Bioremediation of trichlopyr butoxyethyl ester in surface soil treatment bioreactor

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Abstract

Trichlopyr Butoxyethyl Ester (TBEE) is a pyridine-based herbicide, which is widely manufactured and used in agriculture for controlling weeds. The residual TBEE has been found to cause contamination in the soil - water environment during agricultural applications or when partially treated wastes are disposed off. Natural biodegradation in the environment is a slow process, hence the degradation using cow – dung consortium has been carried out in the specially designed bioreactor at varying concentration viz. 25ppm, 50ppm and 100ppm respectively. The bioremediation conditions such as pH, temperature, moisture, DO and nutrients has been monitored & maintained and the degradation is carried out under aerobic conditions. The presence of inorganic in soil and cow dung biomass was found to be good source of nutrients for microbial growth and multiplication which in turn has effectively degraded TBEE under controlled environmental conditions at varying concentrations over a period of time. The technology developed for the treatment of TBEE contaminated surface soil would be an effective technique for bioremediation of pesticide wastes for detoxifying the environment.

Keywords: Trichlopyr Butoxyethyl Ester, Bioremediation, Cow – Dung Biomass

Introduction

In agriculture, only about 2% of the applied pesticides is actually used for preventing and controlling pests and weeds; rest find their ways into environment causing surface water / ground water pollution and soil contamination. The residual pesticide causes health hazards by entering into the food chain and even destroys microbial community, which is essential for maintaining the ecological balance. The natural biodegradation of pesticides takes longer time in soil – water environment.

Trichlopyr Butoxyethyl Ester (TBEE) is a pyridine-based herbicide used for control of woody and broadleaf plants in forests, industrial lands, and parks. TBEE is a clear amber colour liquid at room temperature having a molecular weight of 356.6. It is highly soluble in organic solvents whereas solubility in water is upto 6.8ppm only. TBEE has the tendency to strongly adsorb to soil and organic particle and is relatively immobile [1].

Chemical Structure of Trichlopyr Butoxyethyl Ester

The pesticides are found persisting in the surface soil either by present methods of disposal or during agricultural applications. Pesticides reaching the soil, sediment or water ecosystem can be degraded by biological agents. These hazardous compounds are disintegrated through oxidation, reduction and hydrolysis, the sum total of a biological phenomenon. Microorganisms are known to be the principal agents, which can cleave and modify the complex pesticide moiety. The biodegradation of TBEE in the soil environment is rapid. The major route of biodegradation is via hydrolysis by using microorganisms. Data shows that increase in temperature and moisture increases microbial activity, which in turn accelerates degradation of TBEE [2].

In this laboratory technique, a treatment unit has been designed for bioremediation of soil contaminated with TBEE using activated cow dung consortium. The degradation potential of microbial consortium towards TBEE and its intermediates is studied in this experiment. The aerobic bioremediation by activated microbial under controlled environmental conditions has proved to be an effective technique for biodegradation of TBEE in soil.

Materials and Methods

The technical grade Trichlopyr Butoxyethyl Ester (TBEE) has been procured from pesticide manufacturing industry for developing a technique for bioremediation of TBEE contaminated surface soil. The soil (alluvial) was collected from Palghar, 200 km away from Mumbai city. A Surface soil treatment Unit has been designed (22cm x 10cm x 6cm) for bioremediation. Cow dung mixed with water in the ratio of 1: 1 was filtered to remove suspended particles and was aerated and activated for a period of week. The soil and cow – dung slurry was characterized for physico – chemical [3,4] and microbial parameters. In the experimental set – up, TBEE was spiked to alluvial soil (1kg) at different concentrations viz. 25ppm, 50ppm and 100ppm in the respective surface soil treatment unit [5]. A control treatment unit was also set up which contained zero concentration of pesticides. The cow – dung slurry was added to TBEE contaminated soil as a source of biomass for bioremediation. A surfactant Tween 80 (0.05%) was added to the soil as a surfactant to prevent adsorption of TBEE to soil particles. The Bioremediation conditions were monitored and maintained in the surface soil treatment unit. Soil samples (10gm) were collected after every 24hrs hours over a period of seven days. The soil samples were dried at room temperature and the biodegraded compounds were extracted from soil into acetone by using Soxhlet Extraction assembly [6]. Soil sample extract was analyzed by Gas chromatography / mass spectroscopy (GC-MS) (Hewlett Packard GC-MS instrument Model No. G1800A) for TBEE and its intermediates. The instrument is equipped with electron ionization detector. Conditions maintained for the quantitative & qualitative analyses are: oven temperature – 100 ^{0}C, Injection temperature – 250 ^{0}C, detector temperature – 280 ^{0}C.

