EX-SITU BIOREMEDIATION OF TRICHLOROETHYLENE (TCE) CONTAMINATED SOIL UNDER ANAEROBIC CONDITIONS

ADAM WORSZTYNOWICZ, DOROTA RZYCHOŃ, TOMASZ SIOBOWICZ, SEBASTIAN IWASZENKO, GRAŻYNA PŁAZA, KRZYSZTOF ULFIG

Institute for Ecology of Industrial Areas, ul. Kossutha 6, 40-844 Katowice, Poland

BIOREMEDIACJA *EX-SITU* GRUNTU SKAŻONEGO TRICHLOROETYLENEM W WARUNKACH ANAEROBOWYCH

Grunt, sztucznie skażony trichloroetylenem (TCE), został oczyszczony za pomocą bioremediacji przebiegającej w środowisku redukcyjnym, w warunkach beztlenowych. W celu określenia optymalnych parametrów procesu bioremediacji przeprowadzono badania laboratoryjne. Na ich podstawie wybrano mieszaninę osadów pochodzących ze ścieków komunalnych jako inoculum. Zaprojektowano i zbudowano bioreaktor przeznaczony do oczyszczania gruntu z rozpuszczalników chlorowanych. Jego komora o objętości 6 m³, została wyposażona w układy recyrkulacji gazu i odcieku oraz system akwizycji danych pomiarowych. Bioreaktor pracował jako reaktor ze złożem stałym o ciągłym przepływie gazu. Podczas 28 tygodni 4 Mg gruntu skażonego trichloroetylenem o stężeniu 350 mg/kg gruntu zostały całkowicie oczyszczone w warunkach beztlenowych. Oznaczone w próbkach gazowych i ciekłych stężenia TCE, dichloroetylenu (DCE), chlorku winylu (VC) i etylenu (ETH) wskazują, że w bioreaktorze nastąpiła stopniowa dehalogenacja trichloroetylenu do etylenu. Potwierdza to również wzrastające stężenie jonu chlorkowego w odcieku.

Summary

TCE artificially contaminated soil was cleaned under anaerobic, reductive conditions. A laboratory scale treatability studies were carried out to determine optimal physico-chemical and microbiological parameters for bioremediation process. Upon treatability studies results a sewage sludge mixture was chosen as a microorganism's source. The chlorinated solvents contaminated soil bioreactor (CSCS bioreactor) was designed and built. It consists of a 6 m³ reactor vessel, a gas recirculation system, a leachate recirculation system and a data acquisition system. The bioreactor vessel was designed as a continuous gas flow packed bed reactor. During 210 days 4 Mg of soil containing approximately 350 mg TCE/kg of soil has been completely remediated under anaerobic conditions. The obtained results indicate that the stepwise dechlorination of TCE to ETH occurs in the bioreactor. Increasing amounts of chloride in the leachate were correlated with dechlorination.

INTRODUCTION

Chlorinated solvents such as perchloroethene (PCE), trichloroethene (TCE), trichloroethane (TCA) and carbon tetrachloride (CT) are commonly used as degreasing agents in manufacturing, maintenance and service facilities worldwide. Past disposal and usage practices of these VOCs often resulted in environmental contamination, particularly into soils and groundwater [1].

The goal of this study was to design and construct a portable, efficient and readily deployable system for treating small quantities of solid waste contaminated with chlorinated solvents. Bioreactor systems occurred to be efficient, cost-effective, and safe to handle technology dedicated for *ex situ*, on-site batch remediation, suitable in the case of relatively small contaminated sites.

Though the system was designed to treat TCE contaminated soil, it could also be used to process other contaminated solid wastes and organic contaminated soils.

