BIOTRANSFORMATION OF TERTIARY BUTYL MERCAPTAN IN SOIL MICROCOSMS

S. Kalainesan[1], L.E. Erickson[1], S. L. L. Hutchinson[2] and R. Karthikeyan[2]
Department of Chemical Engineering[1]
Department of Biological and Agricultural Engineering[2]
Kansas State University
Manhattan, KS 66506.

ABSTRACT

Tertiary butyl mercaptan (TBM) is a malodorant that is added to natural gas in small quantities to aid in its detection. The objective of this study is to investigate the biodegradation of TBM in soil under aerobic conditions.

Soil microcosms were set up using two different soils and their headspace was sampled during a 62-day period of study. In the first set containing soil from Fort Riley, there was significantly greater reduction in TBM and production of carbon dioxide in the treatments compared to the sterilized controls. Further it was observed that there was more carbon dioxide production in the microcosms without TBM rather than those with TBM. In the second set of experiments using soil-containing methyl tertiary butyl ether degrading microorganisms, the reduction in TBM level was almost equal in the treatments and the sterilized controls. In this soil, there was more carbon dioxide in the treatments with TBM compared to those without TBM. The sterilized controls showed minimum carbon dioxide production. In both sets of experiments, an intermediate product di-tertiary-butyl disulfide was identified. The disulfide production was observed to be greater in the treatments than in the sterilized controls.

The experimental results show that TBM appears to be biodegradable in soil-water systems under aerobic conditions and may be considered for appropriate uses with appropriate environmental management.

INTRODUCTION

A review of the published literature was made to determine the biodegradation characteristics of tertiary butyl mercaptan (TBM). As no experimental studies were found, two sets of microcosm studies were carried out following the methods that have been used in other studies (2, 3, 7). In addition the value of Henry's constant for TBM was estimated experimentally. Using the Henry's constant value, solubility of TBM in water was also estimated.

EXPERIMENT I: BIODEGRADATION OF TBM IN SOIL MICROCOSMS

Introduction

The first set of experiments used soil from Fort Riley for biodegradation studies. The microcosms for the first set of experiments were set up using 160 mL serum bottle consisting of 10 g soil, 10 mL water and 10 μL TBM each. The microcosms were set up for a 62-day period. They consisted of treatments, sterilized controls and carbon dioxide controls. The

volume of the serum bottles where chosen such that the headspace in the microcosms was sufficient for aerobic biodegradation.

Soil Characteristics

The Fort Riley soil was from the field site where sediments from the vehicle wash water sedimentation basin had been deposited for a phytoremediation field study (5, 6). The organic content of the soil included residual petroleum hydrocarbons; however, these were a small fraction (less than 3%) of the organic matter in the soil.

Table 1*. Soil Analysis Results for Experiment I

Sample	pH	Organic Matter %	Sand %	Texture Silt %	Clay %	Total N %	Total C %
1[a]	7.7	4.4	24	45	31	0.141	3.48
2[b]	7.6	4.2	18	50	32	0.151	3.49
3[c]	7.7	4.5	20	48	32	0.154	3.59

a. Soil taken from the treatments that contained unamended soil and TBM
b. Soil taken from the carbon dioxide controls that contained unamended soil and no TBM.
c. Unamended soil
*Soil analysis results obtained from soil testing laboratory, Kansas State University.

Experimental Setup

Treatments

Treatments were set up to monitor the reduction of tertiary butyl mercaptan (TBM) in soil water system due to biodegradation. The treatments consist of 160 mL serum bottles sealed with a Teflon lined septum, containing 10 gram soil and 10 mL water. After the bottle was closed with the septum and crimped, 10 μL TBM was injected through the septum. The treatments were setup on “Day 0” on Sep 22, 2003. Three replicates of each treatment were setup for sampling on days 1, 4, 6, 8, 12, 16, 20, 26 and 33. Treatments set up for day 1 were sampled on days 0, 1, 49 and 62. The remaining sets of bottles were sampled only once on one of the days mentioned above.

Controls

Controls help to establish that reduction in TBM is primarily due to biodegradation. In the absence of microorganisms there should be no significant reduction in the level of TBM.

The controls consisted of the same soil as treatments but in this case it was sterilized. Sterilization of soil was done as follows: the required amount of soil was packed in an aluminum foil and placed in an autoclave at 250 °F for 50 minutes. The soil packed in the aluminum foil was allowed to cool. It was then incubated at 30°C for 2 hrs. Then the soil was again autoclaved as before. This process was repeated three times to ensure maximum destruction of microorganisms. Sterilized serum bottles were used for these controls and any instrument used for setting up the controls such as spatula, pipettes, beakers etc., were also sterilized. The controls were set up using 10 g sterilized soil (sterilized as mentioned above), 10 mL de-ionized water and 10 μL TBM in 160 mL serum bottle. The de-ionized water was not sterilized before adding to the controls. Controls were setup and sampled on the same days as the treatments.

CO_2 Controls

The carbon dioxide controls were setup to see the extent of microbial activity in the absence of TBM. They helped to identify if TBM increased or decreased the microbial activity. The CO_2 controls consisted of 10 g of the soil and 10 mL water in 160 mL serum bottle. No TBM was added to these CO_2 controls which were set up for analysis on days 4, 12, 26 and 62.

EXPERIMENT II: BIODEGRADATION OF TBM IN SOIL MICROCOSMS CONTAINING MTBE DEGRADING MICROORGANISMS

Introduction

In the second set of microcosms, experimental soil containing methyl tertiary butyl ether (MTBE) degrading microorganisms was used. This soil had been used in earlier experimental work to investigate MTBE biodegradation (10). Microcosms were set up using 26 mL serum bottles consisting of 1 g soil, 50 μL water and 2 μL TBM each. Microcosms included treatments, sterilized controls and carbon dioxide controls and they were set up for a 78-day period. The experimental setup was designed for aerobic biodegradation.

Soil Characteristics

The soil used in this study was a mixture of soil taken from different soil strata. The characteristics of the soil used in the experiment are tabulated below:

Table 2*. Characteristics of Each Soil Stratum			
Features	**Top soil**	**Middle Soil**	**Bottom Soil**
Depths at Actual Site	Surface soil	15-40 cm	Below 40 cm
Representative Depths in Channel (cm)	0-15	15-25	25-35
Sand Content (%)	90	91.5	96
Silt Content (%)	10	8.5	4
Organic Carbon Content (%)	1.8	1.0	0.3
Nitrate Level (ppm)	13	7	1
Phosphate Level(ppm)	103	63	42
Potash Level (ppm)	220	115	85

*Table adapted from Muralidharan, 1994.

Table 3*. Gravimetric Water Content Values (g water / g dry soil)			
Chamber Depth (cm)	**Distance Along the Length of the Channel from the Inlet (cm)**		
	20	80	160
0-10	0.186	0.179	0.178
10-20	0.222	0.231	0.211
20-30	0.231	0.243	0.222

* Table adapted from Muralidharan, 1994.