Results and Discussion

The use of pesticides has become an integral and indispensable part of modern agricultural practice. Their indiscriminate and injudicious use has led to serious environmental & health concern as pesticides are detected in soil - water environment at alarming concentration. Research regarding pesticide transformations in natural environment and under laboratory conditions has established the importance of biological degradation as a major mechanism of pesticide detoxification. Bioremediation methods for destruction of pesticides are being extensively explored and adopted instead of physico – chemical methods on account of their low cost, high efficiency and environmental friendliness. In the present laboratory investigation, a surface soil treatment unit has been designed wherein, technical grade Trichlopyr Butoxyethyl Ester (TBEE) was amended in alluvial soil at three different concentrations viz. 25ppm, 50ppm & 100ppm and bioremediation of TBEE is carried out using activated cow dung consortium. The soil and cow dung slurry were characterized for physico – chemical parameters. The soil was found to have the following conditions; pH = 7.6,Moisture= 4.5 %, alkalinity /100gms = 0.6meq, dissolved oxygen = 6 ppm, temperature = 26 ^{0}C, cation Exchange Capacity /100gms = 108meq, % organic carbon = 1.08, phosphorus = 0.25ppm, Kjeldahl nitrogen = 2100ppm, sulphate = 2.5ppm, COD = 220ppm and BOD = 470ppm. Likewise, the cow – dung slurry was also characterized for pH = 7.4, alkalinity /100gms = 1.2meq, dissolved oxygen = 9ppm, temperature = 28 ^{0}C, % organic carbon = 0.34, phosphorus = 0.78ppm, kjeldahl nitrogen = 8.6ppm, sulphate = 26ppm, calcium = 8.6ppm, chloride = 6ppm, potassium = 161ppm, sodium = 92.8ppm, magnesium = 147ppm, COD = 200ppm and BOD = 880ppm. The data shows presence of inorganic nutrients in cow – dung slurry and soil that served as a nutrient source for the growth of microbial consortium. The microbial characterization of cow – dung slurry and soil shows presence of a varied class of microorganisms. Microbial characterization of soil showed total viable microbial count/g as 1,920 and *Actinomycetes* count/g as 1,340. Presence of *Pseudomonas, S.aureus, Flavobacterium, Alcaligen, Bacillus, Serratia* and *Mucor* were detected whereas the microbial characterization was cow – dung slurry showed total viable microbial count/g as 65 x 10^9 , total coliform count /g as 189 x 10^7 , total Yeast & Mould count/g as 72 x 10^3 , *Pseudomonas* count/g 59 x 10^3, *Actinomycetes* count/g as 83 x 10^4, *E.coli* count/g as 23,600. Presence of

Streptococcus, Sarcina, Serratia, Nocardia and *Mucor* were found. The presence of nutrients as well as a diverse class microorganisms in cow – dung slurry and soil has been found responsible for the bioremediation of surface soil contaminated with TBEE at varied concentrations.

The bioremediation conditions like pH, moisture, temperature, dissolved oxygen and nutrient level (C: N: P) were monitored and maintained in surface soil treatment unit. Variation in bioremediation conditions of surface soil contaminated with TBEE during bioremediation was pH = 6.5 – 8.0, temperature = 25 – 28 ^{0}C, moisture = 60 – 80%, dissolved oxygen = 10 – 12ppm and the C:N:P ratio was maintained in the range of 100:10:1.Variation in COD and BOD during the course of bioremediation of TBEE amended surface soil has been observed. The research data shows that COD has direct correlation with the performance of microorganisms in biodegrading toxic compounds [7]. Changes in COD of TBEE - amended soil during the bioremediation in a surface soil treatment unit is shown in figure 1. The decrease in COD with increasing duration of bioremediation was observed. The percentage decrease in Chemical Oxygen Demand (COD) measured during the bioremediation shows 61.8% COD reduction in the case of 25ppm TBEE amended soil, 55.5% COD reduction for 50ppm TBEE amended soil and 50.3% reduction in the COD for 100 ppm TBEE amended soil while in control soil the percentage COD decrease was around 64.5% (Fig 1). Figure 2 shows the variation in BOD of the experimental soil. Variation in the biological oxygen demand of the soil indicates activity of microorganisms during bioremediation. Similarly, percentage variation in Biological Oxygen demand (BOD) measured during bioremediation shows 6.05% decrease in BOD in the case of 25ppm TBEE amended soil, 7.39% decrease in BOD in the case of 50ppm TBEE amended soil and 8.11% decrease in BOD in the case of 100ppm TBEE amended soil, while in control soil the percentage increase in BOD was 4.5% (Fig 2). The decrease in BOD concentration is attributed to the consumption of oxygen by the microflora for their growth and maintenance, which are primarily responsible for biodegradation of TBEE.