There are many reports in the literature on reductive dechlorination of perchloroethene (PCE) and TCE to ethane or ethene by anaerobic mixed cultures of bacteria both in the laboratory and in the field conditions. Many studies have also stated that selection of an appropriate electron donor may play crucial role in developing a healthy population of microorganisms capable of dechlorinating PCE and TCE [2, 5, 7]. A typical TCE reductive dechlorination process is presented in Fig. 1.
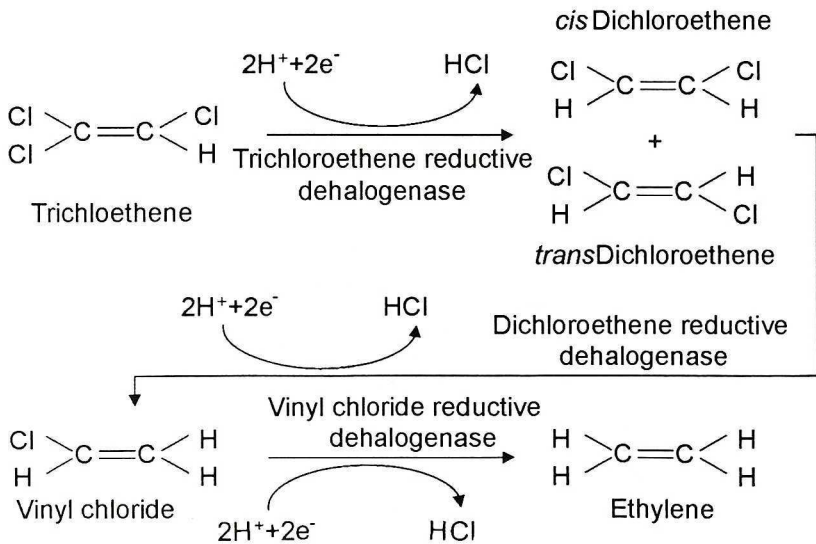

Fig. 1. Anaerobic trichloroethene dehalogenation pathway map

Treatability study to determine optimal conditions for TCE biodegradation has been carried out. Data from the treatability study [4] provided information which served as a basis for determining the physico-chemical and microbiological parameters for the remediation of TCE-contaminated soil in a bioreactor. It was found that bacteria present in the mixture of

selected sewage sludges from two municipal wastewater treatment plants are capable of complete dechlorination of TCE to ethylene under anaerobic conditions and no accumulation of vinyl chloride in the final product mixture was observed. In relation to these findings it was decided that:

- the bioreactor should be adapted to soil cleanup under anaerobic conditions,
- methanol as an electron donor for TCE dechlorination was selected for the remediation test in the bioreactor,
- substrate and products in the gaseous phase can be monitored to determine remediation progress to minimize handling.

BIOREACTOR SYSTEM DESIGN AND CONSTRUCTION

Upon results gained during treatability study bioreactor system has been designed and built and then bioremediation test on TCE contaminated soil has been conducted. The bioreactor consists of a reactor vessel, a gas recirculation system, a leachate recirculation system and a monitoring system. The most important features of the CSCS (Chlorinated Solvent Contaminated Soil) bioreactor system are its air-tightness (ambient air is not allowed to get inside the bioreactor vessel during process), its capability of either aerobic or anaerobic operation, and its ability to completely flood the soil, if required to create anaerobic conditions in contaminated soil.

The bioreactor vessel was designed as a continuous gas flow packed bed reactor. It has a shape of a container of 6 m³ volume (3.30 x 1.65 x 1.00 m outer dimensions) and was made of carbonated steel. A grate was mounted inside the vessel to support the soil bed. Connections were made for gas recirculation, leachate recirculation and probes for data monitoring. In order to prevent the bioreactor soil bed from freezing during winter, a light and easy to remove enclosure made of wood and 15 cm thick pressed polystyrene foam sheets was built around the bioreactor. Inside the enclosure two heaters, 2 kW each, were installed for heating the reactor when required.

The leachate recirculation system (Fig. 2) consists of a tank, a pump, valves, perforated hoses and process piping. This system has one influent and one effluent connection to the reactor vessel. The effluent process piping, equipped with a shutoff valve, is connected to a tank vessel located beneath the bottom of the bioreactor vessel. Leachate from the tank is pumped through a system of parallel, perforated hoses located above the soil bed, allowing the leachate to be spread uniformly on the upper surface of the soil surface. The leachate recirculation system can be used for addition of nutrients as well as controlling soil moisture.

The gas recirculation system consists of a blower equipped with an inlet air filter, a bypass pipe with a control valve and an inlet air nozzle. The bypass pipe allows for the introduction of inert gas (e.g., nitrogen) or substrate gas (e.g., methane) into the bioreactor. Nitrogen from a gas cylinder can be blown through the tank vessel to prevent ambient airflow into the system.

