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Landfill leachate treatment using bacto-algal co-culture: An integrated approach using chemical analyses and toxicological assessment

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ABSTRACT

The present study aims to evaluate the feasibility of leachate treatment using a synergistic approach by microalgae and bacteria. Leachate from one of the landfill of Northern India showed the presence of various toxic organic contaminants like naphthalene, benzene, phenol and their derivatives, napthols, pesticides, epoxides, phthalates and halogenated organic compounds. ICP-AES analysis revealed high concentrations of Zn, Cr, Fe, Ni, and Pb beyond the maximum permissible limit of discharge. Bacto-algal co-culture was found to be the most efficient in removal of toxic organic contaminants and heavy metals. Further, detoxification efficiency of bacto-algal treatment was evaluated by Methyl tetrazolium (MTT) assay for cytotoxicity and alkaline comet assay for genotoxicity using hepatoma HepG2 cells. Reduction in toxicity was confirmed by an increase in LC50 by 1.9 fold and reduction in Olive Tail Moment by 40.6 fold after 10 days of treatment. Results of the study indicate bioremediation and detoxification potency of bacto-algal co-culture for leachate treatment.

1. Introduction

Urbanization and rapid growth in population are pivotal in generating waste and unscientific management of waste leads to health hazards and degradation of the ecosystem. This is a pressing issue in emerging economies like India where the wastes are disposed off in open dumps. Presently, there are 4 functioning landfill sites in Delhi-Ghazipur, Okhla, Bhalswa and Narela of which the first three are unengineered sites and already oversaturated with waste (Ghosh et al., 2015). Approximately 9000 t of Municipal Solid Wastes (MSW) daily is disposed in the three unengineered landfill sites of Delhi (Talyan et al., 2008). Absence of base liners in the unengineered landfills results in continuous groundwater contamination. Close proximity of these landfills to river Yamuna also results in polluting the river. Furthermore, no environmental impact assessment has been carried out prior to selection of these sites. This method of open dumping results in the formation of leachate, beneath the landfill as a result of infiltration processes.

The composition of leachate varies considerably among landfills depending on various factors such as hydrogeology, amount of rainfall, age of the landfill as well as waste composition and degradation stage of waste (Kjeldsen et al., 2002). For general purpose, pollutant load of leachate can be divided into four major groups of contaminants, namely, dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds (Kjeldsen et al., 2002). Various organic contaminants such as polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's), pesticides, phenols and phthalate esters are also present in leachate in trace concentrations.

The synergistic, additive or antagonistic effects of the contaminants present in leachate may lead to toxicity, carcinogenicity or estrogenicity (Matejczyk et al., 2011). The genotoxicity of landfill leachate has been observed using the comet and micronuclei tests in erythrocytes from peripheral blood and gill cells from goldfish (*Carassius auratus*) (Deguchi et al., 2007). It is likely that these genotoxic effects are, to some extent, brought about by free radicals, since oxidative damage has also been described in cells from the heart, kidney, spleen, brain and liver (Li et al., 2006 a, b) of mice. Cytogenetic analysis has also confirmed that landfill leachates can induce genetic damage in meristematic cells from the roots of *Allium cepa* (Srivastava et al., 2005), *Vicia faba* (Sang and Li, 2004) and *Hordeum vulgare* (Sang et al., 2006). Ghosh et al. (2014) reported cytotoxicity, genotoxicity and EROD induction in mammalian cells treated with landfill leachate. Thus, these bioassay studies clearly indicate that if leachates are not treated and disposed safely, they could contaminate nearby soil, ground water and surface water (Mor et al., 2006). Therefore, it is important to find a sustainable option to treat leachate effectively before discharging it into the environment.

A wide range of treatment processes have been explored for

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leachate including physico-chemical and biological techniques with their own advantages and disadvantages. Various physico-chemical methods such as flotation, coagulation-flocculation, adsorption, air stripping, membrane filtration, chemical precipitation, and chemical oxidation have been used for leachate treatment (Renou et al., 2008). Physico-chemical methods of leachate treatment are considered efficient. However, their disadvantages like high cost and sludge generation cannot be ignored (Kapoor and Viraraghavan, 1995). Biological treatment is often preferred due to its reliability, simplicity and high cost-effectiveness and provides many advantages in terms of biodegradable matter.

Studies have reported that the synergistic relationship between a microalgae-bacteria consortium can be very advantageous for the removal of hazardous contaminants in an economical way (Tchobanoglous et al., 2003). Microalgae may play an active role in the purification processes, including heavy metal and nutrient removal, as well as organic matter assimilation and degradation (Tam and Wong, 1989). Microalgae not only enhances the removal of nutrients, heavy metals but also furnishes oxygen to heterotrophic aerobic bacteria to mineralize the organic pollutants. Microalgae in turn use the carbon di-oxide released from bacterial respiration. Photosynthetic aeration is therefore important to reduce operational costs and limit the risks of pollutant volatilization under mechanical aeration. Thus, the synergistic relationship of microalgae and bacteria can be applied for efficient leachate treatment.

