

BTEX Biodegradation in Soil-Water System Having Different Substrate Concentrations.

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Abstract: BTEX are aromatic hydrocarbons which create a major pollution problem in the ground water. The BTEX group of chemical contaminants consists of benzene, toluene, ethyl benzene and three isomers of xylene and they make up a significant percentage of petroleum hydrocarbons. However they get bio-degraded with time by microorganisms. The natural bioremediation process is quite slow. The objective of this study was to find out the degradation kinetics of Toluene in soil-water system at different initial concentration of toluene. The batch experiments were conducted for different concentration of toluene at room temperature; the batches contain 50% contaminated ground water which is collected from oil refinery near Panipath, 50% wastewater and 10 grams of sand. The air phase samples were taken and analysed using Gas chromatography to find the degradation rate. Rate of biodegradation shows the Linear or first order kinetic upto 100 ppm and after that it follows Monod's Kinetics. Value of μ_{max} (maximum utilization of substrate) was approximately 7 ppm/hr and thus K_s (Half saturation coefficient) were 3.5 which is half of the μ_{max} . These values were used to calculate specific growth rates (μ) at higher toluene concentrations. The slope $-dc/dt$ is calculated and it shows an increase with increasing initial substrate concentrations upto 100ppm after that it shows declination in degradation rate.

Key word: BTEX, Bioremediation, Monod's Kinetics, Linear Kinetic.

1. Introduction:

Contamination of groundwater by petroleum hydrocarbons is a major concern. This is caused due to water and soil pollution by organic and inorganic pollutants and is a major public health concern. Non-aqueous phase liquids volatile hydrocarbon exist as a separate, immiscible phase when in contact with water and/or air. These can be classified as either light non aqueous phase liquids (LNAPLs), having densities less than that of water or dense non aqueous phase liquids (DNAPLs), which have densities greater than that of water (Newell 1995).

LNAPL includes BTEX (benzene, toluene, ethyl benzene, xylene), which is a common contaminant in the environment (Fitts 2002). BTEX are monoaromatic hydrocarbon which is of particular concern because of their spread in (sub) - surface widely (Margesin et al.2003). The common sources of BTEX in the ground water and soil are the spills including release of petroleum products such as gasoline, diesel fuel and lubricating oil from the leaking tanks mainly underground. A considerable amount of gasoline enters into the environment as a result of leakage from underground oil storage tanks, accidental spills or improper waste disposal practices (*Bowlen and Kosson, 1999*).

When gasoline is in contact with water, benzene, toluene, ethyl benzene and the xylene isomers (BTEX) accounts for as much as 90% of the gasoline components that are found in the water soluble fraction (*saeed and Al-mutairi, 1999*). Their presence in the water and soil can create a hazard to public health and environment. These compounds have a very high pollution potential, because of their high concentration in gasoline, relatively high solubility in water and chronic toxicity (*corseuil et al., 1996*). These pollutants have been found to cause many serious health side effects in humans (e.g. skin and sensory irritation, central nervous system depression, respiratory problems, leukaemia, cancer, as well as disturbances of the kidney, liver and blood systems) and, therefore, their removal from groundwater and surface waters are essential (*Aivalioti et al., 2010*).

2. Kinetics of degradation

In the biodegradation of toluene, there are a series of biodegradation pathway (known as stoichiometries pathways) during which complex substances are transformed into simpler daughter compounds (mono substituted) (Yadav et.al,2011). There are different degradation kinetics models that can used for determination of rate of degradation of toluene. These are constant or zero-order, linear or first-order, Monod or Michaelis-Menten, Logistic, Logarithmic models.

A general expression of hydrocarbons depletion in soil, in which only microbial densities and the contaminant concentration determine the degradation kinetics, can be written as (Lyman et al. 1992):

$$-\frac{\partial C}{\partial t} = \mu_{max} C \frac{C_0 + X_0 - C}{K_s + C}$$

where μ_{max} is the maximum growth rate, C is the contaminant concentration at time t , C_0 is the initial contaminant concentration, X_0 corresponds to the contaminant required to produce initial microbial density, K_s is the half saturation constant also known as growth limiting concentration.