The monitoring system is designed to collect data, support the control of the bioreactor working parameters and evaluate the bioremediation rate. As reductive conditions have to be maintained in the reactor, there is no opportunity for taking soil samples from the reactor when it is operating. Information on the biodegradation rate has to be obtained from changes in the composition of leachate and soil gases.
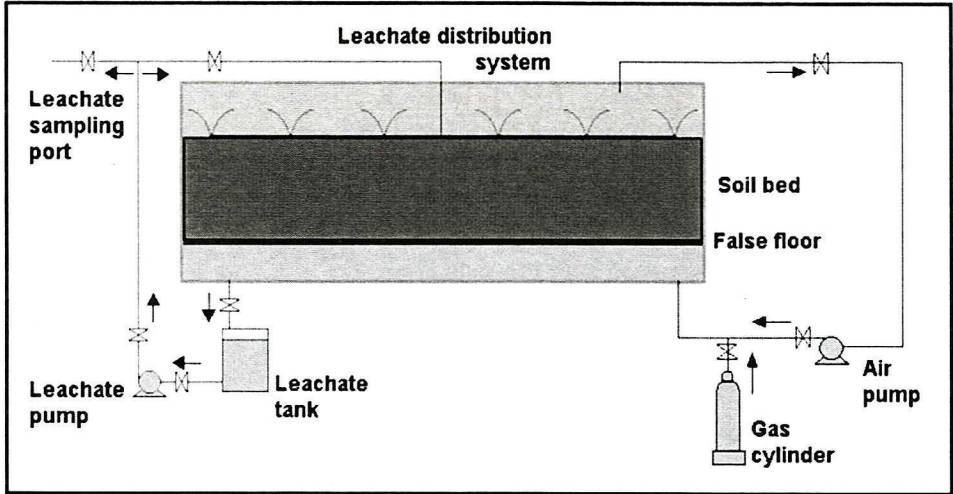
Fig. 2. Leachate and gas circulation systems

The gas sampling system consists of 5 probes distributed evenly along the main axis of the reactor vessel, about 30 cm below the surface of the soil bed. Each probe has a separate connection to the point where soil gas samples are taken.

Bioreactor is fitted with instruments to measure temperature and redox potential of soil, temperature, humidity, oxygen content and pressure of gas in the bioreactor vessel (Fig. 3). Soil redox potential is measured using five platinum electrodes, each one coupled with one of two silver-silver chloride reference electrodes. Each redox electrode is located near one of the soil gas probes. Before installation, the electrodes were calibrated using standard
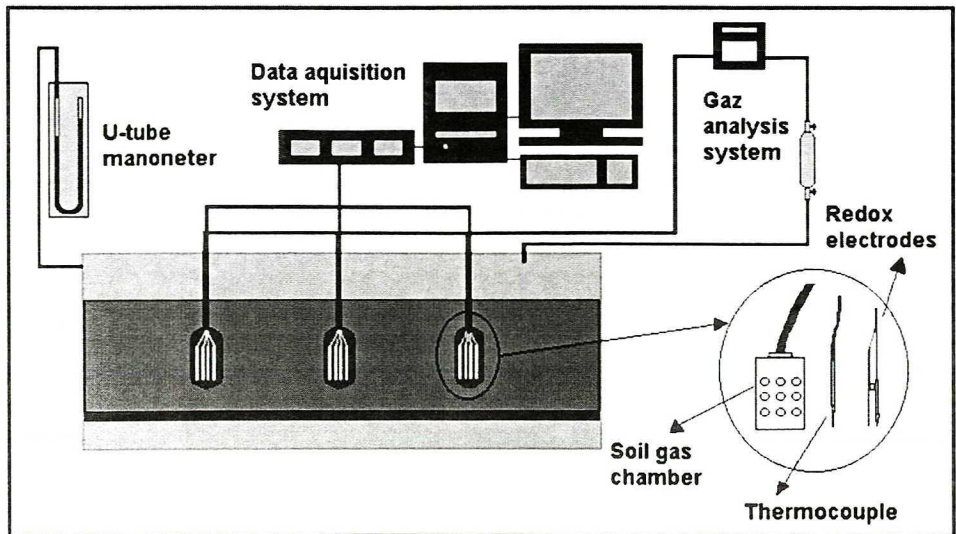

Fig. 3. Monitoring and data acquisition systems

solutions. A set of thermocouples of type K was used for soil temperature measurement. Oxygen content in gases inside the vessel was measured by DRC XT253 Microbac sensor. Bioreactor pressure was controlled through a U-tube manometer fixed to one of the gas outlets.

