near Pune, India, it had carried out the (mandatory within Philips) Stage I and Stage II site assessments at this site. One of the findings of the Stage II assessment was the presence of TCE in ground water and the soil at the site. The study concluded that about 1800 kg of TCE was present as Dense Non-aqueous Phase Liquid (DNAPL) on the bedrock base at about 15 m below the soil surface concentrated in an area of about 32 sq. m. where historically vapour degreasing of metal parts was carried out. (Fig 1)

The proposal of the consultants for the Stage III activities was to take up remediation based on “Pump and Treat” (P & T) method to be carried out for a period of 18 months to 24 months with a provision for extending the period of treatment to 60 months. Another option proposed by the consultants was to carry out *in-situ* Chemical oxidation (ISCO) by permanganate solution for a period of over 60 months. As the manufacturing unit was in operation (not a closed site) the P & T and the ISCO methods were not considered desirable (too many pumps, pipelines, pumping out of water from more than eight bore-

wells, treatment facilities, dedicated personnel for the operations etc., might distract the normal work of the factory) and we decided to carry out the remediation using microbes, specifically using the Probiotics.

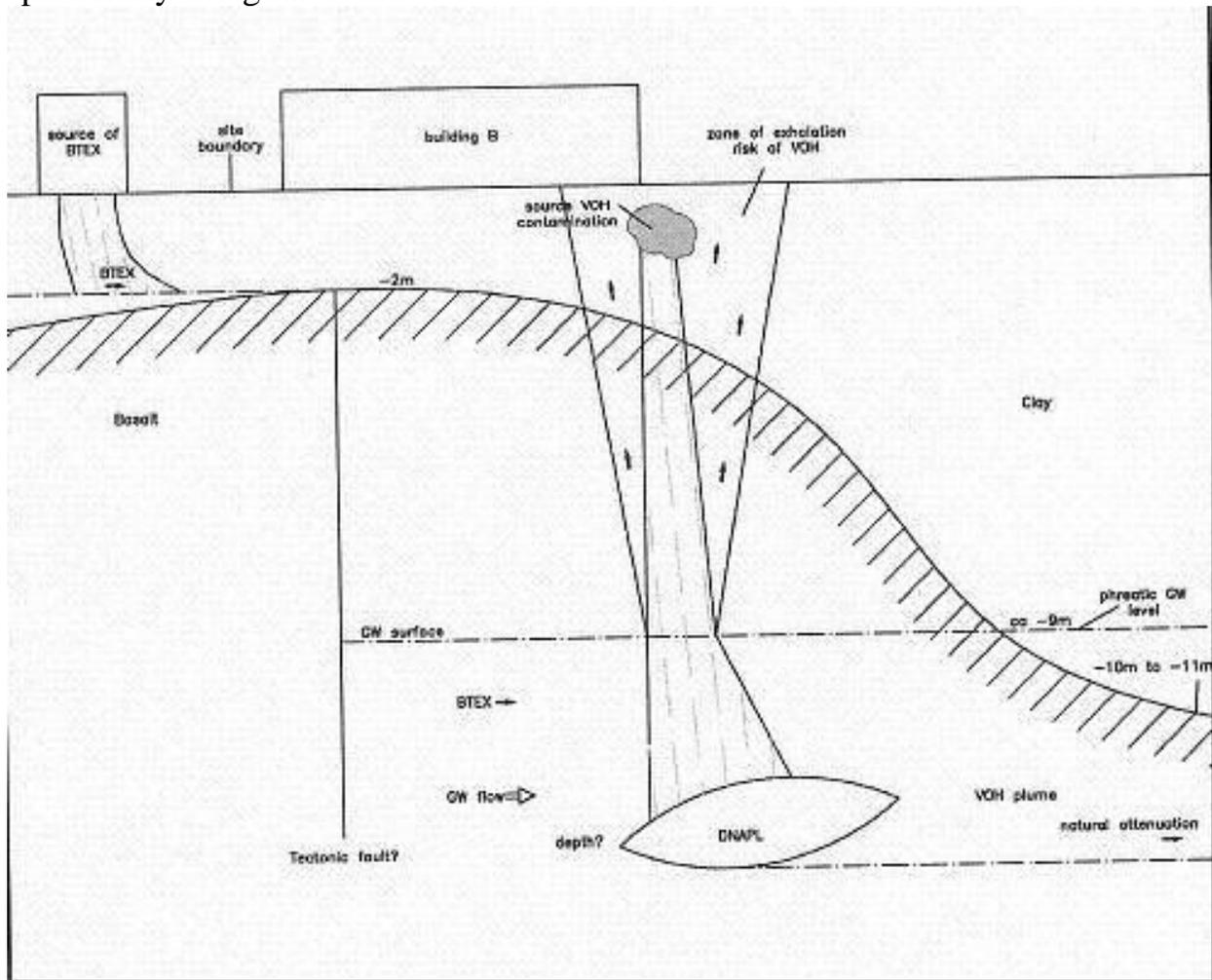


Fig 1: Conceptual picture of TCE contamination at the site (Courtesy: Philips Environmental Services)

