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# A two-phase partitioning bioreactor system for treating benzene-contaminated soil

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**Abstract**

Benzene-contaminated topsoil, with an organic content of 42%, was treated by an air volatilization process, followed by a two-phase partitioning bioreactor to allow benzene mineralization. The effects of moisture content and temperature on the adsorption and desorption of benzene on to soil were investigated, and 95% of the benzene (at a concentration equivalent to 3.7 kg benzene $m^{-3}$ $soil^{-1}$) was removed at 50 °C by air volatilization. When 30 g soil was contaminated with 1000 mg benzene (a concentration 3 times higher), 93% of the benzene was removed by the air volatilization technique, of which 91% was consumed in a two-phase partitioning bioreactor within 2 h.

## Introduction

Benzene, a well known carcinogen, is commonly found in soils contaminated by gasoline and other petroleum products (Chang *et al.* 1993). Benzene adsorbs readily on to soil particles and is more strongly adsorbed on to clay than to either sand or silt (Cole 1994). Various *ex situ* and *in situ* treatments have been suggested to clean up benzene-contaminated soils. *Ex situ* methods are adequate for small contaminated sites while *in situ* methods are also widely used because of lower costs and less disruption to the surface (Cole 1994). Incinerating evacuated soil and the use of a sludge bioreactor are examples of *ex situ* clean up methods. Soil vapour extraction and enhancement of the aerobic biodegradation of pollutants by aeration of the soil are well known *in situ* clean-up technologies. The evaporated pollutants from *in situ* treatments must be treated before release into the atmosphere. Charcoal adsorption or catalytic incineration are often used to treat the extracted benzene. Granulated charcoal filters effectively clean the vapour but the filters must often be replaced and are expensive. The catalytic converters oxidize benzene vapours to carbon dioxide and water but this method has a high maintenance cost (Cole 1994, Urlings *et al.* 1991). Biofilters are especially useful if the emitting pollutant concentration is low, however they are generally limited to volatile organic carbon (VOC) concentrations of a few mg $l^{-1}$ (Hodge & Devinny 1995). Maintaining proper temperature, pH and moisture in biofilters, while dealing with high pressure drops and varying VOC concentrations, are also challenging problems (Shareefdeen & Baltzis 1994).

Collins & Daugulis (1999) showed that a two-phase partitioning bioreactor (TPPB) could be successfully applied to clean up benzene, toluene, and *p*-xylene (collectively called BTX) contaminated sand. TTPBs (as described in Collins & Daugulis (1996), and seen as a component of the overall experimental apparatus depicted in Figure 1) are comprised of a cell-containing aqueous phase on which an immiscible and biocompatible organic phase resides. Very high concentrations of toxic or inhibitory substrates can be dissolved in the organic phase, and these substrates partition, based on thermodynamic equilibrium, into the aqueous phase at sub-inhibitory levels, and in response to the metabolic demand of the cells. Collins & Daugulis (1999) washed BTX-contaminated sand with a selected organic solvent (oleyl alcohol, a low-

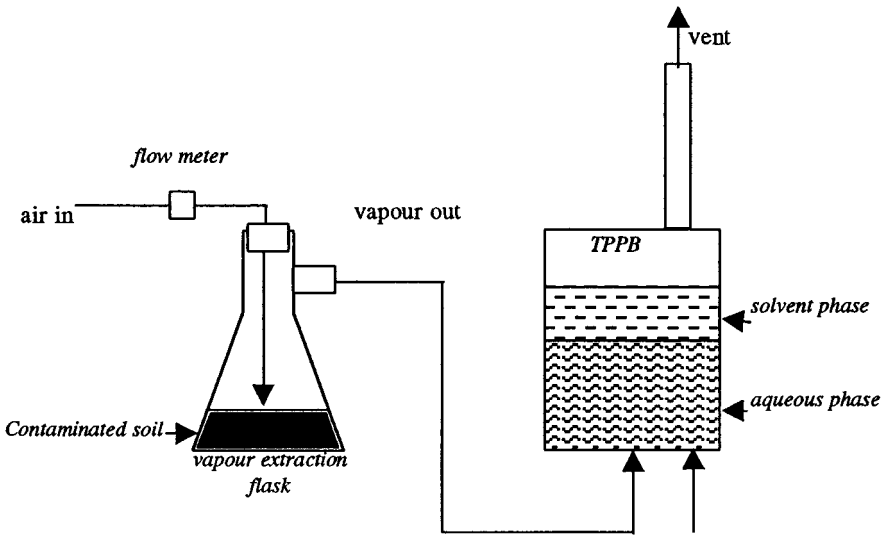


*Fig. 1.* Schematic diagram of experiment apparatus used to degrade benzene-contaminated topsoil.