All electronic sensors and measurement equipment were connected to PC computer through a set of Advantech ADAM 4000 modules. ADAM-4017 and ADAM-4018 modules were used for temperature measurements and analog – digital conversion of voltage signals (redox electrodes and oxygen sensor output). Modules communicate one another via RS-485 interface. An ADAM-4522 module was used as a gate between RS-485 and RS-232 protocols to send gained data to PC computer. Data acquisition process is controlled by software created under Advantech VisiDaq environment. Measurements results are written in text log files and then converted into MS Excel and MS Access data formats for further analysis.

BIOREMEDIATION TEST

About 4 Mg of soil taken from the Institute for Ecology of Industrial Areas (IETU) yard was sieved and mixed with 200 dm³ of fresh hard-wood chips. Basing on the data from treatability study, selected sewage sludges were used as a source of innocula. The soil was mixed with approximately 200 kg of each of dewatered sewage. Then the mixture was loaded to the bioreactor. After loading measurement equipment had been installed and the vessel was tightly closed. The inside air was flushed out with nitrogen to obtain a reductive atmosphere. Then 1 dm³ of TCE (about 350 mg/kg) and 3 dm³ of methanol were mixed with 20 dm³ of water and applied to the bioreactor using the leachate circulation system. Gas recirculation and the registration of measurement parameters were initiated after a 3-day stabilization period. The soil gas was circulated through the bioreactor for 8 hours a day with a flow rate of about 1 m³/hr. Leachate was circulated daily for about 8 hours with a flow rate of about 10 dm³/hr. Gas cylinder nitrogen was attached to the gas circulation system so that bioreactor can be flushed, if necessary (oxygen concentration was monitored continuously).

Gas samples for TCE, DCE, VC, ethylene and ethane analyses were taken by gas pipettes from the gas space above the contaminated soil and at the soil sensor locations. Gas was pumped up out of the bioreactor's bed. After transition through the connecting pipes, the gas samples were washing gas pipette. The pipettes were washed several times and then the gas sample was delivered to the IETU's laboratory. The samples were analyzed for TCE, DCE, VC and ETH by direct sample injection into HP 6890 Gas Chromatograph (with Rtx-Volatiles capillary column and a GS-Gastro column) with FID detector. The gas that had washed the pipette was circulated back into the bioreactor.

The sampling cycle was adjusted to reflect intensity in changes in sample composition. During the first week of the test, gas samples were collected daily but, because their composition did not differ considerably, a biweekly, and, in the final process stage, a weekly air sampling cycle was found adequate to monitor biodegradation rates.

A sampling port was used to collect leachate samples from the leachate recirculation system. Both the soil and the sludge were analyzed before and after finishing the soil clean-up process. The leachate pH, TCE, DCE, VC, TPH, COD, nutrients, and chloride were monitored on a weekly basis. TCE, DCE, VC in the headspace samples of the leachate were

also analyzed by HP 6890 Gas Chromatograph with FID detector. Nutrients (N, P) were analyzed accordingly to Polish standards using CARY 1 VARIAN UV-Vis. Chloride ion concentrations were determined by silver nitrate titration method PN-75/C-04617.02.

Redox potential and temperature in the soil bed as well as humidity, temperature and pressure in the gas above the soil bed were measured continuously.

