ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1401-1404

ORIGINAL RESEARCH ARTICLE

Biodegradation of Pharmaceutical Wastes Using Different Microbial Strains

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Received 25 Jun 2011; Revised 01 Oct 2011; Accepted 12 Oct 2011

ABSTRACT

The enormous amount of the pollutants being dumped constantly from the various industries causes severe damages to the environment. Among various components released from different industries, the soluble products like benzene and toluene appears to be more hazardous than other insoluble products present in the wastewater. This hazardous pollutant can be removed by various conventional methods like removal, alteration and isolation of pollutants. However these technologies are more expensive; do not completely destroy rather transforming them form to another. As an alternative, Bioremediation is used to eliminate the above contaminant completely from the environment with very low operation cost. There are many microbial species, which are found to be efficient in the degradation of benzene and toluene. Among those populations, *Pseudomonas fluorescence* and *Bacillus megatherium* and fungi such as *Aspergillus* species, *Rhizopus* species and *Penicillium* species were proven to degrade the above pollutants. These organisms were found to grow in the medium supplemented with 100ppm of the benzene and toluene. The results recorded were significant and tolerant.

Key words: Pharmaceutical, waste water, benzene, toluene and biodegradation

INTRODUCTION

Benzene and toluene isomers are major constituents of gasoline (petroleum) [1,2] and also serve as important industrial solvents [3]. They are used as fuels and solvents at the rate of millions of metric tons per year, and are among the seventeen most hazardous chemical pollutants listed in the Toxic Release Inventory of the USEPA [4]. Leaky storage tanks and other inadvertent releases place benzene and toluene compounds among the most frequently detected hazardous compounds in industrially polluted groundwater and soil [5]. Dumping of these chemicals in huge amount in the environment leads to the serious irreparable damages in the environment by means of biomagnifications [6]. Remediation by air stripping of groundwater [7] and venting of soils [8] both produce waste air streams that require further treatment.

The development in the field of biotechnology has led to the close focus on the environmental pollution under degradation by microorganisms. There are many industries, which work for the removal of many hydrocarbons through by bioremediation process. The various study conducted on the degradation of benzene and toluene suggest that the rate of degradation is not significant when single organisms used as inoculum. It is also known that use of microbial populations of various substrate specificity enhances the rate of degradation significantly [9, 10]. It is also studied that the maximum ability of degradation and the rate at which occur depends on interaction between environment, number and type of microorganisms present and chemical nature of the contaminants to be degraded.

Among various solvents, benzene and toluene is used extensively in the pharmaceutical industries and petrochemical industries. As well as it's also present in many industrial effluents as a common pollutant. Moreover, the presence of these pollutants in the environment appears to be serious health hazardous to the human beings as well as aquatic forms. It acts as a depressant for central nervous system and even causes mental retardation. This paper aims to examine on the biodegradation of pharmaceutical wastes

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containing benzene and toluene under aerobic conditions.

MATERIALS AND METHODS

Collection of the samples

Owing to isolate, the efficient strains of benzene and toluene degrader from the phytochemical industry situated at near Mettur, Salem District, Tamilnadu was chosen. The wastewater samples were collected from the many streams in sterile BOD bottles aseptically and it was kept in an icebox immediately. The bottles were brought to the laboratory within 6 hours of sample collection. The physicochemical parameter like pH, temperature, acidity, alkalinity and COD were analyzed.

Isolation of benzene and toluene

In order to isolate the efficient degrader minimal mineral salt agar medium and Rose Bengal agar medium prepared by supplementing with 0.01% of benzene and toluene for the isolation of bacterial and fungal species respectively. One gram of contaminated soil sample was taken from various pollutant environments and diluted with 100ml of sterile distilled water.

Then it was diluted up to 10-6 dilutions. From the each dilution 0.1 ml were pipette out and inoculated on the above said two medium by means of spread plate techniques. The seeded plates were incubated at 28°C for 24 hours. The plates were observed for the growth of microbial species at the end of incubation period.

Analyzing the ability of benzene and toluene degradation

The isolates obtained by the above procedures were tested to ability to degrade maximum concentration of benzene and toluene. The minimal broth and Rose Bengal medium were supplemented with varying concentration like 10ppm to 100ppm of benzene and toluene. The test tubes were incubated at 28° C for 20 days and observed for microbial growth at the intervals of 2 days.

RESULTS

The analysis of biochemical properties of wastewater showed that the pH 5.6, Temperature 22^0C and COD- 89,000ppm (**Table 1 & 2**). The bacterial species isolated includes *B.megatherium*, *P.flourescense,* and fungi species includes *Pencillium* Species, *Rhizopus* Species and *Aspergillus* Species illustrated in (**Table 3-7**).

The analyzing of the ability of benzene and toluene degradation showed only up to 100ppm concentration. The growth of benzene and toluene degraded were depicted in the table 3-7. It was clearly indicated that the steady growth up to 100ppm concentration. The growth curve showed short lag phase during the first two hours and increased exponentially later.

In the present study, it was aimed to remove benzene and toluene from the wastewater to the harmless level. The Bioremediation of wastewater using different strains of hydrocarbon degrader has shown significant reduction in acidity, alkalinity and COD along with removal of benzene and toluene. It was recorded that benzene and toluene was removed completely from the environment with 20 days of incubation. There was maximum growth at 20 days of incubation. Change in the pH of wastewater after the treatment was significant. Acidic nature of the pH was changed to neutral. In the present study it was recorded that COD reduced from 89,000ppm to 600ppm/litre.