cost industrial solvent) then transferred it into the TPPB along with washwater used to recover any adsorbed solvent. BTX was released from the solvent into the aqueous phase of the TPPB based on phase equilibrium partitioning, and was consumed by the microorganisms present. Since the TPPB is basically a simple fermentor, it is very easy to control all environmental factors such as oxygen level, temperature and pH. Although the pollutant concentration added to the bioreactor may be very high, the inhibitory effect of the contaminant is greatly mitigated by being adsorbed into the organic phase. Also, the organic phase can serve as a barrier against benzene loss by air stripping (Daugulis & Yeom 2001). However, the process described by Collins & Daugulis (1999) has several drawbacks. Other pollutants, organic matter and indigenous microorganisms could migrate from the soil to the aqueous or organic phases and affect the performance of the TPPB, since the soil and solvent come into direct contact with one another. Also, the process requires labour for washing the soil and regenerating the organic solvent.

In this study, we suggest air volatilization combined with a TPPB bioreactor to treat benzene-contaminated topsoil. Since benzene is evaporated readily by air blowing, non-volatile organic matter, usually abundant in soil, should not affect the process performance. Also, since the microorganisms in the soil do not contact the TPPB directly (i.e., the solvent used to 'capture' the VOCs does not directly contact the soil), the process should easily maintain a pure culture.

## Materials and methods

### *Microorganism and solvent*

*Klebsiella* sp. isolated from oil-contaminated soil was used in this system. This microorganism can degrade benzene, toluene, and phenol (Yeom & Choi 1998). Through a systematic solvent selection procedure described previously (Collins & Daugulis 1997), 1-octadecene was chosen as the best organic solvent for this system. This solvent has a high mass partitioning coefficient for benzene over water, and is biocompatible with *Klebsiella* sp. but is non-bioavailable.

### *Soil*

Topsoil from a local nursery was used as the soil in all experiments, as this would provide the highest levels of organic content. The organic content was determined to be 42% by measuring the weight difference after 12 h in a 600 °C muffle furnace. The moisture content was determined to be 29% by measuring the weight difference after 12 h in a 100 °C oven.

### *Growth medium*

The medium formulation (at a pH 6.8) for the bioreactor was: 5 g $K_2HPO_4$ $l^{-1}$, 4.5 g $KH_2PO_4$ $l^{-1}$,

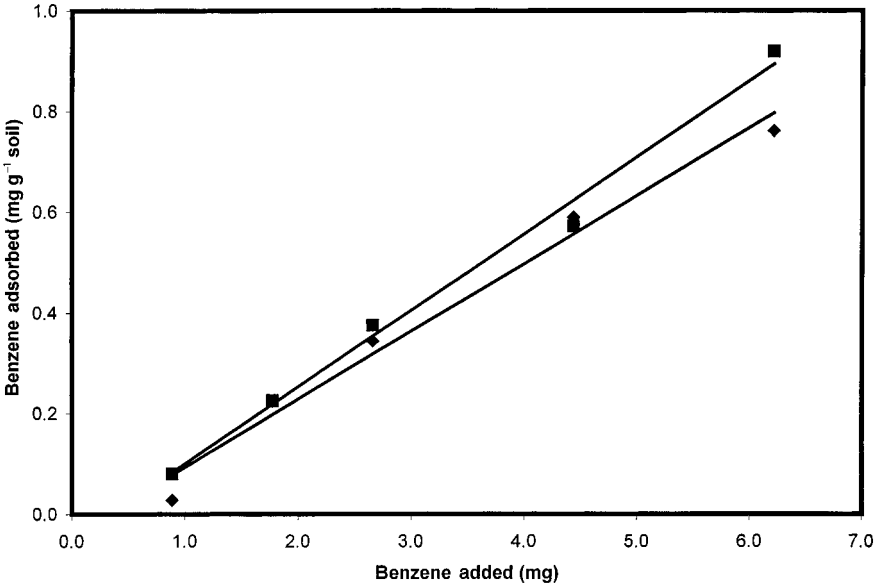


*Fig. 2.* Isotherm for adsorption of benzene onto topsoil with respect to moisture content. ■: 29% moisture, ♦: 100% moisture.