Experimental Setup

Treatments

As in the previous study, treatments were set up to monitor the reduction of tertiary butyl mercaptan (TBM) in soil water system, due to biodegradation. The treatments consisted of 26 mL serum bottles sealed with a Teflon lined septum, containing 1 g soil and 50 μL water. After the bottle was closed with the septum and crimped, 2 μL TBM was injected through the septum. The treatments were setup on "Day 0" on Feb 2, 2004. Six sets of treatments with three replicates each were set up. The first set consisting of three replicates were sampled on days 2 and 20, second set was sampled on days 4 and 26, third set was sampled on days 6 and 33, fourth set was sampled on days 8 and 49, fifth set was sampled on days 12 and 62 and the sixth set was sampled on days 16 and 78.

Controls

Controls help to establish that reduction in TBM is primarily due to mineralization. The controls consisted of the same soil as treatments but in this case it was sterilized; hence, there should be no significant reduction of TBM due to biodegradation in the controls. Sterilization of soil was done as follows: the required amount of soil was packed in an aluminum foil and placed in an autoclave at 250 °F for 50 minutes. The soil packed in the aluminum foil was allowed to cool. It was then incubated at 30°C for 2 hrs. Then the soil was again autoclaved as before. This process was repeated three times to ensure maximum destruction of microorganisms. Sterilized serum bottles were used for these controls and any instrument used for setting up the controls such as spatula, pipettes, beakers etc., were also sterilized. The controls were set up using 1 gm sterilized soil (sterilized as mentioned above), 50 μL de-ionized water and 2 μL TBM in 26 mL serum bottle. The de-ionized water was not sterilized before adding to the controls. Controls were setup and sampled on the same days as treatments.

CO_2 Controls

The carbon dioxide controls were setup to determine the extent of microbial activity in the absence of TBM. They helped to identify whether the presence of TBM enhanced or decreased the microbial activity. The CO_2 controls consisted of 1 gm soil (the same soil as used in the treatments and controls) and 50 μL water in 26 mL serum bottle. No TBM was added to these microcosms. Two sets of CO_2 controls were set up with three replicates in each set. First set was sampled on days 4, 20, 33 and 62 and the second set was sampled on days 12, 26, 49 and 78.

IDENTIFICATION OF TBM AND CO_2 PEAKS

Headspace from the microcosms was analyzed for TBM using a 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) with Chemstation© integration software. The GC was equipped with a flame ionization detector. The column used was HP1 (J & W Scientific, Folsom, CA) mega bore column with a length of 30 m, internal diameter 0.53 mm and 5 μm film thickness. The fuel and carrier gas was H_2 (99.999%), the make up gas was N_2

(99.999%) and the support gas was zero-grade dry air. The column head gauge pressure was maintained at 0.34 atm (5 psi). The temperature program began at 50°C for 2 minutes, ramped at 10°C / min to 150°C and was then held at 150°C for 3 minutes. The injection port and detector temperatures were at 150°C and 280°C respectively. TBM was analyzed by injecting 10 μL (split less) of headspace gas from the serum bottle manually using a gas tight Hamilton syringe. The chromatogram obtained showed a TBM peak appearing at about 2 min. The peak areas were obtained through automatic integration.
CO_2 was measured using a Shimadzu gas chromatograph with a thermal conductivity detector. A sample containing 0.5 mL of gas phase was injected and the carbon dioxide peak appeared around 0.6 minutes. The peak area was calculated automatically.

DISULFIDE DETECTION

During the analysis of headspace gas on the GC, another significant peak appeared around 12 min, in addition to the TBM peak. Analyzing sulfur chemistry suggested that TBM oxidizes to form di-tertiary-butyl disulfide. As the microcosms were designed for aerobic biodegradation, the second peak was expected to be di-tertiary-butyl disulfide, an intermediate product of degradation. Mass spectrometer was used to analyze the GC results and the peak that appeared at 12 min was confirmed to be di-tertiary-butyl disulfide.
Another unidentified peak appeared around 4 minutes. Though this peak could not be exactly identified using GC/MS it may be a trisulfide or one of the oxidation products of TBM.

ESTIMATION OF HENRY'S CONSTANT

Henry's Law and Henry's Constant

When a vapor mixture and liquid solution exist in equilibrium at a temperature T and pressure P, the relationship for vapor-liquid equilibrium of a pure species "i" is given by (9): -

$$\hat{f}_i^v = \hat{f}_i^l$$
$$\hat{f}_i^v = y_i \hat{\phi}_i P$$
$$\hat{f}_i^l = x_i \gamma_i f_i$$

Where,

- $\hat{f}_i^v$ is the fugacity of species "i" in vapor mixture.
- $\hat{f}_i^l$ is the fugacity of species "i" in liquid solution.
- y_i is the mole fraction of species "i" in vapor.
- $\hat{\phi}_i$ is the fugacity coefficient of species "i" (equal to unity if vapor phase behaves as ideal gas).
- P is the total system pressure.
- x_i is the mole fraction of species "i" in liquid.
- γ_i is the activity coefficient of species "i" (equal to unity for an ideal solution).
- f_i is the standard state fugacity of species "i".

If

$$\hat{f}_i^v = \hat{f}_i^l$$

$$y_i \hat{\phi}_i P = x_i \gamma_i f_i$$

Assuming ideal vapor and liquid phases

$$y_i P = x_i f_i$$

Or,

$$f_i = \frac{y_i P}{x_i}$$

Also,

$$f_i = \frac{\hat{f}_i}{\gamma_i x_i} = \frac{\hat{f}_i}{x_i}$$

$$f_i = \frac{y_i P}{x_i} = \frac{\hat{f}_i}{x_i}$$

The standard state f_i must be chosen based upon the liquid phase composition of the system. Henry's law behavior arises when the liquid mixture of interest is very dilute in component "i" and a direct proportionality is observed between the fugacity of the species "i" and its mole fraction in the liquid phase. Then f_i becomes equal to the Henry's constant, H_i which may be expressed as below (4, 9): -

$$H_i = \lim_{x_i \to 0} \left[\frac{y_i P}{x_i} \right] = \lim_{x_i \to 0} \left[\frac{f_i}{x_i} \right]$$

The above equation is the statement of Henry's law as it applies to real solutions (Smith and Van Ness, 1987). The Henry's constant is the partial pressure in the gas phase divided by the concentration in the liquid phase.

