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Bioremediation of benzene, toluene and o-xylene by cow dung microbial consortium

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ABSTRACT

Objective: Bioremediation of Benzene, Toluene and o-Xylene (BTX) by cow dung microbial consortium using batch scale process.

Methodology and results: Cow dung microbial consortium collected from cow shed and continuously aerated at the rate of 0.9 - 1.2 m^3/h was used as in bioremediation of BTX in batch scale process under controlled environmental conditions of mesophilic temperature (25 – 27°C) and neutral pH (7.4 - 6.6). Benzene and Toluene were separately bioremediated at 100, 250 and 500 mg l^{-1} whereas o-Xylene was bioremediated at 50, 100 and 250 mg l^{-1}. The bioremediated compounds were assayed hourly for up to 6 h and thereafter every 24 h up to a period of 168 h. Benzene and Toluene were degraded 100% at a concentration of 100 mg l^{-1}, whereas o-Xylene was degraded 97% at a concentration of 50 mg l^{-1}. When Benzene was bioremediated at 250 and 500 mg l^{-1} the degradation was 67.5 and 9%, respectively. Toluene was biodegraded by 75 and 10 % at an exposure of 250 and 500 mg l^{-1}, respectively. In case of o-Xylene the degradation was 75.9 and 10 % for concentrations of 100 and 250 mg l^{-1}, respectively. The rate of decrease in chemical oxygen demand (COD) was directly proportional to the bioremediation of each compound studied. The decrease in biological oxygen demand (BOD) level in case of lower concentrations of contaminant also indicates the potential of cow dung microbial consortium to degrade the BTX.

Conclusion and application of findings: The present study demonstrates the potential of cow dung microbial consortium in the biodegradation of BTX in a batch process under controlled environmental conditions. The technology developed in this pilot scale study can be effectively applied in petrochemical and other chemical industries for treatment of toxic wastes.

Key words: Bioremediation, Benzene, Toluene, o-Xylene, Cow dung microorganisms

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INTRODUCTION

Technological revolution has brought new changes in industrial products and processes, and the waste generated is of concern to environmentalists. The present treatment technologies do not efficient nor effectively treat the contaminants to the acceptable level as per the Environmental Protection Act, 1986 (India). Accelerated growth of industrial activities has increased hazardous waste, with the main sources of contaminants being the chemical industries, e.g. petrochemical industry which involves the production of several chemicals, basic raw materials, and key intermediates, and generates complex hazardous waste. The organic

compounds commonly found in the waste include benzene, toluene, and xylene (BTX), which are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA) (Eriksson *et al*., 1998). The EPA estimates that approximately 11 million gallons of gasoline are lost per year due to leaking underground storage tanks (Barbaro *et al*., 1992). Human and environmental exposure to BTX is a global environmental problem.

Conventional methods of treatment of volatile organic wastes have been largely physical or chemical but these can also lead to secondary effluent problems. The ecologically acceptable disposal of organic waste remains a major challenge to the petrochemical industry. Recent advances in bioremediation technology for the treatment of contaminant are significant because new innovation in waste treatment technology is more effective.

Bioremediation is an increasingly popular alternative to conventional methods for treating waste compounds with the possibility to degrade contaminants using natural microbial activity. This method uses relatively low-cost techniques, which generally have a high public acceptance and can often be carried out on site (Vidali, 2001; Fulekar, 2005). The present research studied the bioremediation of organic compounds such as BTX by activated cow dung microbial consortium in a batch process under controlled environmental conditions.

MATERIALS AND METHODS

Chemicals: The benzene, toluene, o-xylene and n-hexane were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai (India). All salts used as nutrients were obtained from Himedia Laboratories Pvt. Ltd Mumbai (India).