3. Materials and Methods

3.1 Materials

The groundwater was collected from a hand pump near oil refinery in Panipat, India that was contaminated with toluene. The samples were kept in refrigerators in cans of 20 litres. All used chemicals were of analytical grade. Toluene, mercury chloride and methanol were all obtained from Merck. Toluene was used as the contaminant that was biodegraded. Mercury chloride was used for sterilization of groundwater to create sterile controls. It was having a concentration of 10 g/l. Methanol was used to clean the used syringes. Calibration curve was prepared with double distilled water. Clean sand (Quartz sand) used in the experiments had grain sizes between 0.1 and 1.0 mm. A stock was prepared by dissolving toluene with 99.9% purity in distilled water to its solubility limit. The batches were 120 ml glass bottles. The batches were capped with a viton stopper and a 20 mm aluminium seal. Toluene solution was injected using gas tight syringes (25 μ l and other volumes, Hamilton). The groundwater was sampled using a 1000 μ l capacity micropipette (Thermo fisher scientific, Finland). Samples were properly sealed using a sealing machine. Vials of 4 ml volume used to prepare different volumes of toluene in distilled water to prepare calibration curve.

3.2 Methodology

The groundwater samples are collected from the domestic hand pumps near by the panipat oil refinery with four or five metre depth. Type of sampling was Random. Samples are collected in the can of 20 litres. After the sampling it was storage in Lab's refrigerator. Waste water was collected from IIT, Delhi's boys hostel canal in the can of 20 litres and store in lab. Batches of groundwater and soil samples were prepared with different toluene concentration. Groundwater and soil in different volumes was taken in batch bottles. Bottles were sealed with viton stopper. Then different volumes of Toluene stock (490 ppm) were injected in each bottle to make different concentrations of toluene in groundwater. Batches of different concentrations were prepared in duplicates. The batches were shaken by hands before taking the first sample. The batches were put horizontally on a shaker at approximately 100 rpm to ensure complete mixing of the system till the next reading is taken. Groundwater and soil in different volumes was taken in batch bottles. Bottles were sealed with viton stopper. Then

different volumes of Toluene stock (490 ppm) were injected in each bottle to make different concentrations of toluene in groundwater. Batches of different concentrations were prepared in duplicates. The batches were shaken by hands before taking the first sample. The batches were put horizontally on a shaker 150 rpm to ensure complete mixing of the system till the next reading is taken.

3.3 Sample Analysis

In this experiment we use gas chromatograph NUCON 5765 for analysis of all samples i.e. determination of toluene concentration. It was use of two detectors FID (Flame Ionization Detector) and TCD (Thermal Conductivity Detector). The principal use of FID (Flame ionization detector) works on the principle that the electrical conductivity of gas is directly proportional to the concentration of charged particles within the gas. The extent of the ionization depends on the nature of the compound, its quantity and temperature of the flame. So from each batch of different concentrations, 10 μ L gas phase samples were injected into GC for analysis.

4. Result and discussion

4.1 Calibration curve

Area was determined from the chromatogram for each concentration that was made for the calibration curve, is as follows:

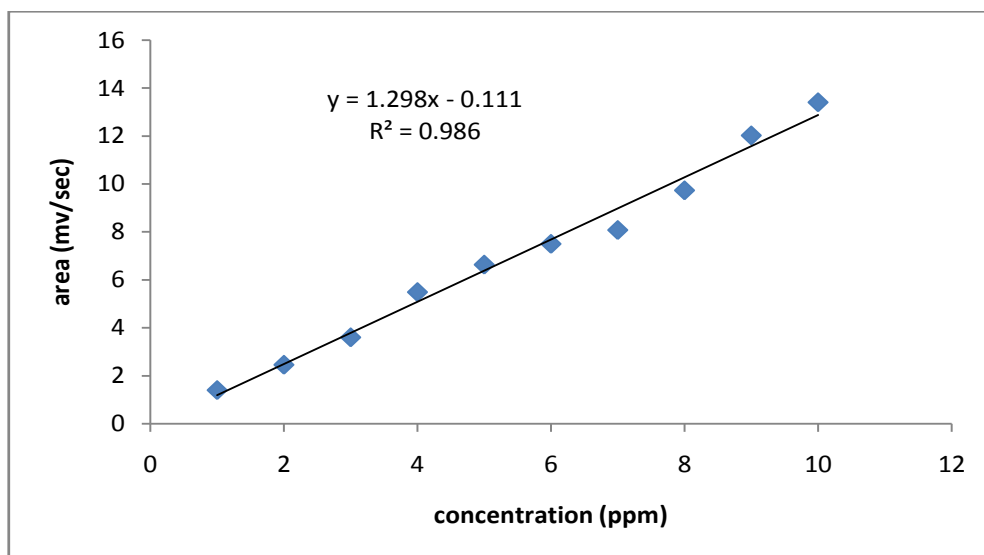


Figure 1: Calibration curve of Toluene

4.2 Batch experiment of groundwater with different concentrations of toluene

Table 1: Results of batch experiments

Conc.(ppm) →	5 ppm	8 ppm	10 ppm	20 ppm	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm
Time(Hr) ↓									
0	4.848	7.736	-	-	34.01	22.28	23.49	26.53	26.92
1	3.825	6.097	-	14.71	27.95	15.2	22.96	26.43	25.89
2	3.342	5.684	8.958	9.26	26.75	13.21	22.78	25.94	25.93
3	3.122	5.281	5.838	8.73	24.02	12.79	21.13	23.23	24.71
4	3.001	5.021	4.028	7.65	23.45	11.38	19.67	23.09	22.45
5	2.783	4.981	3.673	7.23	22.01	10.02	17.83	21.77	20.9
6	2.621	4.764	3.211	7.12	21.785	9.34	15.49	20.68	20.44
7	2.503	4.567	2.986	7	21.354	8.86	14.79	19.45	20.13
8	2.387	4.451	2.675	6.75	19.9	-	-	-	-
9	2.211	4.073	2.452	6.56	19.454	-	-	-	-
10	2.094	4	2.031	6.12	18.78	-	-	-	-
18	1.023	3.762	1.006	6.001	13.203	-	-	-	-
19	0.987	3.563	0.987	5.76	12.92	-	-	-	-
20	0.873	3.123	-	5.12	10.78	6.99	8.29	12.38	16.81
21	0.653	2.907	-	5.012	7.47	6.49	7.78	10.44	15.97
22	0.544	2.564	-	4.57	-	5.73	4.01	8.93	15.01
23	-	2.22	-	-	-	4.7	3.65	7.01	13.6
24	-	1.98	-	-	-	3.23	3.22	5.12	12.77
25	-	-	-	-	-	1.29	2.05	2.53	10.73
26	-	-	-	-	-	-	1.72	0.37	8.81

Below graph plotted from the above reading of toluene degradation at different concentration:-

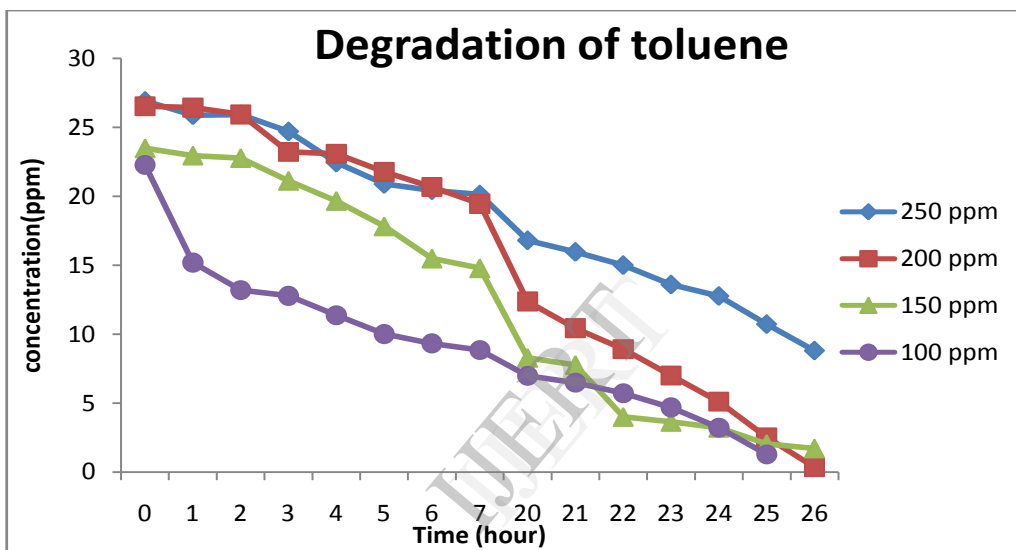
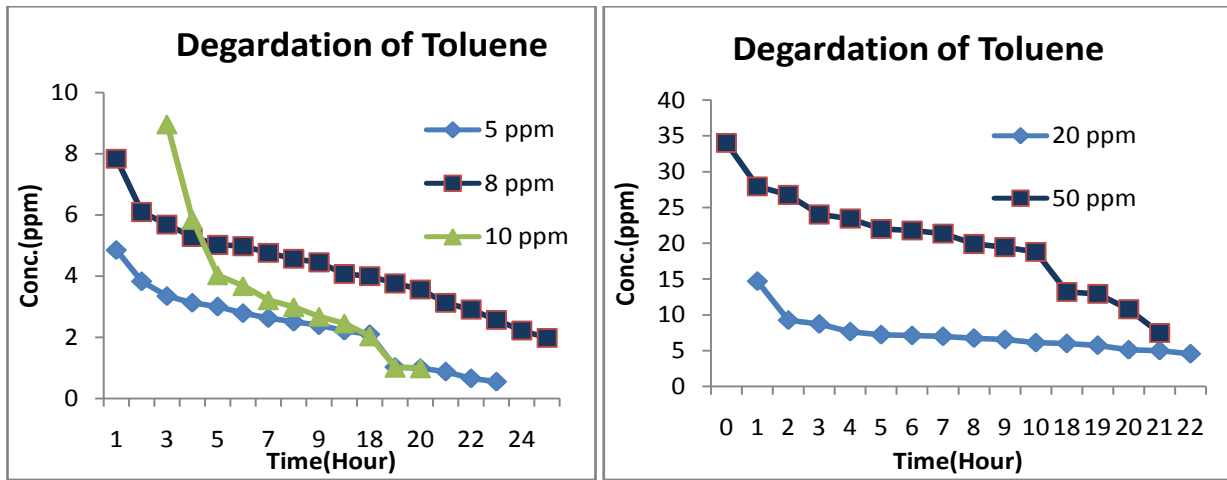