Method of Estimation of Henry's Constant

Standards were prepared by adding 10, 20, 30, 40 and 50 μL TBM into 160 mL serum bottles containing 100 mL deionized water each. The standards were allowed to come to equilibrium. Then the headspace gas and the liquid phase from each bottle were analyzed for TBM using a gas chromatograph. The injection volume was 10 μL for headspace gas analysis and 1 μL for liquid phase analysis. The analysis results obtained in terms of peak areas are as tabulated below:

Table 4. Headspace Analysis of Standards for TBM	
Concentration of TBM μL/100 mL Water	**Peak Area**
10	394532
20	791617
30	1186591
40	1579388
50	2080666

Table 5. Liquid Phase Analysis of Standards for TBM	
Concentration of TBM μL/100 mL Water	**Peak Area**
10	142036
20	263731
30	386274
40	475088
50	543727

From the standards prepared, let us consider the standard where 30 μL TBM was added to 100 mL water. At equilibrium 30 μL TBM added would have partitioned into 100 mL aqueous phase and 60 mL headspace. Assuming that: -

Gram moles of TBM in 100 mL water = M_w

Gram moles of TBM in 60 mL head space = M_v

From analysis by Gas Chromatograph,

Peak area obtained for (aqueous phase) 1 μL injection = 386274

Peak area obtained for (head space) 10 μL injection = 1186591

For the evaluation of Henry's constant we use the principle that the same amount of TBM injected into the gas chromatograph should result in a peak with the same peak area.

$$\frac{386274/1}{\left(\frac{M_w}{100X10^{-3}}\right)}\frac{\left(Peakarea/\mu L\right)}{\left(\frac{gmoles}{\mu L}\right)} = \frac{1186591/10}{\left(\frac{M_v}{60X10^{-3}}\right)}\frac{\left(Peakarea/\mu L\right)}{\left(\frac{gmoles}{\mu L}\right)}$$

$$\frac{M_w}{M_v} = \frac{386274\,X\,100\,X\,10^{-3}\,X\,10}{1186591\,X\,60\,X\,10^{-3}}$$

$$\frac{M_w}{M_v} = 5.43 \quad \ldots\ldots (1)$$

Total volume of TBM = 30 μL

Mass of TBM = $30X10^{-3}\,(mL)X0.7943(g/mL) = 0.02383\ g$

Moles of TBM = $\frac{0.02383}{90.19} = 2.6422X10^{-4}\ gmoles$

$$\therefore M_w + M_v = 2.6422X10^{-4}\ gmoles \quad \ldots\ldots (2)$$

Solving equations (1) and (2) we get

$$M_v = 4.11X10^{-5}\ gmoles$$

$$M_w = 2.23X10^{-4}\ gmoles$$

$$P^* = \frac{nRT}{V} = \frac{4.11X10^{-5}\,(gmol)\,X\,1(atm)\,X\,22400(ml)\,X\,297(K)}{60(mL)\,X\,273(K)\,X\,1(gmol)}$$

$$P^* = 0.0167\ (atm)$$

$$H = \frac{P^*}{C} = \frac{0.0167\,(atm)}{\left(\frac{2.2310\,X\,10^{-4}}{100\,X\,10^{-6}}\right)\left(\frac{gmoles}{m^3}\right)}$$

$$H = 7.49\,X\,10^{-3}\left(\frac{atm\,m^3}{gmole}\right)$$

Similarly the Henry's constant value was calculated using vapor and aqueous phase peak areas obtained from the analysis of standards 20 μL / 100 mL water and 10 μL / 100 mL water.

Table 6. Henry's Constant Evaluation	
Standard Concentration (μL TBM / 100 mL Water)	**Henry's Constant (atm m^3 / gmol)**
30	7.47 X 10^{-3}
20	7.31 X 10^{-3}
10	6.77 X 10^{-3}

The average Henry's constant value calculated using the above three values of Henry's constant is:

$$H = 7.19\,X\,10^{-3}\ (atm\,m^3 / gmol)$$

The above value of Henry's constant was evaluated at 24°C.

It may be noticed that the calibration for the gas phase concentration of TBM is very close to linear but in the case of liquid phase we see that there is a tailing off of the peak area with increase in concentration and the results are not as linear as in the case of gas phase concentration. Hence for the estimation of Henry's constant only the lower part of the calibration curve, which is more linear, has been considered. The value of Henry's constant appears to be in the lower range of 6.77×10^{-3} to 7.31×10^{-3}, as the calibration is highly linear in this range. This experimental value is compared to predicted values from the published literature in Table 7.

Table 7. Comparison of Estimated Henry's Constant Value with Literature Values	
S.No	**Henry's Constant atm m^3 / gmole**
1*	7.19 x 10^{-3} @ 24°C
2**	6.11 x 10^{-3} @ 25°C
3***	5.12 x 10^{-3} @ 25°C

* Estimated value of Henry's constant from this experiment

** Henry's constant value from EPI Suite estimation software (bond estimate), Syracuse Research Center, Syracuse, New York.

*** Henry's constant value from literature (Yaws et al., 2003).

Henry's Constant and Solubility

The estimated value of Henry's constant as explained above was 7.19×10^{-3} atm m^3 / gmole at 24°C. To evaluate the value of Henry's constant at 20°C we use the data in Table 8.

Table 8. Vapor Pressure of TBM at Different Temperatures			
Vapor Pressure Mm mercury	**Log(Vapor Pressure)**	**Temperature K**	**1/T 1/K**
181[a]	2.2577	298	3.3557×10^{-3}
305[b]	2.4843	311	3.2154×10^{-3}
760[c]	2.8808	338	2.9586×10^{-3}

a - value from EPI Suite Estimation Software, Syracuse Research Center, Syracuse, New York.
b - value from Atofina Chemicals Inc., Tertiary Butyl Mercaptan MSDS, 2002.
c - value from literature (Yaws et al., 2003).

Using a plot of log (vapor pressure) vs. 1/T the value of vapor pressure at 293 K was evaluated to be approximately 144.5 mm mercury. Then assuming solubility to be constant, Henry's constant at 20°C was estimated to be 5.74×10^{-3} atm m^3 / gmole. As the biodegradation studies were carried out at 20°C, this estimate was used for the calculations.

Using the above estimate of Henry's constant the solubility of TBM in water was calculated from the equation:

$$S = P / H$$

Where,

S is the solubility in mg/L
P is the vapor pressure in atmospheres
H is the Henry's constant in atm L / mg
The solubility of TBM was thus estimated to be 2988 mg/L at 20°C.

RESULTS AND DISCUSSION

Experiment I

TBM Disappearance with Time

In the first set of TBM biodegradation studies it was seen that the concentration of TBM in the treatments decreased more rapidly than that in the controls. Figure 1 shows the behavior of TBM during a 62-day period for the controls and treatments. It may be seen from Figure 14, that TBM reduction in the treatments is much higher than the controls, which had sterilized soil. The controls have sterilized soil and hence ideally there should have been no reduction of TBM in the controls. But the graph shows that there was some reduction in TBM in the controls. This may be due to the fact that the contents might not have been sterilized completely. There might have been some form of bacterial contamination that led to the reduction in TBM in the controls. Nevertheless, reduction of TBM is more in the case of treatments than in the controls. So there is some evidence of biodegradation. There is also a possibility that the loss of TBM may be partly due to chemical reaction. There was no evidence of a lag period for the reduction of TBM in the unsterilized soil. For these treatments more than 99% of the TBM disappeared during the first 33 days of the experiment.