Microbial biomass preparation and growth medium: The cow dung taken as a source of biomass was diluted with water in the ratio 1:25 and filtered through sieve (20µm) to remove suspended particles. The prepared cow-dung slurry was aerated (continuous aeration at the rate of 0.9 - 1.2 m3/h) and activated in a glass vessel for a week (Singh *et al*., 2007). The physico-chemical (Jackson, 1973) and microbial characteristics of the cow dung were determined after the activation process. Basal salt medium $(NH_4)_2 SO_4$ - 0.1 mg/ml, dextrose - 0.2 mg/ml, K_2HPO_4 - 0.1 mg/ml, KH_2PO_4 - 0.1 mg/ml, was added as a source of inorganic nutrients (C: N: P) for growth and proliferation of microbial biomass.

Experimental setup for BTX degradation: The biodegradation of benzene, toluene and o-xylene was performed in 160-ml capacity glass bottles containing 100 ml of the activated cow dung slurry, kept at 25 °C and continuous shaking at 100 rpm (Arvin *et al*., 1989). The glass bottles were tightly sealed with Teflon-coated rubber septa and screw cap to prevent the release of BTX by evaporation (Lee *et al*., 1995). In a lab setup 100, 250 and 500 mg l^{-1} concentrations of benzene and toluene; and 50, 100 and 250 mg l^{-1} concentration of o-Xylene, were added into activated cow-dung slurry.

Biodegradation was assessed by comparing the disappearance of BTX in sample compared to the controls over time. Sterile controls containing benzene, toluene and o-xylene were prepared for each concentration to determine volatilization and adsorption losses. BTX concentrations were monitored over time in order to compare lag periods and biodegradation rates for different concentrations. The lag period was determined as the time during which BTX concentrations remained relatively constant. This was followed by an abrupt decrease in BTX concentrations relative to the sterile controls. The bioremediation conditions were monitored through out the experiment. Initially, samples (10 ml) were withdrawn hourly for up to 6 h; and afterwards every 24 h over a period of 168 h (7 days). Samples were transferred to 10 ml capacity vials and capped with Teflon-coated septa prior to high-performance liquid chromatograph (HPLC) analysis.

Sample preparation and analytical procedure: Samples were centrifuged (10 min, 10000 rpm) to separate cell mass and the supernatant. The samples were extracted in organic solvent (n-hexane) for analysis. The extracted samples were injected in a high-performance liquid chromatograph system (HPLC, model- Jasco LC 2000 plus, Japan) equipped with a UV-VIS Detector and C – 18 column. The samples were analyzed using the following programme: mobile phase Acetonitrile-water 75: 25, wavelength 254 nm, flow rate 1 ml / min, isocratic run for 10 min (Lee *et al*., 1995).

RESULTS AND DISCUSSION

Cow dung is basically the digested residue of herbivorous matter which is acted upon by symbiotic bacteria residing within the animal's rumen. The resultant fecal matter is rich in minerals and contains diverse classes of microorganisms. The physicochemical characterization of cow dung microbial consortium (table 1) indicates the presence of C, N, P, K, S, Ca, in high concentration and these served as nutrients to the microbial consortium. The activated cow dung microbial consortium included bacteria, fungi and actinomycetes, e.g. *Pseudomonas* sp., *Streptococcus* sp., *Sarcina* sp., *E.coli* sp., *Penicillium* sp., *Rhizopus* sp., *Mucor* sp. and *Nocardia* sp. (table 2).

Table 1: Physico–chemical characterization of activated cow dung slurry.

Parameter	Content/Unit *	Parameter	Content/ Unit *
pH	7.4	Phosphorus	0.13 mg l^{-1}
Dissolved Oxygen	6.3 ppm	Kjeldahl Nitrogen	14 mg l^{-1}
Temperature	25.4 ^{o}C	Sulphate	34 mg l^{-1}
% Organic Carbon	0.31%	Calcium	9.8 mg l^{-1}
Biological Oxygen demand (BOD)	9.50 mg l^{-1}	Chloride	6 mg/l
Chemical Oxygen demand (COD)	184 mg l^{-1}	Potassium	152 mg/l
Total viable count /100 ml	104 X 10^{7}	Sodium	89.7 mg/l
		Magnesium	143 mg/l

* Values indicates the average of triplicate samples

Table 2: Constituents of the cow dung microbial consortium.