## PROBIOTIC TECHNOLOGY

The program design primarily aims at using a sustainable and eco-friendly technology which is already in use in many parts of the world. This technology is SCD Probiotics developed by Sustainable Community Development, LLC; dba SCD Probiotics, U.S.A<sup>(3)</sup>.

Efficient Microorganism (EM) is a generic term used for innumerable combinations of over 80 strains of beneficial and efficient microorganisms that have been pioneered by Dr. Teuro Higa, University of Ryukyus, Japan, in the 1970s and 1980s<sup>(10)</sup>.

SCD Bio Klean is the product developed by SCD Probiotics, which essentially consists of four types of bacterial strains: (a) Phototrophic bacteria, (b) Lactic Acid bacteria, (c) Yeast and (d) Actinomycetes<sup>(11)</sup>. The fundamental principle of this technology was the introduction of a group of beneficial microorganisms to improve the soil conditions, suppress putrefying (disease inducing) microbes and improve the efficacy of organic matter utilization by crops<sup>(10)</sup>. SCD Probiotics is a mixed, probiotic culture of beneficial microorganisms which works to repopulate environments with good bacteria<sup>(11)</sup>. This is a natural process, harnessing powerful and beneficial microbes to achieve results. Even though originally developed for use in agriculture to replace fertilizers and chemicals, over the last twenty years many more applications of EM technology were explored successfully; these new applications include effective treatment of industrial hazardous wastes and industrial effluents. The SCD Probiotics uses microbes which are safe, natural, non-GMO and OMRI listed for use in organic production.

Dr. Margarita Correa, the Project Leader of the project, has been working on applications of Probiotics for over 17 years for various fields including agriculture, aquaculture, animal husbandry, environmental rehabilitation, waste management, composting and human health, in all over the world.

## EXPERIMENTS

### SCD Bio Klean

The decontamination (bio-remediation) of the soil and ground water from TCE was carried out using the SCD Bio Klean solution based on SCD Probiotics obtained from Sustainable Community Development, LLC, dba SCD Probiotics, Kansas City - USA ([www.SCDProbiotics.com](http://www.SCDProbiotics.com)).

### Equipments

1000 litre black HDPE container (Sintex) with cover (with a tap at the bottom)

Stirring Rod (s)

Weighing balance (s)

Digital pH meter

Beakers

Measuring Jars

Filter Cloth

Polythene buckets

## Treatment Scheme

The treatment scheme involved the dripping of the SCD Bio Klean solution into five holes (diameter 15 cm, with a rigid PVC pipe diameter 10 cm inserted in the hole) dug in the soil in the area where TCE contamination had taken place (see figure 2). Of the five holes, four holes were located at the four corners of a rectangular area of 2 metres length and 1.5 metres width and the fifth hole was located at the centre of the rectangle. The depth of the five holes was 2 metres, 4 metres, 6 metres, 8 metres and 10 metres. A sixth hole, up to a depth of 2 metres, was dug after six month's of experimentation and it was located outside the building in the open.

The structural framework constructed for the experiment is presented in Fig. 2. It consisted of three 1000 litre black HDPE containers, one for the SCD Bio Klean solution being dripped, the second for the fermented solution ready for use and the third for the solution under preparation. All the three containers were connected to a main distribution pipeline (made of stainless steel pipe of 3.8 cm diameter) through the tap (stainless steel ball cock valve) at the bottom of each of the containers.



Fig 2. Structural set-up for carrying out dripping of SCD Bio Klean into holes

Dripper lines, made of flexible plastics pipes (Plastro HDPE pipes) were connected to the main distribution line through stainless steel half cock valves; at the dripping end of the dripper line “on line” 4L and 2L drippers were fixed. The whole set-up had been placed on a steel framework capable of withstanding more than 5000 Kg load. The whole set up was kept in a dark area (room) devoid of direct Sun light.