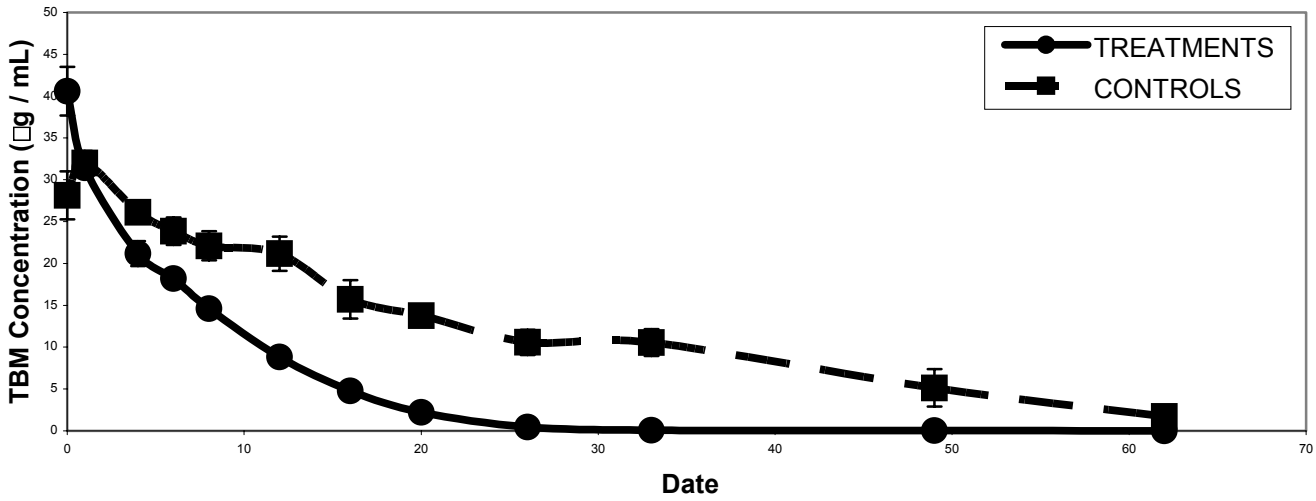

Figure 1. Gas Phase Concentration of TBM in the Headspace for the Treatments and the Controls

CO_2 Production with Reduction in TBM

Figure 2 shows carbon dioxide production in treatments, controls and carbon dioxide controls from day 0 to day 62. It may be seen that there is significantly more carbon dioxide produced in the treatments than in the sterilized controls. The production of CO_2 in the controls and treatments may result from the biodegradation of TBM, from the mineralization of organic carbon content in the soil, or from chemical conversion of TBM. For the unsterilized treatments, the headspace concentration of carbon dioxide was about 17 gm /L after 62 days. This accounts for about 16 percent of carbon that was supplied as TBM.

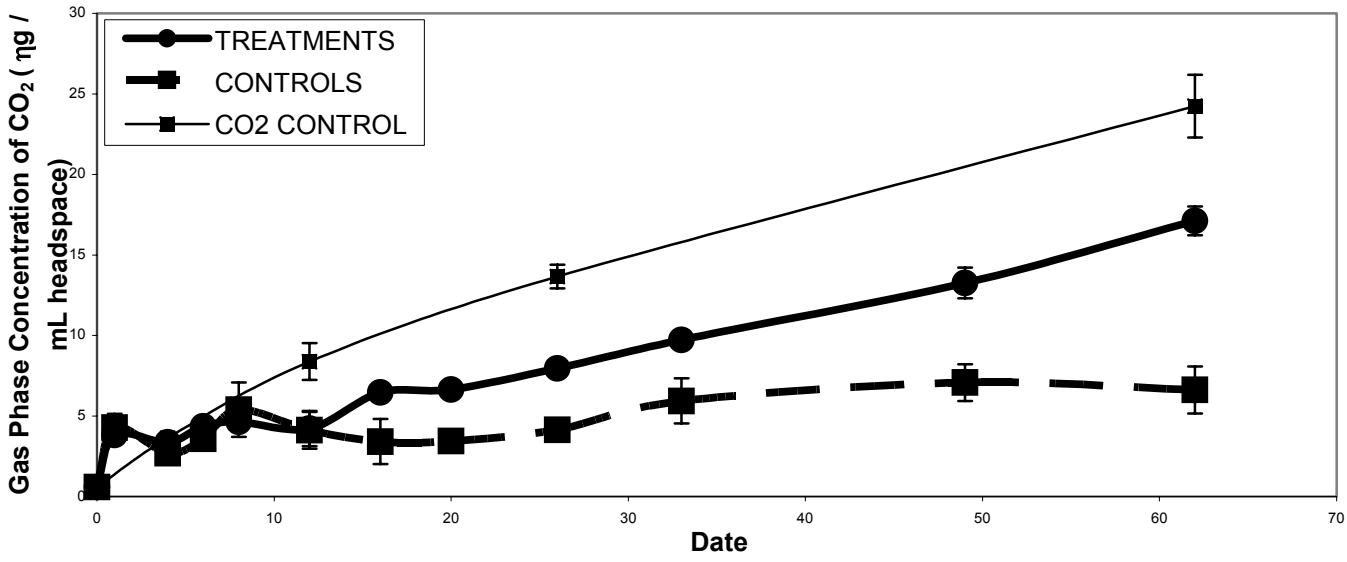

Figure 2. Gas Phase Concentrations of CO_2 in the headspace of the Treatments, Controls and CO_2 Controls.

Disulfide Production

Figure 3 shows disulfide production in the controls and treatments. It may be seen from Figure 16 that there is more disulfide production in the treatments than in the sterilized controls. While this provides some evidence in favor of bioconversion of TBM to disulfide, disulfide production may also be due to the chemical conversion of TBM.