Table 1: Influence in the pH of the Wastewater due to Microbial Inoculum

S. No	Time intervals (Hrs.)	pH of the Waste water
1	0	5.2
2	2	4.8
3	4	4.7
4	6	4.6
5	8	4.8
6	10	5.6
7	12	6.4
8	14	6.8
9	16	7.0
10	18	7.0
11	20	7.0

Table 2: Impact of Bacterial inoculum in the Reduction of COD of the wastewater

S. No	Time intervals (Hrs.)	COD ppm/Lit.
1	0	89,000
2	2	87,310
3	4	70,010
4	6	63,740
5	8	40,150
6	10	22,300
7	12	8,040
8	14	5,320
9	16	4,920
10	18	1,025
11	20	600

Table-3: Ability of *Psuedomonas flourescense* to grow on the various concentrations of Benzene and Toluene

S. No	Time interval	Bacterial growth at 580nm		
	(Hrs)	50 ppm	75ppm	100ppm
1	0	0	0	0
2	2	0.04	0.04	0.06
3	4	0.17	0.28	0.35
4	6	0.23	0.5	0.7
5	8	0.26	0.58	0.73
6	10	0.4	0.8	1.16
7	12	0.42	0.81	1.23
8	14	0.5	1.0	1.44
9	16	0.7	1.46	2.04
10	18	1.0	1.8	3.2
11	20	1.1	2.17	3.2

Table-4: Ability of *Bacillus megatherium* to grow on the various concentrations of Benzene and Toluene

S. No	Time interval (Hrs)	Bacterial growth at 580nm		
		50 ppm	75ppm	100ppm
1	0	0	0	0
2	2	0.03	0.05	0.08
3	4	0.15	0.22	0.37
4	6	0.21	0.39	0.68
5	8	0.23	0.49	0.89
6	10	0.37	0.76	1.08
7	12	0.45	0.86	1.16
8	14	0.52	1.15	1.34
9	16	0.68	1.34	2.58
10	18	0.95	1.69	3.1
11	20	1.12	1.98	3.3

Table-5: Ability of *Aspergillus* species to grow on the various concentrations of Benzene and Toluene

S. No	Time interval (Hrs)	Bacterial growth at 580nm		
		50 ppm	75ppm	100ppm
1	0	0	0	0
2	2	0.01	0.03	0.05
3	4	0.14	0.20	0.31
4	6	0.20	0.38	0.62
5	8	0.28	0.56	0.92
6	10	0.40	0.69	1.02
7	12	0.49	0.82	1.15
8	14	0.60	1.08	1.26
9	16	0.86	1.28	2.49
10	18	1.15	1.74	3.19
11	20	1.39	2.36	3.89

Table-6: Ability of *Rhizopus* species to grow on the various concentrations of Benzene and Toluene

S. No	Time interval (Hrs)	Bacterial growth at 580nm		
		50 ppm	75ppm	100ppm
1	0	0	0	0
2	2	0.01	0.03	0.08
3	4	0.10	0.21	0.32
4	6	0.20	0.32	0.61
5	8	0.27	0.44	0.84
6	10	0.32	0.71	1.07
7	12	0.41	0.82	1.11
8	14	0.56	1.02	1.30
9	16	0.67	1.22	2.53
10	18	0.90	1.58	3.13
11	20	1.10	1.79	3.35

Table-7: Ability of *Penicillium* species to grow on the various concentrations of Benzene and Toluene

S. No	Time interval (Hrs)	Bacterial growth at 580nm		
		50 ppm	75ppm	100ppm
1	0	0	0	0
2	2	0.05	0.08	0.15
3	4	0.10	0.29	0.45
4	6	0.28	0.36	0.72
5	8	0.29	0.46	0.94
6	10	0.39	0.63	1.01
7	12	0.47	0.98	1.25
8	14	0.59	1.20	1.48
9	16	0.67	1.59	2.24
10	18	0.92	1.73	3.07
11	20	1.29	1.92	3.22

DISCUSSION

The toluene can serve as substrate for different species [11]. The study also emphasized that both benzene and toluene can act as a sole carbon source. Our results were also strongly correlated with Worsey and Williams [11]. The *Pseudomonas* can grow in the benzene contaminated environment predominantly [12]. Another study conducted by Babu *et al.* [13], isolated *Pseudomonas marginalis* from the contaminated water sample.

The reasons for having maximum growth in the test tube supplemented with 100ppm of benzene and toluene were due to the ability of microbes to breakdown the both components. The removal of chemical contaminants from soil and ground water is feasible by using microorganisms having the ability to use them as a sole carbon source [11]. The plasmid was responsible for the degradation of toluene and benzene [14]. The research further reported that plasmids encode the catabolic enzymes able to degrade both benzene and toluene. Many organisms are known to utilize hydrocarbons as a growth substance [15]. The specific gene isolated and found to encode enzyme degrade benzene and toluene [16]. The exact mechanism of toluene degradation could be by the incorporation of molecular oxygen into the aromatic nucleus to form cis 1, 2, dihydoxy 3 methyl hexane [17]. The reduction of the pH is being kept in the growth medium, which produce organic acids [18]. But soon after it was increased in the pH, could be due to the degradation of organic acids by mixed populations. Hence the present study reported that increased biomass in the environment is able to reduce the COD in the greater extend.

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