6 g $(NH_4)_2SO_4$ $l^{-1}$, 0.9 g $MgSO_4 \cdot 7H_2O$ $l^{-1}$ and 500 $\mu$l $l^{-1}$ of trace element solution. The trace element solution consisted of 16.2 g $FeCl_3 \cdot 6H_2O$ $l^{-1}$, 9.44 g $CaHPO_4$ $l^{-1}$, 0.15 g $CuSO_4 \cdot 5H_2O$ $l^{-1}$ and 40 g citric acid $l^{-1}$.

*System configuration*

Either 125 or 500 ml side-armed flasks were used as the benzene-contaminated soil containers. The side-arm port was connected to the inlet air port of the bioreactor. The TPPB has been well described in earlier work (Collins & Daugulis 1996). The working volumes of organic and aqueous phases were 0.5 l and 1 l, respectively in the TPPB. A condenser was installed on the bioreactor off-gas port to decrease benzene loss, and the bioreactor was operated at 30 °C. A schematic diagram of the system is shown in Figure 1.

*Analytical procedures*

Aqueous samples were taken from the bioreactor and centrifuged to remove cells. The liquid benzene concentration was analyzed by directly injecting 2 $\mu$l of the liquid sample into a gas chromatograph. To measure gas-phase benzene, 250 $\mu$l gas was directly withdrawn (from bottles or an off-gas port of bioreactor, as described later), and injected into the GC. The concentration was determined from the peak area as compared with a previously prepared calibration curve. The operating conditions of the GC were: 250 °C injection port, 50 °C oven and 200 °C detection port temperature. The cell concentration was measured turbidometrically at 660 nm and compared to a calibration curve.

*Adsorption equilibrium*

Two g (wet wt) of soil was put into each of several 160 ml serum bottles. Various amounts of benzene were added into each bottle and the bottles were closed with butyl-rubber septa and aluminum cramp caps. After 4 h at 30 °C on a shaking incubator, the benzene concentration in the gas phase was measured and the amount of benzene adsorbed onto the soil was calculated by mass balance.

Benzene adsorption onto soil soaked with water was determined as follows. Two g (wet wt) of soil was put into each of several 160 ml serum bottles containing 30 ml of water. Under these conditions, the soil was completely immersed in the water. Different amounts of benzene were added to each bottle and the bottles were closed with butyl-rubber septa and aluminum cramp caps. After 4 h at 30 °C on a shaking incubator, the gas phase benzene concentration was measured directly. After withdrawing liquid samples and centrifuging to settle any suspended soil particles, 2 $\mu$l of the liquid sample was injected into the GC. The amount of benzene adsorbed onto soil was calculated through mass balance.