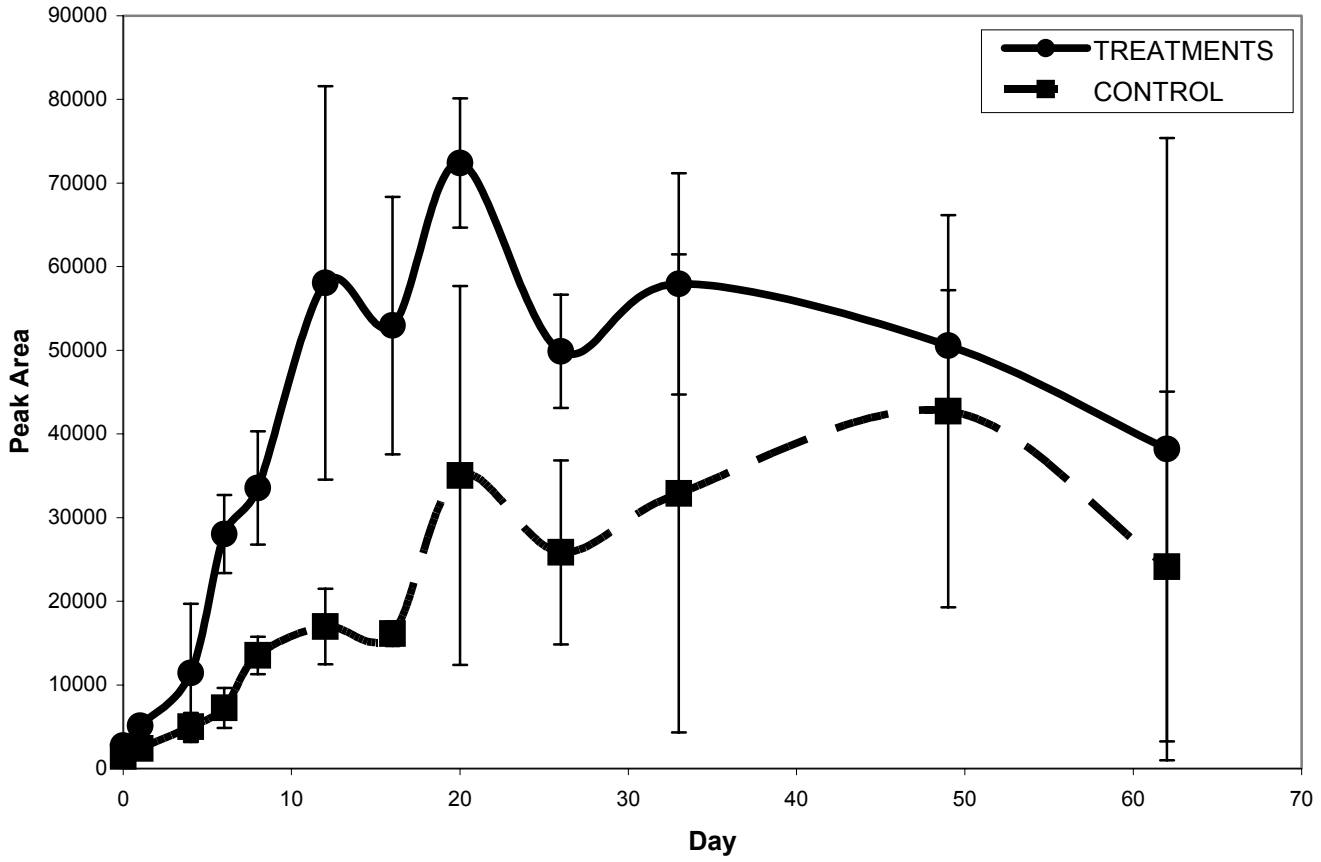

Figure 3. Gas Phase Concentration of Di-teriary-butyl Disulphide Produced from TBM, for the Treatments and the Controls

It was seen earlier by testing that the Teflon lined septum could withstand multiple punctures with negligible leakage. Also the treatments, sterilized controls and CO_2 controls were placed in dark locations away from any light. Hence the reduction in TBM due to photo degradation or leakage is negligible.

Kinetics and First Order Rate Constant

The natural logarithm of the gas phase concentration of TBM divided by the initial gas phase concentration of TBM was plotted versus time in order to determine the reaction rate

constant for TBM decay in the treatments and the controls. The slope of the straight line fitted to the data in the above mentioned plot gave the negative of the first order rate constant.

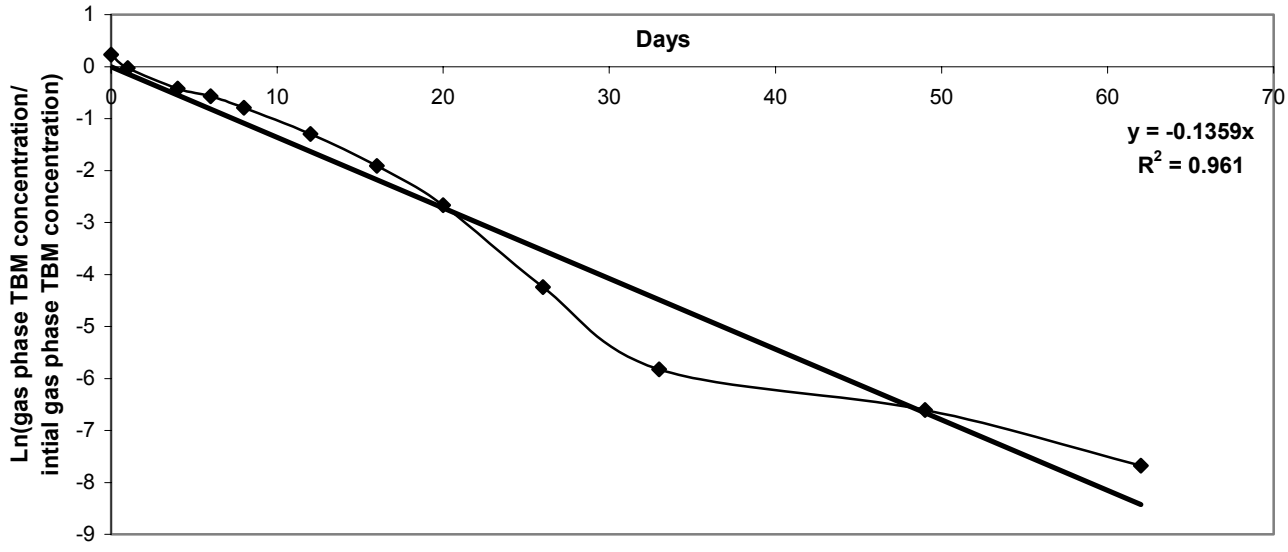

Figure 4. Determination of First Order Rate Constant for the Disappearance of TBM in the Treatments in Experiment I

The first order rate constant thus estimated was found to be 0.0384 day^{-1} for the sterilized controls and 0.1359 day^{-1} for the treatments. It may be seen that first order rate constant for the treatments is greater than that for the controls. No lag period is observed in the case of Experiment I. The half life, which is the time taken for TBM to reduce to half its initial concentration was found to be 5.1 days for the treatments and 18.1 days for the controls.

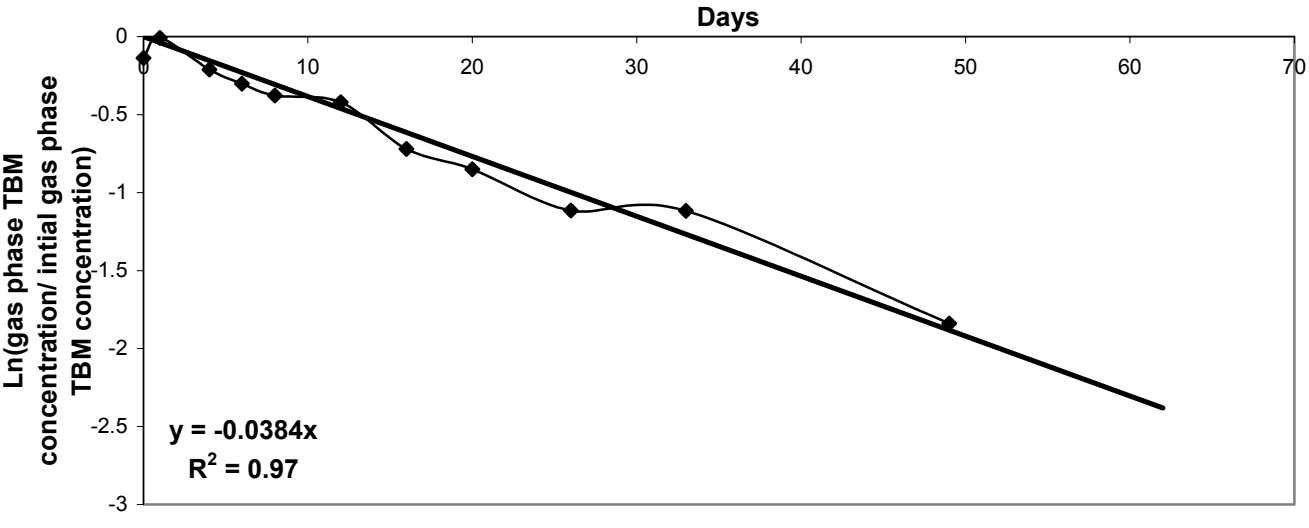

Figure 5. Determination of First Order Rate Constant for the Disappearance of TBM in the Controls in Experiment I

Experiment II

TBM Disappearance with Time

In the second set of TBM biodegradation studies it was seen that the concentration of TBM in the treatments and controls decreased at almost the same rate.