The drip rate of the SCD Bio Klean in each hole was adjusted to about 200 litres/hole/week (i.e. about 18-20 millilitre/minute/hole); it was ensured that the dripping was continuous throughout the day and night and throughout the six months period on all days without any break. After the first six months period the drip rate was brought down to 100 litres/hole/week (i.e. about 9 -10 mililitre/minute/hole) and this rate was continued till the end of the treatment. The process was monitored for the rate of dripping (all the holes; thrice a day), pH (once a day) and leakages (regular inspection of the pipes and joints) / blockages (dripper, valves etc.) and the data were recorded.

## SAMPLING, ANALYSIS AND RESULTS

Sampling of soil samples and ground water samples were carried out by ERM (India) and the analysis of the samples were carried out by Analytico Milieu B.V., the Netherlands for ERM (India). Samples were collected by ERM personnel before the commencement of the SCD Bio Klean treatment, six months after the commencement of the SCD Bio Klean treatment and one year after the commencement of the SCD Bio Klean treatment. ERM organized the analysis of these samples at Analytico Mileu B.V. Soil Gas Survey using Gore Sorber (W.L. Gore & Associates, Inc., USA) modules was conducted by ERM before the commencement of the SCD Bio Klean treatment and one year after the commencement of the SCD Bio Klean treatment.

Table 1. *Analytical data – Ground Water*

1,1,2-trichloroethene (µg/litre)#

	GW1	GW2	GW3	GW4	GW5	GW6	GW7	GW8	GW9	TV	IV
2005 Baseline	77000	30	21000		720	3200	130	130	18000	24	500
2005 Dec	10		300								
2006 July	BDL	4.3	74	BDL	BDL	220	130	14	BDL	24	500

# the sample of July 2006 was also analysed for vinyl chloride and in a few cases the concentration of vinyl chloride was above the intervention value (100 – 9700 microgram per litre); but considering the initial concentration of TCE in ground water, we would

*have expected very high concentration of VC in the ground water if the decomposition of TCE was the sole reason for the presence of VC.*

In the reductive dechlorination process, naturally occurring bacteria gain energy for cell metabolism and growth by removing chlorine atoms from a pollutant and replacing them with hydrogen. Chlorinated solvents amenable to reductive dechlorination include tetrachloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (cis-DCE), vinyl chloride (VC), 1,1,1-trichloroethane (1,1,1-TCA), 1,1,2-trichloroethane (1,1,2-TCA), 1,2-dichloroethane (1,2-DCA), carbon tetrachloride (CT), and chloroform (CF). For example, PCE and TCE can be biodegraded to the non-toxic end product ethene by the following reaction:



Cis-1,2-dichloroethene (cis-DCE) and vinyl chloride (VC) are produced as intermediate compounds in this reaction. However, microorganisms can completely degrade cis-DCE and VC to the nontoxic end-product ethene when sufficient substrate is present. A wide variety of organic substrates can be used to provide hydrogen for reductive dechlorination including glucose, lactate, methanol and molasses. Vinyl chloride can be produced then fully transformed to ethene after approximately 90 days.

This aspect of the analytical result needs further study.

## PERIODIC EVALUATION PERFORMANCE

The first evaluation of the decontamination of the soil and ground water by SCD Probiotics was carried out after six months of initiating the process. Since this testing was meant for checking the effectiveness of the process, the evaluation was done on a sampling basis (cost of testing !!!!) for concentration of TCE in water and soil (see Table 1 for results). More comprehensive testing of water, soil and soil-gas was carried out one year after initiating the project

*Table 2: Analytical Results – Passive Gas Survey (comparison of data before and after one year of SCD Bio Klean treatment, from locations nearer to each other) 1,1,2-trichloroethylene ( $\mu\text{g}$  per Gas Absorber module – 14 days of passive absorption)*

<b>Gore-Sorber Analysis</b>		
Sample No. (*)	TCE Concentration (micrograms)	
	<b>Feb-05</b>	<b>Aug-06</b>
1	205.21	5.82
2	219.95	24.23
3	0.1	2.00
4	11.44	15.08
5	6.04	135.02
6	1.9	1.60
7	0	40.10
8	0.57	2.79
9	0.09	14.80
10	139.06	47.64
11	1	0.18
12	1.41	6.82
13	0.02	26.97
14	0.58	1.69
15	0.13	5.05
16	2.39	0.67
17	1.37	0.90
18	3.05	0.23
19	11.37	0.66
20	4.01	1.23
21	4.34	3.14
22	26.81	2.52
23	35.43	2.39
24	3.29	4.11
25	23.4	0.47
26	98.38	2.53
27	214.38	0.13
28	94.57	0.99
29	108.71	3.04
30	2.75	1.18
31	4.44	0.35
32	62.4	0.88
33	30	1.13
34	32.11	0.24
35	59.15	3.82
36	0	0.09
Maximum Value	219.95	135.02
Mean value	40.28	