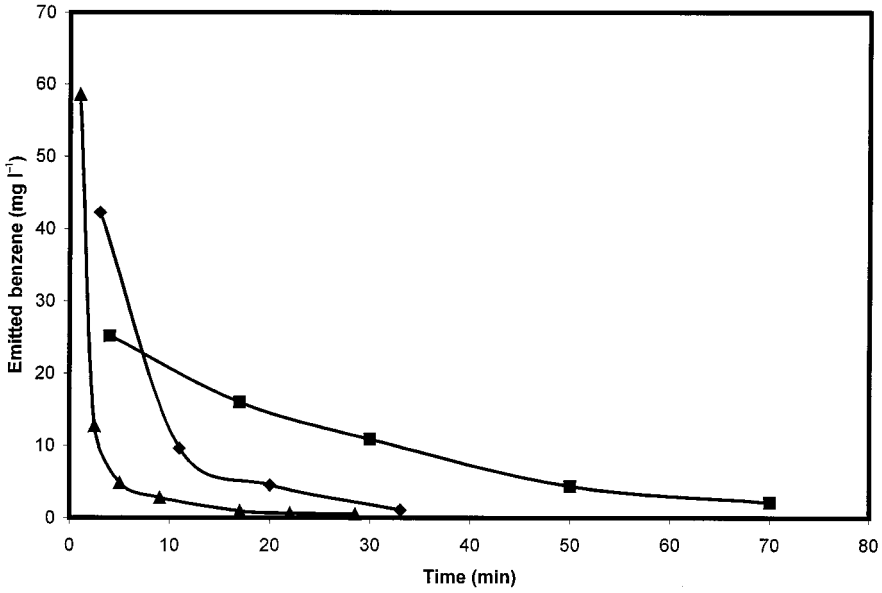


*Fig. 3.* Profile of emitted benzene concentration by air volatilization at various air flow rates. ■: air flow rate of 125 ml min$^{-1}$, ♦: air flow rate of 250 ml min$^{-1}$, ▲: air flow rate of 500 ml min$^{-1}$.

The effect of temperature on benzene adsorption was examined by adding various amounts of benzene to 2 g (wet wt) soil in sealed 160 ml serum bottles, and incubating at various temperatures, with shaking, for 4 h. The benzene concentration in the headspace was then measured, and the amount of benzene adsorbed to the soil was calculated by mass balance.

## Results and discussion

### *Soil adsorption*

Benzene adsorption on to soil is affected by the properties of the soil (particle size, organic content, etc.), environmental factors (temperature, moisture content, etc.) and physical properties of the pollutant (water solubility, soil sorption coefficient, etc.) (Allen-King *et al.* 1996). Since benzene is highly volatile and does not have functional groups to combine or react chemically with soil, volatilization is considered to be a good method to remove it from soil. We have investigated the effects of moisture content and temperature on benzene sorption/desorption onto soil.

Figure 2 shows an adsorption isotherm relationship of benzene at 30 °C. As can be seen, the relationship is linear rather than following a Langmuir-type curve over the experimental range, which suggests that many sorption sites of the soil remained unoccupied by benzene molecules. The results also indicate that about 25% of the benzene was adsorbed onto soil and 75% of the benzene remained in the gas phase. In order to investigate the effect of moisture on benzene sorption onto soil, the extreme case of completely soaking the soil in water was undertaken. The experimental data indicated that 30% of the benzene was in the water and 47% was in the gas phase. Thus about 23% of the benzene was adsorbed onto the soil as shown in Figure 2. Even after vigorous shaking of the serum bottles, almost the same amount of benzene was still adsorbed onto soil; that is, about 75% of the benzene remained unbound to the soil and should be readily removed through volatilization, along with some desorption of bound benzene.

The extent of removal of contaminants that can be desorbed from soil by volatilization can be increased by lowering the pressure (that is, lowering the boiling point of benzene) or increasing the temperature (enhancing the vapour pressure of benzene). Here, we investigated the effect of temperature on benzene adsorption/desorption onto soil. As shown in Table 1, as the temperature was increased from 20 to 50 °C, the portion of the benzene adsorbed onto the soil decreased from approximately 30% to 5%. This suggests that elevating the temperature, as expected, is an effective means of volatilizing benzene, however the trade-off of energy cost and desorption