Bacteria	Fungi	Actenomycetes
Pseudomonas sp.	*Penicillium sp.*	*Nocardia sp.*
Streptococcus sp.	*Rhizopus sp.*	
Sarcina sp.	*Mucor sp.*	
E.coli sp.	*Aspergillus sp.*	

During biodegradation the pH varied between 7.4 to 6.6 (table 3), which is desirable since biodegradation rate is highest at near neutral pH (Salleh *et al.,* 2003). Other environmental parameters that could affect bioremediation, e.g. temperature, dissolved oxygen and macro nutrients (C, N, P) were also monitored throughout the experiment. During the bioremediation process temperature increased from 25 to 27 °C, which tallies with previous findings (Okoh, 2006), that the rate of bioremediation increases with increasing temperature. He reported the highest rate of bioremediation in aqueous environment at 20 - 30 °C.

Dissolved oxygen was observed to decrease during bioremediation, which is a likely indicator of growth and proliferation of microorganisms. The organic carbon (%) decreased from 0.46 to 0.34% during the experiment. The decrease in % organic carbon is one of the key indicators used in bioremediation process.

The observed decrease in BOD (difference in initial and final concentrations of dissolved oxygen) is primarily due to the consumption of oxygen by the microbial biomass for their growth and proliferation. BOD decreased in case of 100 and 250 mg l^{-1} of benzene and toluene; and 50 and 100 mg l^{-1} of o-xylene, whereas BOD increased at 500 mg l^{-1} of benzene and toluene, and 250 mg l^{-1} of xylene. Increased BOD at the higher concentration levels of the chemicals suggests that growth of microorganisms is inhibited at this concentration. The results indicate that decrease in BOD was proportional to the growth of microorganism upto lower concentrations of contaminants thereafter the BOD increased at higher concentrations (Fig. 1, 2, 3). The decrease in COD was directly proportional to the rate of degradation of the compound studied. The decrease in COD (oxygen concentration) with increasing time duration was observed at 50, 100 and 250 mg l^{-1} of BTX (Fig. 4, 5, 6). There were no significant changes observed in COD at the higher concentrations (500 mg l^{-1} for benzene and toluene; and 250 mg l^{-1} for o-xylene) of

contaminants which indicate that these concentrations inhibit cow dung microbial consortium.

Table 3: Variation of environmental conditions during bioremediation.

Parameters	Before bioremediation*	After bioremediation *
pH	7.4	6.6
Temperature	25 °C	27 °C
DO	6 mg l^{-1}	3 mg l^{-1}
COD	196 mg l^{-1}	148 mg l^{-1}
BOD	9.2 mg l^{-1}	4.35 mg l^{-1}
% Organic Carbon	0.46 %	0.34 %
Phosphorus	0.41 mg l^{-1}	0.22 mg l^{-1}

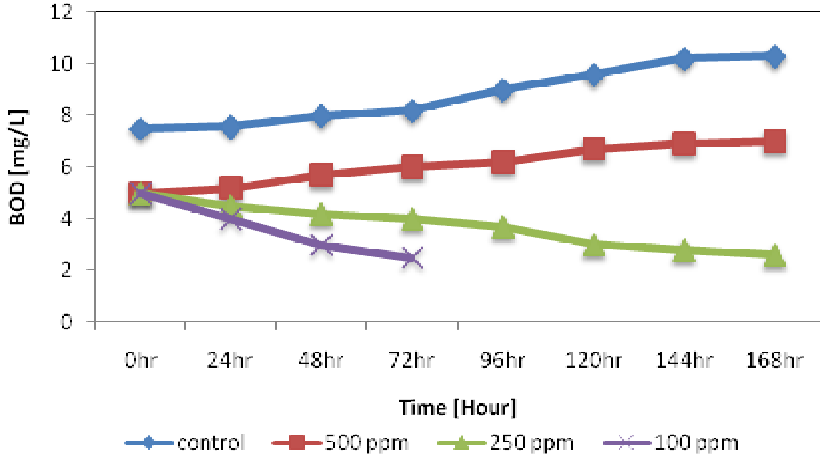
COD: Chemical oxygen demand, BOD: Biological oxygen demand, DO: Dissolved oxygen
'* Values indicate average changes.