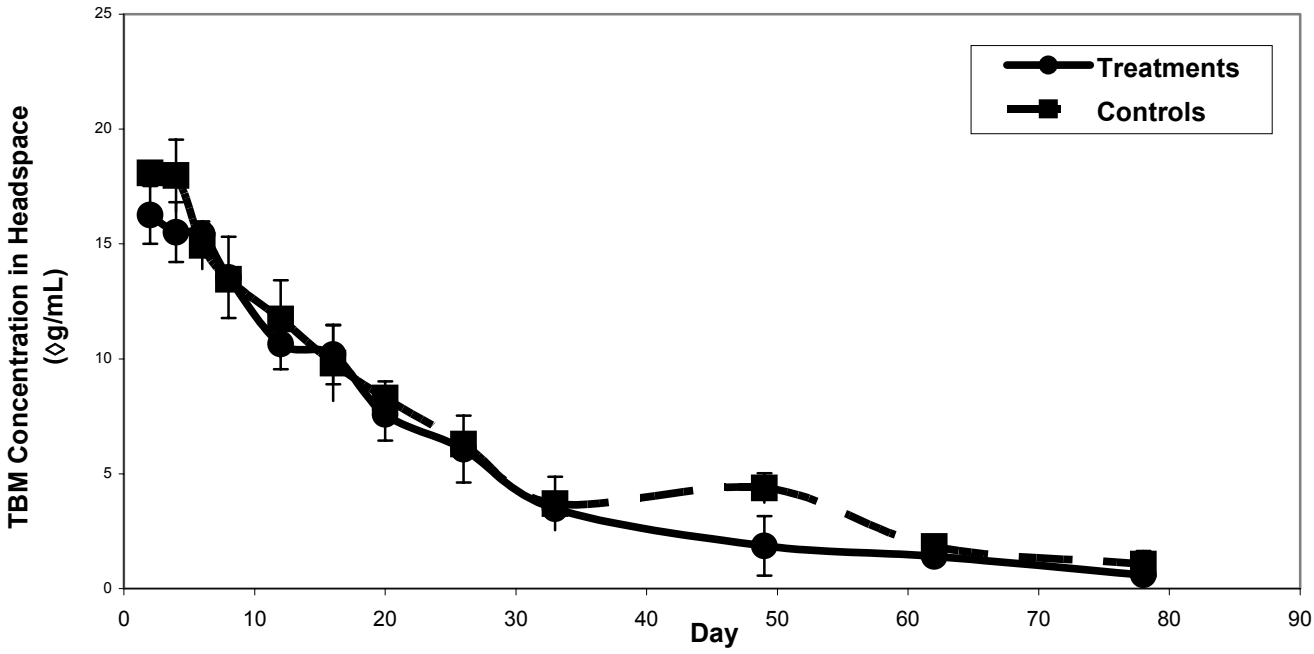

Figure 6. Gas Phase Concentration of TBM in the Headspace for the Treatments and Controls from Day 2 to Day 78

Figure 6 shows the behavior of TBM during a 78-day period for the sterilized controls and the treatments. Ideally there should have been no reduction of TBM in the sterilized controls, but we find TBM reducing at the same rate in the treatments and controls. Though some reduction of TBM in the sterilized controls can be attributed to the lack of effectiveness of the sterilization, the reason for reduction of TBM in the sterilized controls, at the same rate as the treatments, is not very clear. Hence there is a possibility that chemical reaction may also be causing TBM disappearance in addition to biodegradation.

CO_2 Production with Reduction in TBM

Figure 7 shows the production of CO_2 in the soil microcosms from day 0 to day 78. CO_2 may be produced due to biodegradation of TBM, due to mineralization of the organic carbon content in the soil or due to chemical reaction. It can be seen from figure 7 that the production of CO_2 in the sterilized controls is much less than that produced in the treatments, which leads us to believe that the sterilization was effective to a certain extent in destroying the microorganisms. It may also be seen from figure 18 that more carbon dioxide was produced by the treatments than the CO_2 controls which had no TBM in them. This shows that the activity of the microorganisms is enhanced by the presence of TBM and hence we can also say that there is some evidence of biodegradation of TBM.

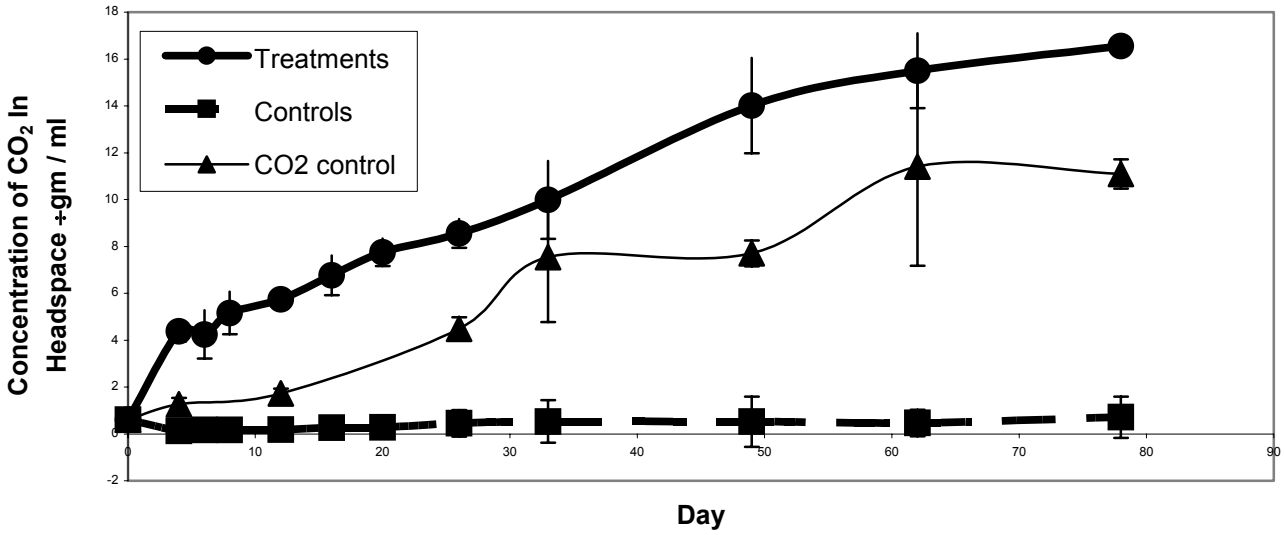

Figure 7. Gas Phase Concentration of CO_2 in the Headspace for the Treatments, Controls and CO_2 Controls from Day 0 to Day 78

For the unsterilized treatments, the headspace concentration of carbon dioxide was 16.56 mg TBM/L after 78 days. This accounts for about 13 percent of carbon that was supplied as TBM.