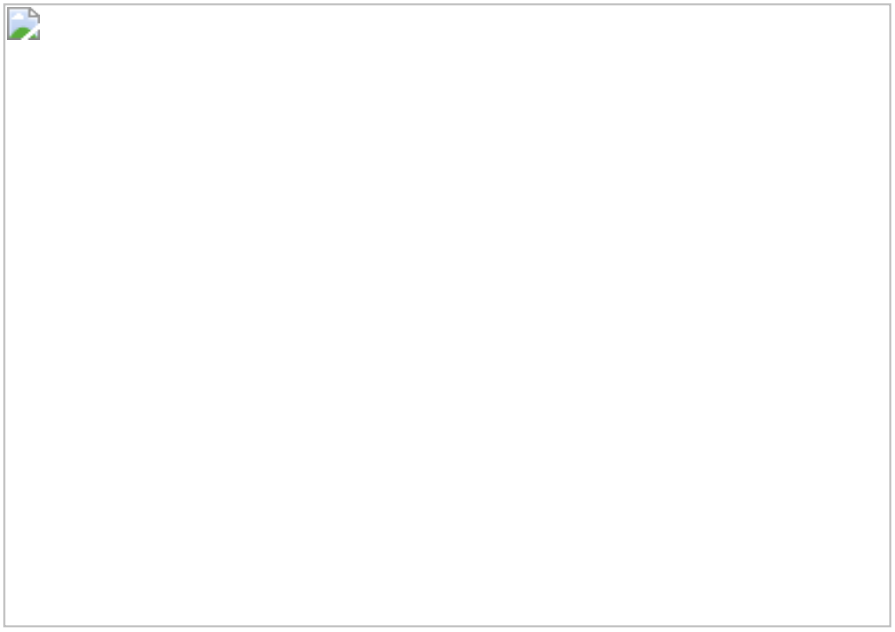

Figure 1. Variation in COD during Bioremediation of TBEE-amended soil

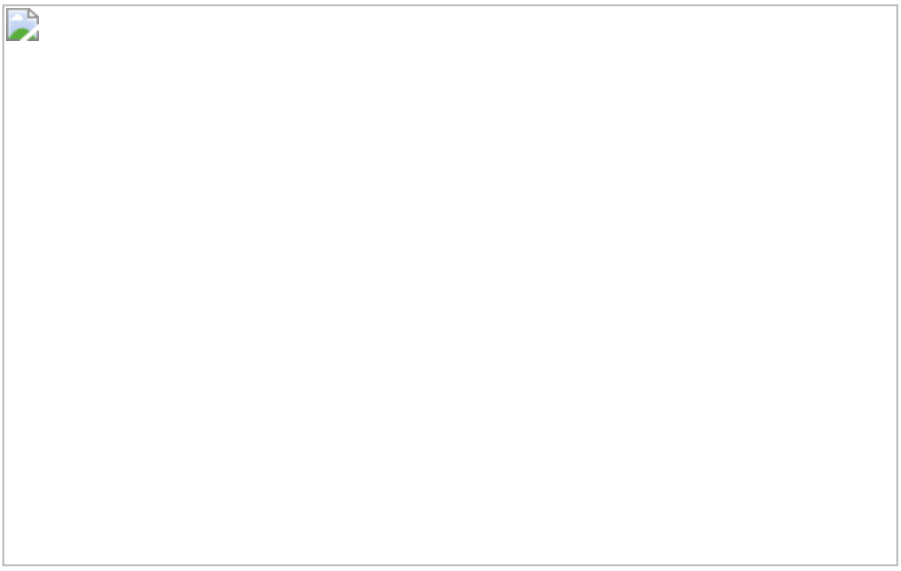

Figure 2. Variation in BOD during Bioremediation of TBEE amended soil

The concentration of TBEE and its intermediates, degradation pattern and pathway during the course of bioremediation of TBEE contaminated surface soil at 25ppm, 50ppm and 100ppm were studied. It is evident from the GC – MS data that TBEE was rapidly broken down into trichlopyr acid via hydrolysis of the ester functional moiety (Table 1 & Fig 3). It was observed that hydrolysis and reduction reactions were the principal mechanisms occurring during the course of bioremediation of TBEE in surface soil treatment unit. The compounds viz. trichlopyr acid and 3,5,6 trichloro pyridinol were found to be the principal metabolites of TBEE biodegradation. In the treatment unit containing 25ppm, 50ppm and 100ppm TBEE contaminated soil respectively, TBEE has been completely biotransformed into trichlopyr acid within 24 hours. An investigation done by Bidlack also states that TBEE disintegrates rapidly into Trichlopyr acid by virtue of hydrolysis with a half-life of three hours [8]. In 100ppm TBEE contaminated soil trichlopyr acid and 3,5,6 trichloro pyridinol (TCP) were found partially degraded and accumulated in soil. Studies carried out by Baskaran. et al. also states that primary metabolite TCP persist for longer duration in soil [9]. Research data shows that TCP will eventually convert to CO_2 [10,11]. Benzene derivatives like 3 methyl Phenol, 1,1-Dimethylethyl benzene and 4 methyl benzene have been formed during the later stages of bioremediation in 25ppm and 50ppm TBEE contaminated soil which are breakdown products of TCP. These would be mineralized further into nutrient, biomass & inorganic on sufficient acclimatization. Thus, activated cow dung biomass has different impact on different concentrations of TBEE under the same environmental conditions. Therefore, the variation in the bioremediation of intermediates and the persistence of some compounds during the bioremediation. The pathway identified for TBEE degradation is demonstrated in Figure 3.