Analyses of the compounds from the biodegradation of BTX indicates that in case of 100 mg l^{-1} benzene, degradation started within 4 h and was degraded below the limit of detection within 72 h (Fig. 7). Clearly, the lag period at this concentration of benzene is very short. At 250 mg l^{-1} of benzene, degradation started after 24 h and 67.5 % degradation had occurred over the 168 h experimental period.. Higher concentration of benzene (500 mg l^{-1}) was relatively toxic to the cow dung microbial consortium and very little decrease in concentration was observed up to 168 h (corrected for volatilization and adsorption losses). The biodegradation rates were about 1.4 and 1.1 mg $l^{-1}hour^{-1}$ for 100 and 250 mg l^{-1} benzene, respectively. Biodegradation rates were estimated as the ratio of BTX removed (corrected for sterile controls) to the corresponding time after the lag period (Alvarez, 1991).

At 100 mg l^{-1} toluene degradation started after 6 h and degraded below detection limit within 72 h (fig. 8). The lag phase was considerably longer (24 h) at 250 mg l^{-1} and 75 % was degraded within 168 h. Higher concentration of toluene (500 mg l^{-1}) was also inhibitory to cow dung microorganism. The biodegradation rates for 100 and 250 mg l^{-1} toluene were 1.5 and 1.56 mg $l^{-1}hour^{-1}$, respectively. Toluene degraded relatively at higher rate than benzene.

Xylene has been reported to be the most recalcitrant among the BTX compounds (Alvarez *et al*., 1991), and this is supported by our findings. At 100 mg l^{-1} xylene was only partially degraded after 168 h. Therefore biodegradation study was carried out with reduced concentration of 50 mg l^{-1}. At this level, a decrease in concentration of o-xylene was observed after 24 h (Fig. 9), which is a relatively long lag phase, and almost 97 % was degraded in 72 h. At 100 mg l^{-1} xylene degradation started in 48 h and 75.9 % was degraded within 168 h. Higher concentration of xylene (250 mg l^{-1}) was inhibitory to cow dung microorganisms and was not degraded at all for the entire 168 h period. At 100 mg l^{-1} o-Xylene degraded at a slower rate (0.63 mg $l^{-1}hour^{-1}$) compared to 1.4 mg $l^{-1}hour^{-1}$ for.benzene and 1.5 mg $l^{-1}hour^{-1}$ for toluene. At 50 mg l^{-1}, o-Xylene was degradation at a rate of 2 mg $l^{-1}hour^{-1}$. Since the microorganisms present in cow dung slurry were not earlier exposed to BTX compounds, a lag period was immediately observed after exposure, which signifies the acclimatization period when microorganisms adapt to the stressing environment. This study has shown that cow dung microorganisms can efficiently biodegrade BTX. Benzene and toluene were observed to be less recalcitrant and toxic than o-xylene as as they degraded at relatively higher rates, and were also less inhibitory even at higher conecentrations. The order of biodegradation was Toluene > Benzene > o-Xylene.

The results of this study demonstrate that cow dung is a potential source of highly effective microbial consortia that can be used in bioremediation of hazardous compound. This potential should be exploited to enhance removal of hazardous compounds from the environment.