Disulfide Production

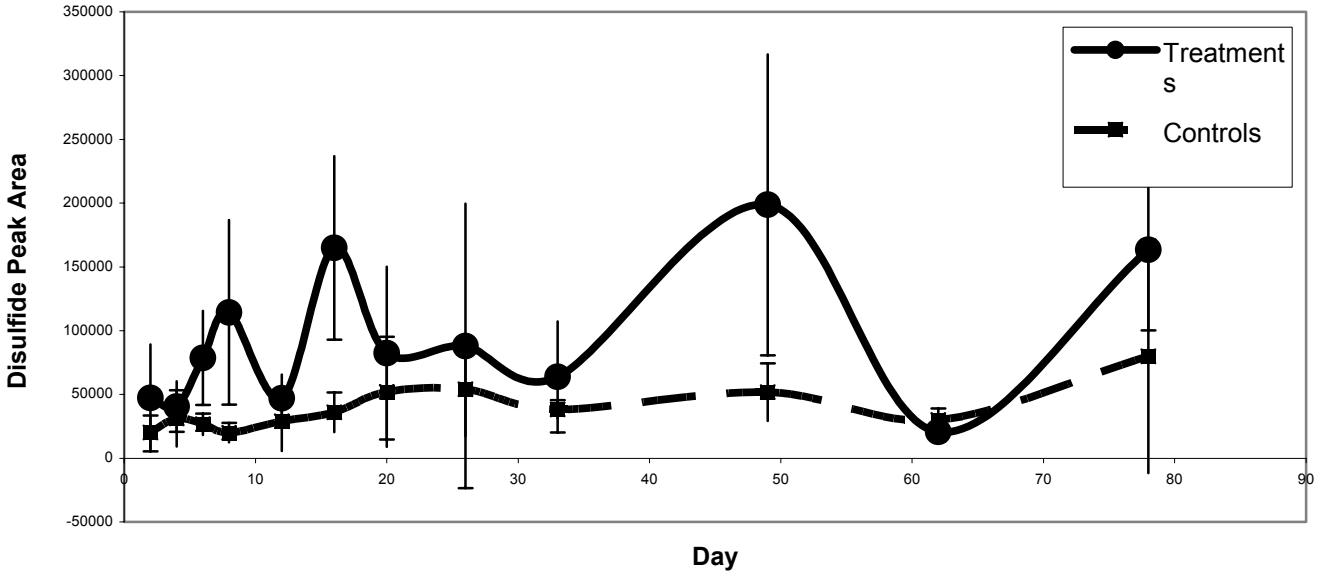

Figure 8. Production of Disulfide in the Treatments and Sterilized Controls from Day 2 to Day 78

As in the case of Experiment I disulfide production was noticed in the second set of experiments. Figure 8 shows disulfide production in the controls and treatments. It may be

seen from Figure 8 that there is more disulfide production in the treatments than in the sterilized controls.

In the case of Experiment II about 80% of the TBM disappeared in 33 days and about 96% TBM disappeared in 78 days.

Kinetics and First Order Rate Constant

The natural logarithm of the gas phase concentration of TBM divided by the initial gas phase concentration of TBM was plotted versus time in order to determine the reaction rate constant for TBM decay in the treatments and the controls. The slope of the straight line fitted to the data in the above mentioned plot gave the negative of the first order rate constant.

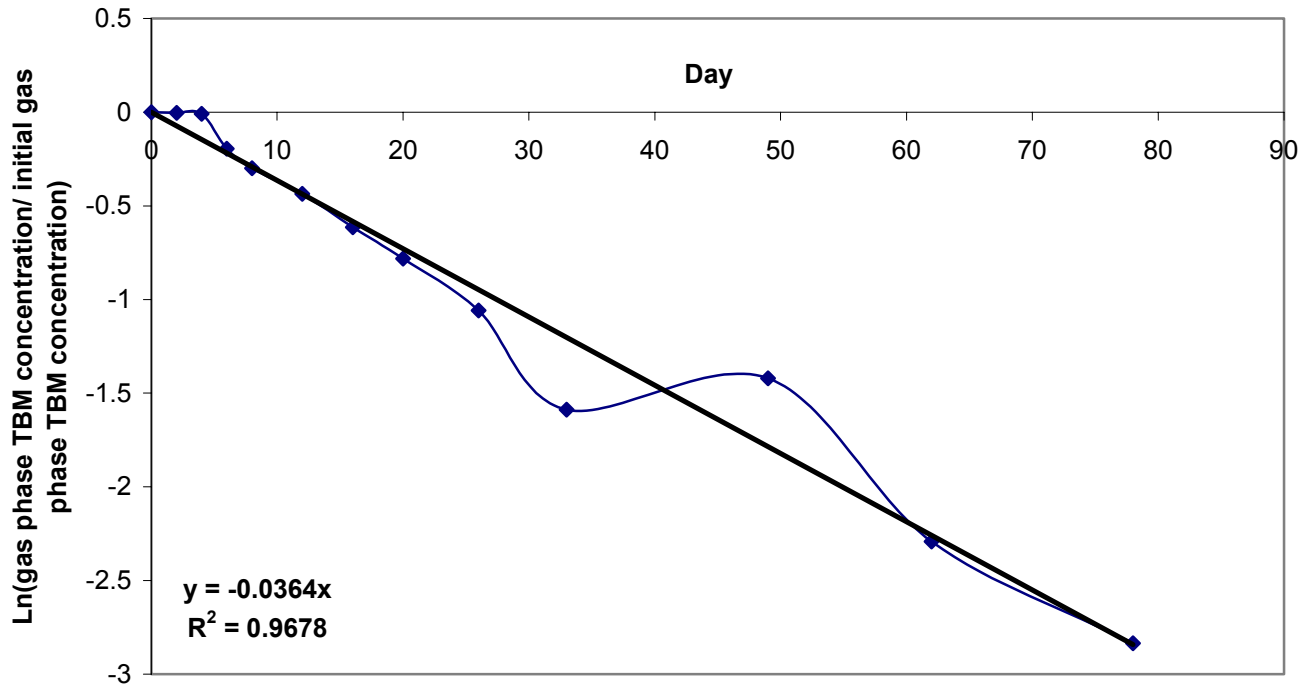

Figure 9. Determination of First Order Kinetic Parameter for the Decrease in TBM in the Headspace of the Controls in Experiment II

The first order rate constant thus estimated was found to be 0.0364 day^{-1} for the sterilized controls and 0.0437 day^{-1} for the treatments. It may be seen that first order rate constant for the treatments is greater than that for the controls. An initial lag period was observed in the case of Experiment II, which was greater for the treatments than the controls. The half life, which is the time taken for TBM to reduce to half its initial concentration was found to be 15.9 days for the treatments and 19.0 days for the controls.