The compounds present in trace amounts remain undetected by chemical analyses and their potential biological effects are underestimated. Currently, mammalian cell line based *in vitro* bioassays are being popularly used for toxicological studies as they produce a wide response to complex environmental samples and can be used to study the efficiency of chemical analysis and bioremediation (Tsarpali and Dailianis, 2012). Hepatic carcinoma cell lines such as HepG2 are model cell lines for toxicological evaluation as liver is the main site of xenobiotic biotransformation due to their ability to synthesize xenobiotic metabolizing enzymes cytochrome P450 (CYP) 1A1 (Chaloupka et al., 1994). The MTT assay for cytotoxicity is a colorimetric assay based on the ability of metabolically active cells to reduce yellow tetrazolium MTT to purple formazan by the action of mitochondrial succinate dehydrogenase (Mosmann, 1983). Alkaline comet assay is a sensitive technique to detect even a very minute level of DNA damage even as low as 0.1 DNA break per 109 Da (Gedik et al., 1992) and is suitable for measuring different types of DNA damages like DNA double-strand breaks (DSB), single-strand breaks (SSB), alkali-labile sites (ALS), DNA–DNA and DNA–protein crosslinks, and SSB associated within complete excision repair (Singh et al., 1988; Zegura and Filipi, 2004). Studies on risk assessment of leachate using chemical and toxicological analyses have been very few, especially in the Indian scenario, where majority of the landfills are unengineered and stringent management practices for pollution control are lacking (Narayana, 2009; Vij, 2012).

The present investigation was designed to assess the production of leachate in an artificially set-up lysimeter resembling an unengineered landfill site and monitor its treatment as well as detoxification using bacteria and algae individually as well as in a co-culture. *In-vitro* biotests for analyzing acute cytotoxicity and genotoxicity of the treated samples was also carried out to evaluate the efficiency of the treatment methods.

2. Material and methods

2.1. Chemicals

All chemicals and cell culture-related reagents were procured from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from Merck (Darmstadt, Germany) and were of HPLC grade.

2.2. Description of landfill site, lysimeter design and sampling

The Ghazipur landfill (28° 37 25.11 N, 77° 19 36.1 E) started in the year 1984, is an unengineered landfill site where wastes are still being dumped in an unscientific manner. It spreads over an area of approximately 3×10^5 m^2 and receives 2200 metric tonnes/day of waste on an average. Wastes were collected in May 2015 from different layers of the landfill (up to 20 cm depth) using a core drill from 6 sampling points within the landfill. Then, the samples from different sampling points were mixed to prepare a composite sample. It was further screened to remove large items such as stones, cans etc. The waste was then placed in the lysimeter to study the leachate characteristics in an open dump. Lysimeter was used to simulate the unengineered landfill concept prevalent at Ghazipur landfill site. Lysimeter consisted of a cube-shaped casing (open at the top) made of 6.4 mm thick steel and polyvinylchloride (PVC). A refuse weight of 30 kg (40% moisture content, initial temperature: 35 °C) was added into the lysimeter. Leachate generated (1.5 L) was collected from the lysimeter monitoring system into glass bottles in August 2015 (post monsoon). The collected sample was filtered and centrifuged at 7000 rpm for 10 min and preserved at 4 °C until further analysis.

2.3. Microorganisms for leachate treatment

An already reported endosulfan degrading bacterial strain *Paenibacillus* sp. ISTP10 (gene bank accession number-KJ946937) isolated from sludge sample of Vasant Kunj sewage treatment plant, New Delhi, India was used for bioremediation of Ghazipur landfill leachate obtained from lysimeter. The bacterial culture was maintained in chemostat with Minimal salt medium (MSM) containing ($g\,L^{-1}$), $Na_2HPO_4 \cdot 2H2O$, 7.8; KH_2PO_4, 6.8; $MgSO_4$, 0.2; $NaNO_3$, 0.085; NH_4 $(CH_3COO)_3$ Fe, 0.05; and $Ca(NO_3)_2 \cdot 4H_2O$, 0.05 at 30 °C. The microalgae identified as *Scenedesmus* sp. ISTGA1 (Gene Bank Accession No. KC 342184) used in the study was isolated previously from Jhiri marble mining site, Alwar, India and grown in BG-11 medium supplemented with sodium bicarbonate ($NaHCO_3$) as a carbon source.

2.4. Shake flask study for leachate treatment

In shake flask experiments, bacterial and algal cultures were set up in the presence of 20% leachate (v/v) either individually or together to study the efficiency of different leachate treatment methods. Inoculums size in MSM-leachate (20% v/v) was kept 5×10^4 CFU ml^{-1} (absorbance measured at 600 nm) for bacteria and (680 nm) for algae. The samples were removed at intervals of 0, 3, 7 and 10 days. All the experiments were set up in triplicates to study degradation efficiency of the microorganisms. Samples were centrifuged at 7000 rpm for 10 min to remove bacterial and/or algal biomass, and the supernatants were processed for GC–MS analysis, ICP-AES analysis, and *in vitro* bioassays for the identification of organic compounds, analysis of heavy metals, and toxicological evaluation, respectively.

2.5. Physico-chemical characterization of leachate

Leachate samples were collected from the lysimeter, stored at 4 °C and subsequently characterized and used for degradation study. Physico-chemical analyses such as pH, electrical conductivity, color and TDS were performed using Cyberscan PC 510 m, COD by open reflux method and color using platinum-