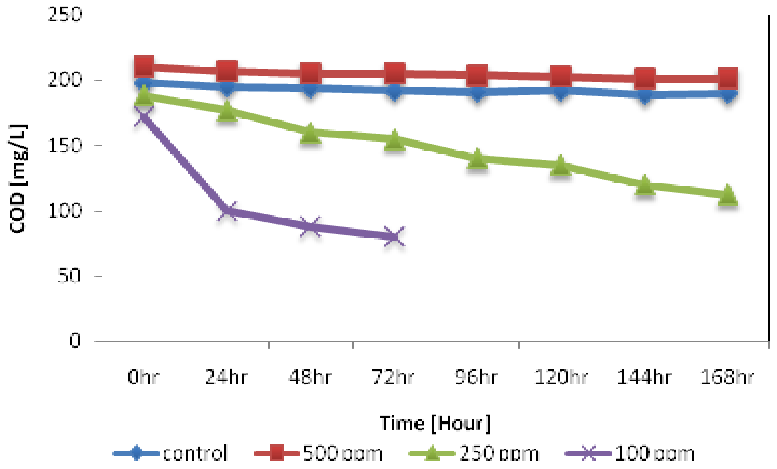
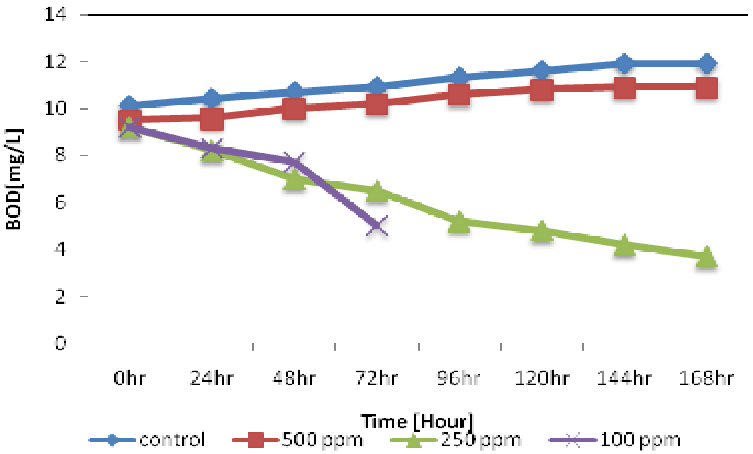


Figure 1 (left): Variation in Biological Oxygen Demand (BOD) during bioremediation of benzene by cow-dung microbial consortium; Figure 2 (right): Variation in Biological Oxygen Demand (BOD) during bioremediation of toluene by cow-dung microbial consortium.



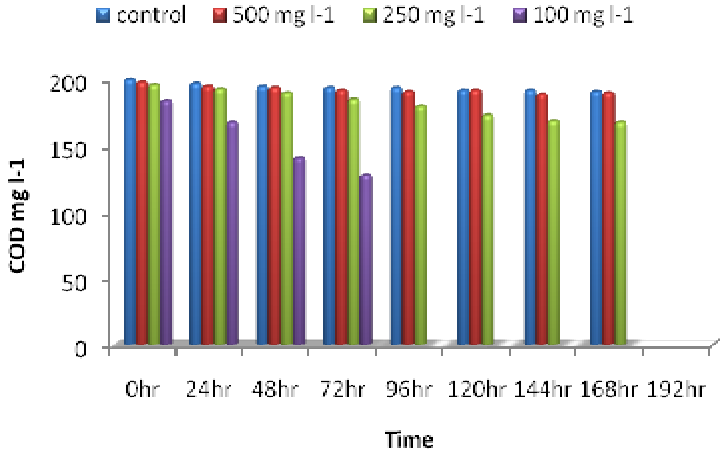
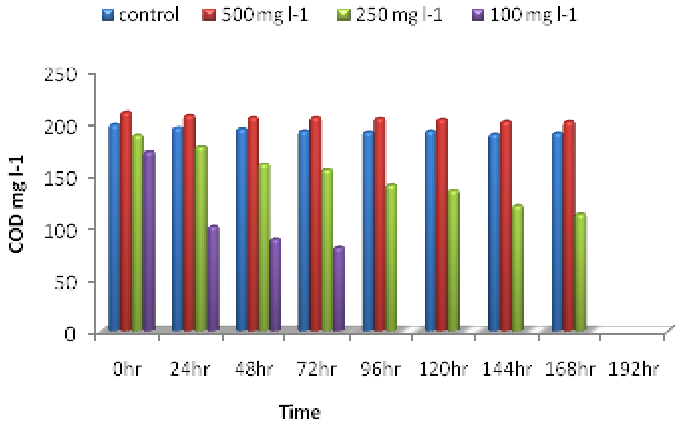


Figure 3 (left): Variation in Biological Oxygen Demand (BOD) during bioremediation of o- Xylene by cow-dung microbial consortium; Figure 4 (right): Variation in Chemical oxygen Demand (COD) during bioremediation of Benzene by cow-dung microbial consortium.