RESULTS AND DATA ANALYSIS

Measurement results of the physico-chemical parameters and the data on the changes of gas composition in the bioreactor are presented in Figs 4–6.
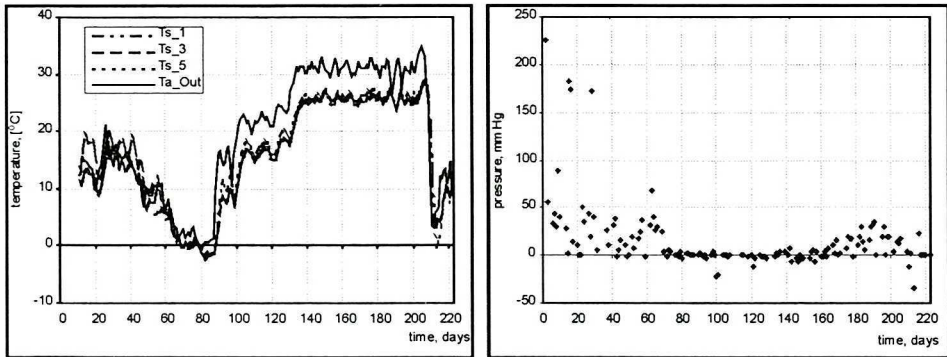

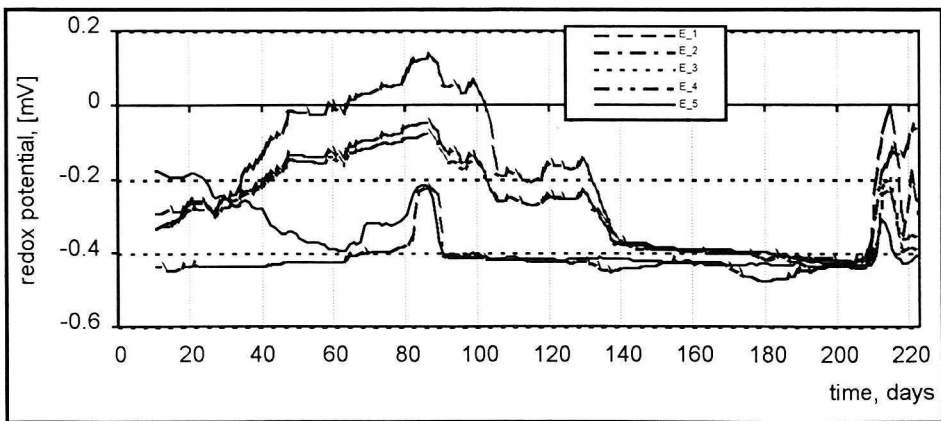Fig. 4. Temperatures and pressure in bioreactor

Fig. 5. Redox potentials recorded by 5 electrodes placed in bioreactor soil

The 220 day bioreactor operation can be divided into three different periods:
1)   days 0 – 80, characterized by:
       – increase of the soil redox potential,
       – decrease of soil and gas phase temperatures, caused by decrease of ambient temperature, and
       – unstable pressure in the bioreactor, which resulted from daily changes in ambient

temperature and/or fermentation processes taking place in the organic matter of the sludge added to the reactor soil.
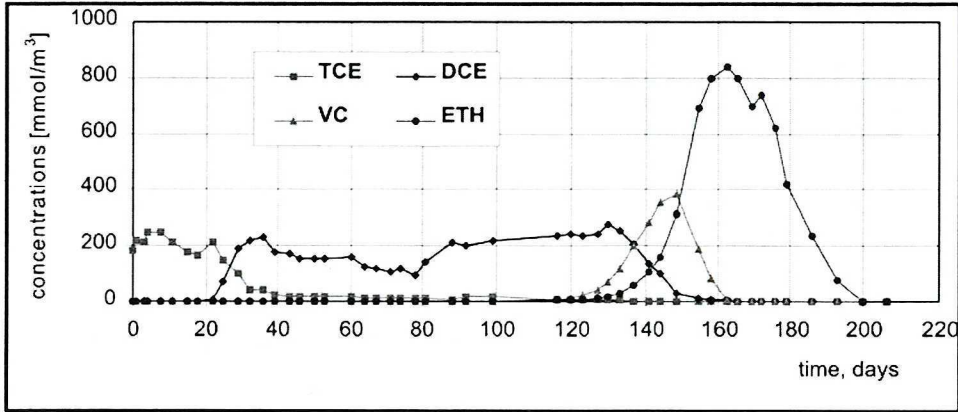

Fig. 6. Average TCE, DCE, VC and ETH mean concentrations in soil gas (mmol/m³)

The concentration of TCE in the gas phase, after initial period of oscillation around constant value ~ 200 mmol/m³, (days 0 – 20), began to decrease sharply, which was accompanied by a distinct increase in cis-DCE concentration. Slow decrease in cis-DCE concentration afterwards, was caused mostly by drop in the bioreactor temperature. The concentrations of VC and ETH were negligible over the whole time. Chloride concentration in the leachate showed a distinct increase at the same time when TCE concentration in gas phase declined.