Table 1. Concentration of Trichlopyr Buyoxyethyl ester (TBEE) and intermediates analyzed every day during the bioremediation in surface soil treatment unit.

100 PPM TBEE amended surface soil								
	0^{th} Day	1^{st} Day	2^{nd} Day	3^{rd} Day	4^{th} Day	5^{th} Day	6^{th} Day	7^{th} Day
A	68	31	22	20	20	19	17	17
B	-	29	34	26	22	22	20	19
C	-	0	12	16	20	24	24	22
D	-	-	Trace	5	-	-	-	-
50 PPM TBEE amended surface soil								
A	34	19	15	15	13	11	10	10
B	-	16	21	18	15	15	12	10
C	-	5	8	11	10	10	9	7
D	-	-	Trace			-	-	-
E	-	-	-	-	5	8	Trace	-

	-							
25 PPM TBEE amended surface soil								
A	16	12	12	10	10	8	8	8
B	-	6	8	8	7	6	6	5
C	-	Trace	Trace	5	5	Trace	Trace	Trace
E	-	Trace	4	4	-	-	-	-
F	-	-	5	Trace	-	-	-	-
G	-	-	-	5	5	5	5	5

A Trichlopyr butoxy ethyl ester
B Trichlopyr acid
C 3,5,6 trichloro pyridinol
D 2,4,6 Trichloro Benzeneamine
E Phenol, 3-methyl
F 1,1-Dimethylethyl, benzene
G 4 methyl, benzene

Trichlopyr Butoxyethyl Ester (TBEE)

Hydrolysis

Trichlopyr Acid

Reduction

3,5,6 Trichloro pyridinol (TCP)

Microbial acclimatization

Nutrient, biomass, inorganic and CO_2

Figure 3. Bioremediation pathway of Trichlopyr Butoxyethyl Ester in Surface Soil Treatment Unit

Conclusions

The surface soil treatment unit developed for the bioremediation of TBEE contaminated surface soil at varying concentration under controlled environmental conditions has been found as an effective treatment technology for pesticides. The present research finding shows that TBEE is biodegraded into intermediates like trichlopyr acid, TCP, 3 methyl Phenol, 1,1-Dimethylethyl benzene and 4-methyl benzene during laboratory experiment. The longer exposure in nature will convert these intermediates into biomass, inorganic nutrients and CO_2. The present technology developed would be an effective treatment technique applicable to pesticide industry for treatment of pesticide wastes and detoxifying the environment.

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