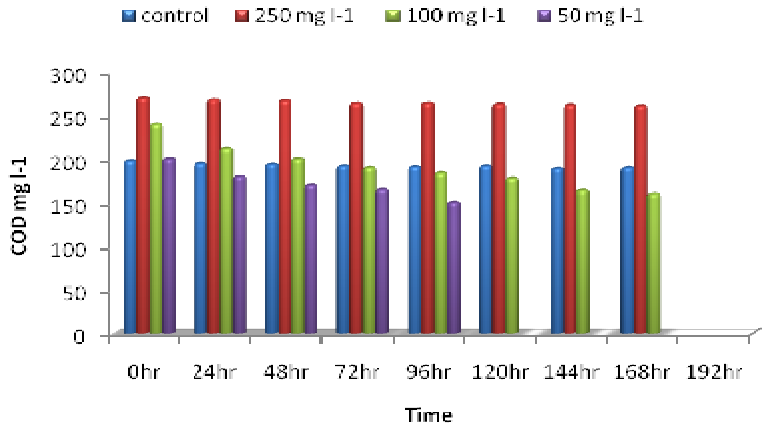
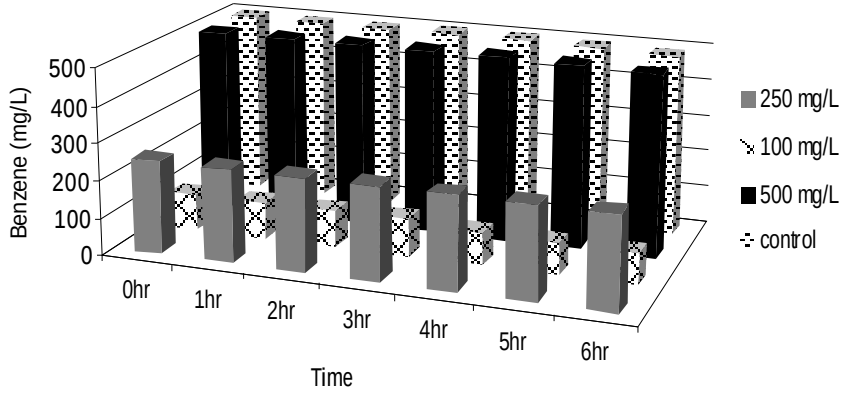


Figure 5 (left): Variation in Chemical Oxygen Demand (COD) during bioremediation of Toluene by cow-dung microbial consortium; Figure 6 (right): Variation in Chemical Oxygen Demand (COD) during bioremediation of o-Xylene by cow-dung microbial consortium.



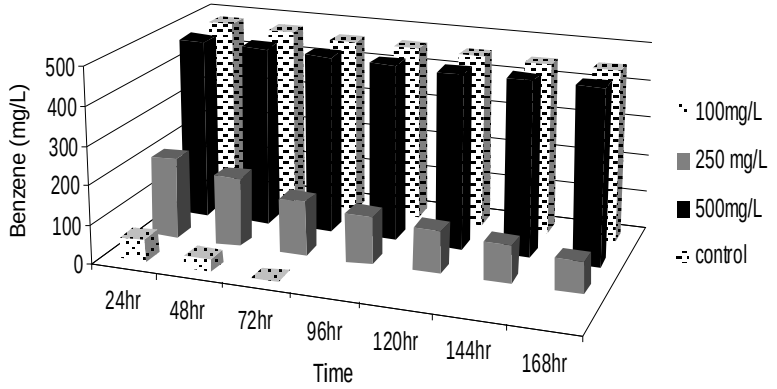


Figure 7: Changes in Benzene concentration during bioremediation by cow-dung microbial consortium, shown in two phases (0 - 6 hr; 24 – 168hr).