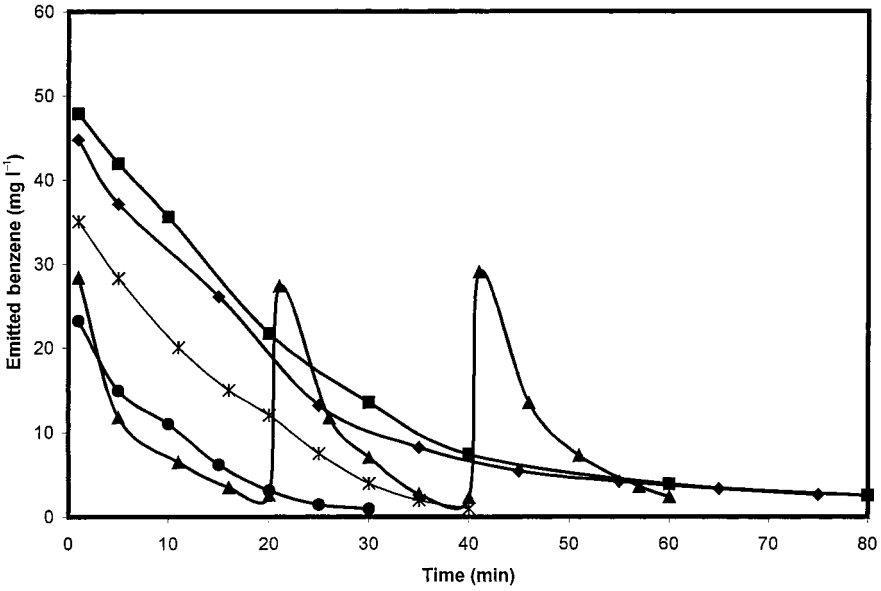


*Fig. 4.* Comparison of air volatilization methods for treating large amounts of benzene-contaminated topsoil. ●: small flask, ⋆: small flask with sparger at half depth of the soil, ▲: three small flasks with sequential desorption, ■: three small flasks connected serially, ♦: large flask.

*Table 1.* Effect of temperature on benzene adsorption onto soil.

| Initial benzene added (mg) | % Benzene adsorbed to soil | | | |
|---|---|---|---|---|
| | 20 °C | 30 °C | 40 °C | 50 °C |
| 1.8 | 27 | 19 | 9 | 5 |
| 4.4 | 31 | 20 | 10 | 5 |
| 6.2 | 33 | 23 | 13 | 6 |

effectiveness should be addressed to determine optimal temperature. Since 95% of the added benzene can be readily vaporized at 50 °C, we decided to continue to use this desorption temperature. After air desorption, the trace amounts of benzene retained by the soil could be treated either by inoculating the soil with benzene-degrading microorganism or by stimulating indigenous ones (Cole 1994).

*Air volatilization*

Air was blown into 125 ml side armed flasks (in a 50 °C water bath) in which 100 mg of benzene had been added to 10 g (wet wt) of soil. This simulated benzene contamination would represent a spill of approx. 3.7 kg benzene per cubic meter of soil (i.e., a high concentration spill). The air flow rate was a main operational parameter to control the emitted benzene concentration (or benzene removal rate) and the required operation time. As shown in Figure 3, when the air flow rate was 125 ml min$^{-1}$, the initial benzene concentration was extrapolated to be 29.5 mg l$^{-1}$ from exponential regression. The total amount of emitted benzene could be extrapolated by integrating under the curve, and 80 min was required to remove 95% of the benzene. Since benzene-laden air will be used as both a carbon and oxygen source in the TPPB, the effect of varying gas flows was examined. (The trade-off here would be that low gas flows would minimize air stripping from the bioreactor, but could lead to oxygen limitation; high gas flows would have the opposite effect.) Figure 3 therefore also shows the desorption rates and anticipated gas-phase benzene concentrations for gas flow rates of 250 ml min$^{-1}$ and 500 ml min$^{-1}$. As anticipated, higher gas flows lead to both significantly higher benzene concentrations in the gas, as well as reduced desorption times. For the integrated TPPB system it was decided to use an air flow rate of 250 ml min$^{-1}$.

In the event that a large amount of soil is to be treated by air stripping, the benzene adsorbed onto the soil may not be removed effectively because the macro pores within the soil become small (due to soil compression), resulting in an increased pressure resisting the penetration of air. To simulate compacted soil, 30 g (wet wt) of soil (81 ml in a 4.3 cm height)