2)   Days 81 – 140, in which:
    – temperature in the reactor was steadily increasing due to external heating and thermal insulation installed on the reactor,
    – redox potential was steadily decreasing and gained very low level,
    – practically no difference between reactor and ambient pressure were noted suggesting that no microbial activity took place during this period of time.

The concentration of TCE was negligible after the initial decline. The concentration of VC and ETH were initially close to zero but demonstrated distinct increases at the end of this period. The concentration of cis-DCE increased slowly in accordance with increase in the reactor temperature, but began to decline, when VC and ETH concentrations began to rise. No clear changes in chloride concentration in leachate were observed over this period of time.

3)   Days 141 – 200, when:
    – temperature in the reactor soil bed stabilized at 25°C,
    – redox potential decreased very slowly,
    – frequent changes in pressure indicated, that gases were produced in the reactor.

TCE concentration remained negligible, the concentration of cis-DCE continued to decrease. The concentration of VC gained a maximum (386 mmol/m³) and then dropped to below 0.1 mmol/m³. The concentration changes of ETH were similar but the maximum was much higher (839 mmol/m³) and shifted in time. Increase in chloride concentration in the leachate was observed all over the time.

As ETH was a final product of the reductive biodegradation of TCE, its concentration should not decrease after maximum has been gained. The drop in ETH concentration observed during the test indicates that ethylene is further processed or leaking of gases from the reactor took place. However, no ETH metabolism products were detected in the soil, thus it was assumed that ETH concentration decrease is caused by gas leakage from bioreactor vessel. Because CO_2 and CH_4 are produced during reductive degradation of sludge added to the bioreactor as inoculum and there were no signs of ambient air intake to the vessel (oxygen concentration had stayed at 0% and the pressure inside vessel was grater than atmospheric pressure) the tightness loss had not influenced bioremediation process conditions.

CONCLUSION

The reductive biodegradation of TCE to ETH in the bioreactor was successfully completed. The soil contaminated with 350 mg of TCE/kg was cleaned below the level suggested for arable soil. It is worth noting that VC reductive conversion to ETH began almost immediately after VC appeared in the bioreactor. The rate constant of VC degradation was higher than the rate constant of DCE conversion to VC. Possible explanation of this can be the following:

1. The microbial consortium present in the wastewater treatment sludge, chosen as an inoculum in the bioreactor test, contains microorganisms capable of VC reductive dechlorination to ETH. This would include bacteria specifically with VC reductive dehalogenases [3].

2. VC degradation to ETH is thermodynamically more favorable than DCE degradation to VC. Although the thermodynamic favorability of a chemical reaction cannot be linked directly to the rate at which the reaction occurs, in biologically mediated reactions this kind of dependency is much more frequent. It seems that in many cases the key role plays a quality of mixing between liquid and gaseous phase. VC being a poorly soluble gas in reaction conditions has tendency to leave liquid phase. On the other hand, most reactions mediated by microorganisms occurred in soil water. In these circumstances, the rate at which VC is degraded is limited partly by the rate at which it is delivered to where the reaction occurs. Circulating both gaseous and liquid phases through the soil bed in the bioreactor intensify this process. As a result, a higher rate of VC conversion to ETH was obtained. The matrix of soil combined with soil in the bioreactor is contributed to additional surface area for biofilm formation to facilitate biodegradation.

In summary, the evaluation at the field scale completed the evaluation which led to quantitative data verifying the success of engineered *ex situ* bioremediation of TCE-contaminated soil. Traditional remediation methods typically monitor only when contaminant concentrations have met regulatory limits, while this evaluation verifies that the VOCs are completely mineralized. From this demonstration it is evident that the bioreactor approach can be applied successfully at small sites where remediation can be accomplished through biodegradation of organic contaminants.

ACKNOWLEDGMENTS

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