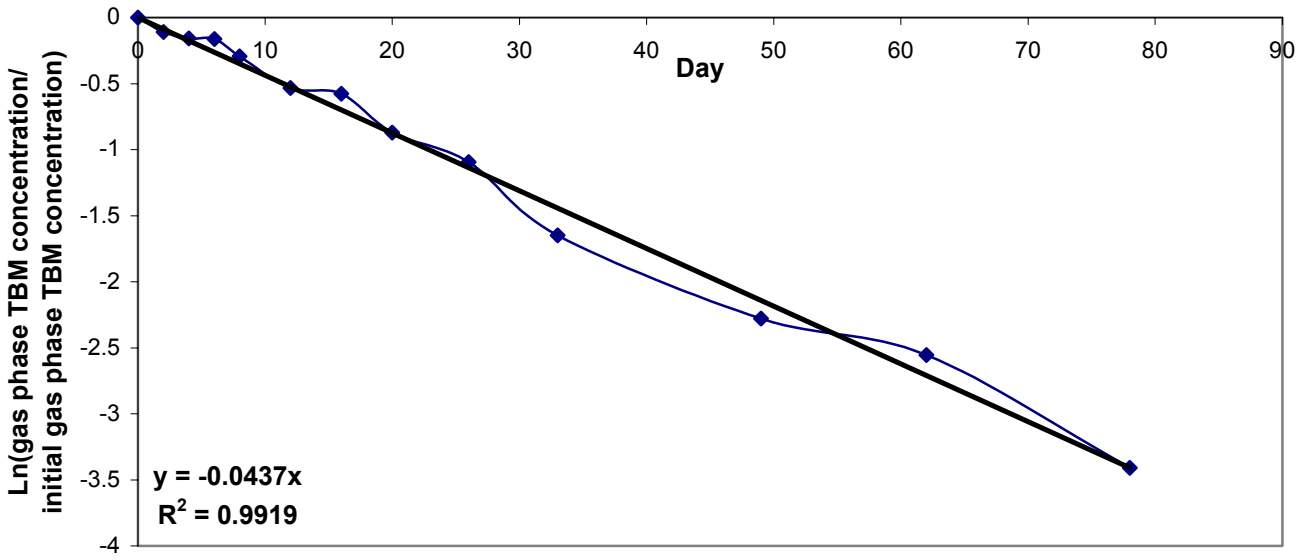

Figure 10. Determination of First Order Kinetic Parameter for the Decrease in TBM in the Head Space of the Treatments in Experiment II

ERROR BARS

Three replicates (each from a different microcosm set up at the same time) were made for each set of treatment, control and CO_2 control sampled on a particular day. The average values of the three replicates are plotted in the Figures 1, 2, 3, 6, 7 and 8. The error bars show one standard deviation of the replicate values in the positive and negative direction from the average value.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Using measured peak areas for both gas phase and liquid phase TBM, the value of Henry's constant was estimated to be 7.19×10^{-3} atm m^3 / gmole at 24°C. This value is in good agreement with predicted values.

From both sets of biodegradation studies it was found that TBM appears to be biodegradable in soil and may be used appropriately with appropriate environmental management. In the first set of biodegradation studies, more than 99% of TBM disappeared in 33 days, which may be due to biodegradation or chemical reaction. In the second set of biodegradation studies, more than 96% of TBM disappeared in 78 days. In experiment I there was no evidence of any lag phase. In both cases carbon dioxide was produced and a disulfide intermediate was also produced. It was also observed that the soil containing MTBE biodegrading organisms showed higher respiration than microcosms without TBM. This shows

that the activity of MTBE degrading microorganisms is enhanced by the presence of TBM and that they thrive well in the presence of TBM.

Recommendations

The biodegradation studies conducted gave some significant results on the biodegradation of TBM. But the experiment was performed in small microcosms using small quantities of TBM. Similar study could be conducted on a large scale to understand the biodegradation of TBM better.

More effective methods of sterilization of the controls could be used to confirm that only microbial degradation is responsible for TBM biodegradation in the system. Also, microbial plate counts can be done at the end of the study to understand the activity of microbes in the soil.

The microorganisms that showed the capacity to biodegrade TBM can be isolated and further experiments can be conducted to see if the same microorganisms are capable of biodegrading other compounds with tertiary butyl group.

Di-tertiary-butyl disulfide was identified as an intermediate in the biodegradation experiments that were conducted. Further work can be carried to understand the pathways of degradation of TBM and the tertiary butyl group in general.

LITERATURE CITED

References

1. Atofina Chemicals Inc., “Material Safety Data Sheet of Tertiary butyl mercaptan,” Philadelphia, PA, USA, (2002).
2. Cookson, J. T., Bioremediation Engineering, McGraw-Hill, New York (1995).
3. Eweis, J. B., S. J. Ergas, D. P. Y. Chang, and E. D. Schroeder, “Bioremediation Principles,” WCB/McGraw-Hill, New York (1998).
4. Howe, G.B., M.E. Mullins, and T.N. Rogers, “Evaluation and Prediction of Henry's Law Constants and Aqueous Solubilities for Solvents and Hydrocarbon Fuel Components: Vol. I: Technical Discussion,” Research Triangle Institute, Tyndall Air Force Base, Florida (1987).

5. Karthikeyan, R., P. Kulakow, and L. E. Erickson, “Vegetated Treatment of Vehicle Wash Sediments,” *Proceedings of the 28th Annual Biochemical Engineering Symposium* [Reilly, P., Editor]. Iowa State University, IA., 101-104 (1998).
6. Karthikeyan, R., P. Kulakow, B. Leven, G. Pierzynski, and L. E. Erickson. “Vegetated Treatment of Vehicle Wash Sediments: A Field Demonstration,” *Proceedings of the 2000 Conference on Hazardous Waste Research* [Erickson, L. E. and M. M. Rankin, Editors]. GP/RM Hazardous Substance Research Center, 101 Ward Hall, Manhattan, KS., 159-166 (2000).
7. Knox, R. C., D. A. Sabatini, and L. W. Canter, “Subsurface Transport and Fate Process,” Lewis Publishers, Florida (1993).
8. Muralidharan, N., “Environmental and Modeling Studies on Bioremediation of Organic Contaminants in the Presence of Alfalfa Plants,” M.S. Thesis, Kansas State University, 1994.
9. Smith, J. M., and H. C. Van Ness, “Introduction to Chemical Engineering Thermodynamics,” 4th ed., McGraw-Hill, New York (1987).
10. Winnike-McMillan, S. K., Q. Zhang, L. C. Davis, L. E. Erickson, and J. L. Schnoor, “Phytoremediation of Methyl Tertiary-Butyl Ether,” *Phytoremediation: Transformation and Control of Contaminants* [McCutcheon, S. C. and J. L. Schnoor (Editors)], Wiley, New York, 663-694 (2003).
11. Yaws, C. L., P. Bajaj, and R. W. Pike, “Solubility and Henry’s Law Constants for Sulfur Compounds in Water,” *Chemical Engineering*, **110**(8), 60-64 (2003).

Web Resources

A. Syracuse Research Center., “EPI Suite Estimation Software,” Syracuse, New York, USA. http://www.syrres.com/esc/epi.htm