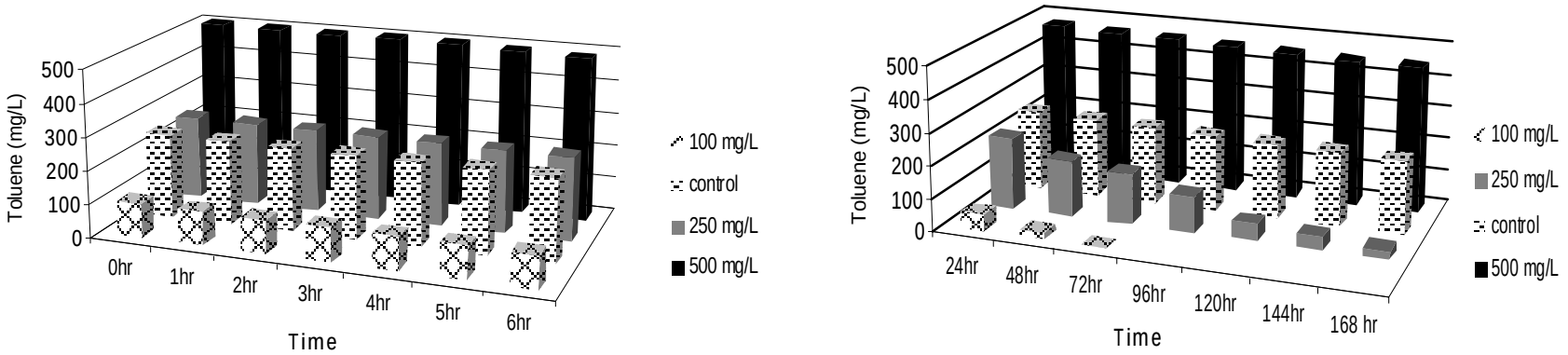


Figure 8: Changes in Toluene concentration during bioremediation by cow-dung microbial consortium, shown in two phases (0 - 6 hr; 24 – 168hr).

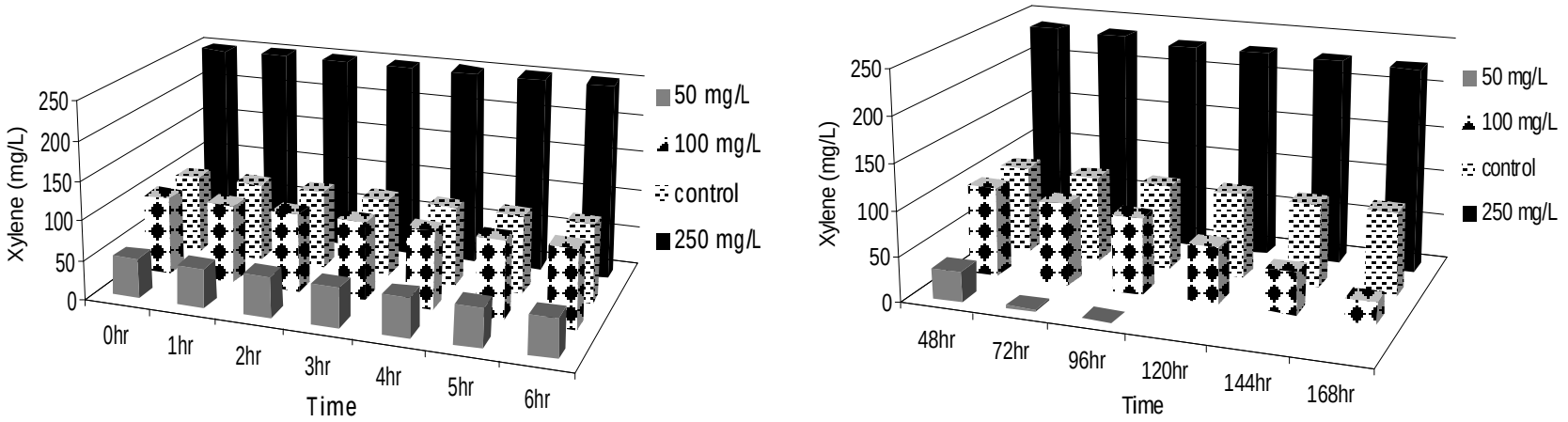


Figure 9: Changes in Xylene concentration during bioremediation by cow-dung microbial consortium, shown in two phases (0 - 6 hr; 24 – 168hr).

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