Figure 2: showing degradation of toluene at different concentration

Table2: Degradation rate at different initial substrate concentration

Initial Substrate Conc.(ppm)	Degradation Rate (ppm/hr)
5 ppm	1.02
8 ppm	1.74
10 ppm	4.12
20 ppm	5.45
50 ppm	6.86
100 ppm	7.08
150 ppm	3.77
200 ppm	2.71
250 ppm	2.26

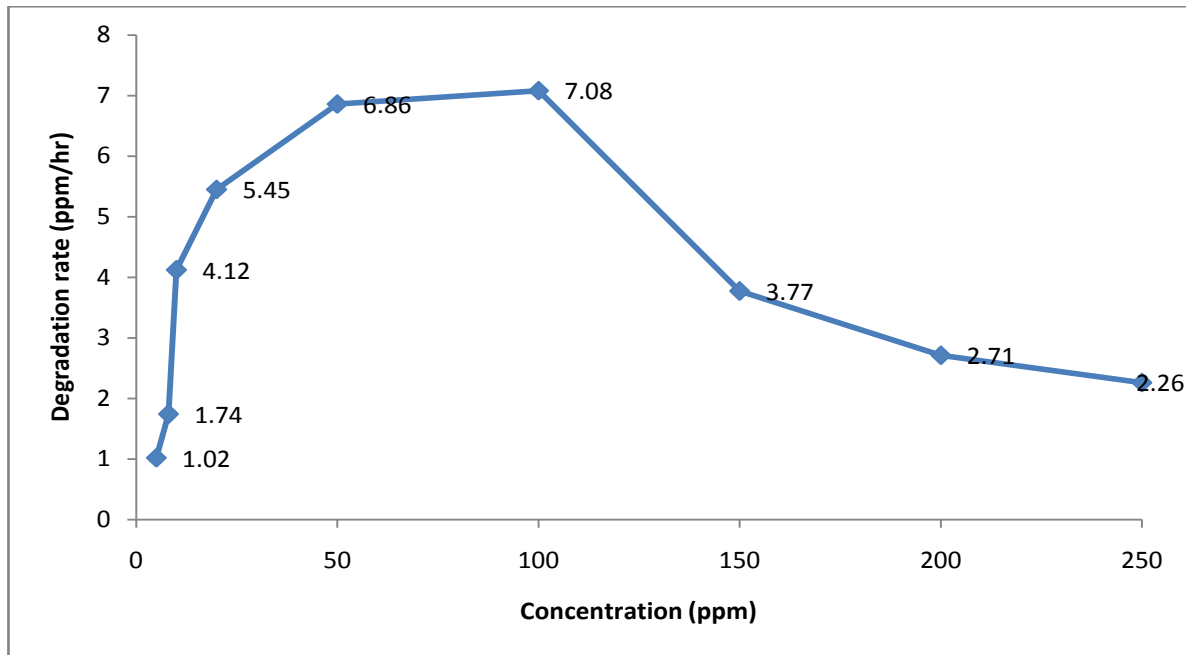


Figure 3: Initial substrate concentration versus degradation rate

5. Discussion:

In this case of different batch experiments were performed to know the degradation pattern at different initial substrate concentration. It has been observed that increase in the initial substrate concentration of toluene led to increase in the rate of degradation as it shows the minimum for 5 ppm that is 1.02 ppm/hr and showing the maximum rate of degradation for 100 ppm that is 7.08 ppm/hr after which with increasing initial substrate concentration there is a decrease in the rate of degradation of toluene as it is showing 2.26 ppm/hr for 250 ppm.

In all batches equal amount of soil- water was used with same amount of waste water for stimulating the growth of microbes. The toluene contaminated water having different population of micro-biota was used for reducing the lag time.

The high rate of degradation of toluene is justified by the use of waste water. The nutrients present in waste water stimulated the micro-biota in degrading toluene.

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