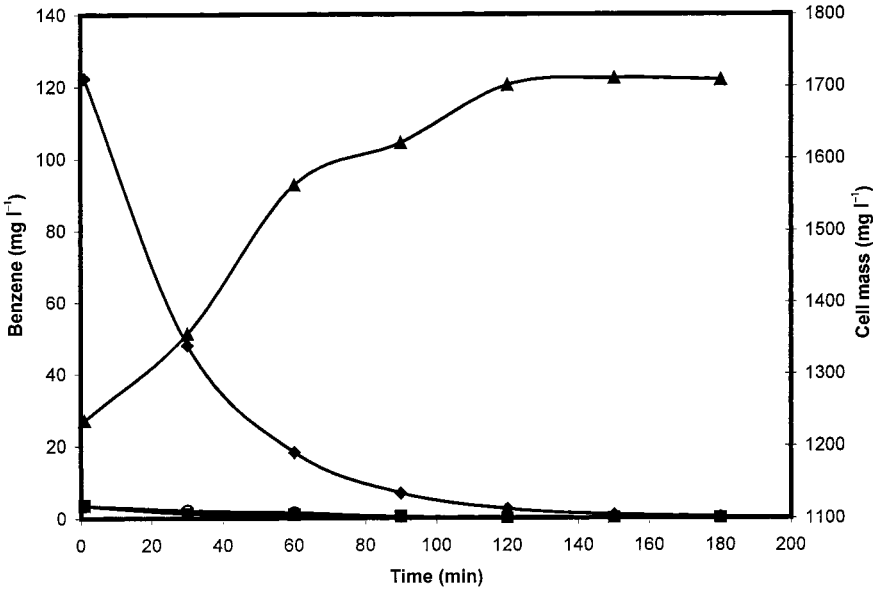


*Fig. 5.* Treatment of 30 g of soil contaminated with 1000 mg of benzene by air volatilization and a two-phase partitioning bioreactor. ♦: inlet gaseous benzene concentration to bioreactor, ■: off-gas benzene concentration from bioreactor, ○: benzene concentration in aqueous phase of bioreactor, ▲: cell mass.

was mixed with 500 mg benzene, and was placed into a 125 ml side-armed flask (11.5 cm height). Air was blown across the surface of the soil, but only 23% of the benzene was removed (integrating under the desorption curve) as shown in Figure 4. By placing an air sparger at the half depth of the soil, 48% of the benzene was removed also as seen in Figure 4.

Since the removal efficiency of benzene by air stripping was low in these cases, other methods were considered. The soil was next divided into three containers (1.3 cm height of soil) and they were treated sequentially in repeated batch mode as shown in Figure 4. In this instance, almost 95% of the benzene was removed using this arrangement, although it may be impractical to divide the soil in this fashion. The three containers were then connected serially and a single stream of inlet air was passed continuously through the 3 flasks. The emitted benzene concentration can be seen to gradually decrease, with 95% of the benzene being removed by this mode in 80 min. By increasing the volume of the container relative to the mass of soil (i.e., using a 500 ml flask with a 17.5 cm height) and treating 30 g of soil with a concomitantly decreased depth (1.7 cm) it was possible to remove almost 93% of the benzene. These results show that the latter two methods gave very similar emitting concentration profiles and that soil depth (or more generally air access to the soil) is a key factor in benzene desorption. Since the results of the serial system and the larger container were so similar, for the sake of convenience in the integrated system, we elected to use the latter approach.

*Bioreactor operation*

Air at 250 ml $l^{-1}$ was blown into a 500 ml side-armed flask containing 30 g (wet wt) topsoil contaminated with 1000 mg benzene. The flask was incubated in a 50 °C water bath. Since the initially-emitted benzene concentration was found to be 125 mg $l^{-1}$, and since 270 mg of benzene would be desorbed from the soil in the first 10 min, it was clear that either a biofilter or a conventional single (aqueous) phase bioreactor of the same size would be severely challenged by this very high benzene loading. However, with the use of the TPPB which could 'capture' the benzene in the solvent phase and release it on demand to the cells in the aqueous phase, the emitted gas stream was injected directly into the aqueous phase of two-phase partitioning bioreactor. As shown in Figure 5, as soon as the benzene-laden air was injected into the bioreactor, the cells began to grow without any lag phase and the off-gas benzene concentration from the TPPB was a mere 1.0 mg $l^{-1}$. Additionally, the benzene concentration in the aqueous phase never exceeded 5 mg $l^{-1}$, a level that is far below that which is toxic to the cells. In

this experiment 93% of the benzene was removed from the topsoil by air blowing and 91% of this benzene was consumed in the TPPB (total removal efficiency of 85.3%) in 2 h operation.

## Conclusion

Benzene-contaminated soil with a substantial organic content can be treated with very high removal efficiency. The released benzene can then be readily degraded in a TPPB system. The bioreactor can also be used for the treatment of released benzene from other sources, and applied to other VOC contaminants.

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