(\*)Sample Nos in two analysis are not exactly identical

## DISCUSSION

Decontamination of soil and water contaminated with halogenated hydrocarbons always posed problems. Studies carried out in the USA and Japan have indicated that microbes can help in decomposing halogenated hydrocarbons<sup>(12)</sup>. Biodegradation of trichloroethylene (TCE) and tetrachloroethylene (PCE) has been demonstrated in pure cultures, mixed cultures, microcosms. Field demonstrations of in situ bioremediation of chlorinated solvents have included biostimulation of indigenous methane-oxidizing bacteria (methanotrophs) and bioaugmentation with a metabolic, nutrient inducer. Both demonstrations were aerobic systems and focused on biodegradation of vinyl chloride (VC), dichloroethylene (DCE), and TCE<sup>(2)</sup>.

There has, however, been no report of actual decontamination on contaminated site; this study appears to be one of those first few studies of large scale in-situ decontamination of TCE in soil and ground-water.

The results of soil and ground water analysis carried out prior to the “decontamination” did indicate that the concentration of TCE in ground water as well as in soil were above the Dutch intervention values.

Since the source of the TCE contamination had been identified by the earlier studies by Royal Haskoning and ERM, it was decided to carry out the decontamination experiments with SCD Probiotics in a small area above the suspected DNAPL. The drip rate used in the first six months was higher than the normally required rate as the chlorine compounds can adversely affect the bacterial life and allowance was made for this adverse effect.

Tests were carried out on samples at the end of six months to check if the trend in decontamination was in the right direction at all. Results did show that the TCE levels in ground water had come down below the Dutch intervention levels. One soil sample, however, had shown a TCE concentration above the intervention value; a new hole for dripping the SCD Bio Klean had been made near the sampling area.

From the sixth month the dripping rate was reduced to the normal rate (i.e, 8 -10 ml/hour/hole) and at the end of one year, analytical data were obtained for both soil and ground water contamination by TCE. The results (*Table 1*) indicate that ALL the ground water samples had TCE in quantities less than the intervention value; similarly all the soil samples shown concentrations below the Dutch intervention value. These data indicate an effective decontamination of TCE from the soil and the groundwater.

Soil gas analysis (*Table 2*) before the beginning of the decontamination project and after carrying out decontamination clearly point out the efficient/effective way the SCD Probiotics had acted on the TCE.

While the whole exercise was carried out to address TCE contamination of soil we also found other beneficial effects of SCD Probiotics on soil and ground water. The heavy metal concentrations in soil and ground water had come down during the period of the SCD Probiotics treatment. During the same period the total hydrocarbon contamination of the ground water had been drastically reduced. These positive effects add on to the main achievement of the SCD Probiotics in reducing TCE concentrations in soil and ground water.

We found many advantages of the Probiotics Technology for decontamination

1. Probiotics is easily available, simple to use and maintain
2. No electricity is required (this is especially important in Maharashtra with many hours of power cut)
3. Easy to monitor (pH)
4. Passive and non-intrusive technique – extremely useful in a factory working for 24 hours
5. No harmful by-product (e.g. hazardous waste)
6. Additional benefits (reduction of heavy metal conc. + total hydrocarbon conc.)

In addition Probiotics offers a cost effective method for decontamination of ground water and soil. The estimated cost of removing TCE by the P & T method at this site was between Rs.32,000,000 to Rs.50,000,000; this excludes the cost of testing of soil and water samples, maintenance and electricity charges. Using the Probiotics the actual cost of TCE removal has been realized at Rs.1,500,000 (excluding testing costs). That is, the decontamination soil and ground water was carried out at less than 5 % of the cost of decontamination by conventional methods.

Another advantage we realized was the time taken for decontamination, while SCD Probiotics gave excellent results by the end of the first year (we continued till 18 months as per our original plan), the estimated time for decontamination of the site by the conventional method was about 24 months for decontaminating the soil and up to 60 months for decontaminating ground water <sup>(6) (7) (8)</sup>.

## CONCLUSION

Our in-situ experiments at the TCE contaminated site at Loni Kalbhor prove that SCD Probiotics can offer an efficient technology for decontaminating the site (both soil and ground water) in a cost effective way.

The results also indicate that the method may be tried for soil and water contaminated with chlorinated pesticides, heavy metals and hydrocarbons.

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