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Involvement and interaction of microbial communities in the transformation and stabilization of chromium during the composting of tannery effluent treated biomass of *Vallisneria spiralis* L.

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ABSTRACT

Tannery effluent treated with aquatic macrophyte *Vallisneria spiralis* L. for 14 d showed significant improvement in physico-chemical properties and reduction in Cr concentration. Accumulation of Cr was found maximum in roots (358 μg g^{-1}dw) as compared to shoot (62 μg g^{-1}dw) of the plant. A laboratory scale composter was designed with the objectives to investigate the physico-chemical changes and role of microbes in stabilization and transformation of Cr in the composting material. Results revealed that the composting process was quick within 7–21 d as indicated by peak time for various physico-chemical parameters and drop in C/N ratio up to acceptable limit. The profile of microbial communities indicated that population of anaerobic, aerobic and nitrifying bacteria increased quickly at the initial phase, and reached a peak level of 4.2×10^6, 9.78×10^8 and 9.32×10^9 CFU g^{-1}, respectively at 21 d; while population of actinomycetes and fungi was found maximum i.e. 3.29×10^7 and 9.7×10^6 CFU g^{-1}, respectively, after 35 d of composting. Overall bacterial population dominated over the actinomycetes and fungi during the composting process. $Cr^{(VI)}$ was transformed to $Cr^{(III)}$ due to the microbial activity during the process. Sequential extraction of Cr fractionation showed its stabilization via changing into organic matter-bound and residual fractions during the composting.

1. Introduction

Phytoremediation is a novel strategy to detoxify and stabilize the organic and inorganic substances; however, phytoremediated biomass is a major outcome of treatment processes, which creates the problem in the success of remediation technology due to the high load of metallic and non metallic pollutants (Cunningham et al., 1995; Rai et al., 1995; Guardia et al., 2002; Wei et al., 2007). Composting of the treated phytomass is an important advance step to enhance the success of treatment options. Phytoremediated biomass of aquatic plant contains high amount of toxic metals, which has important role in biogeocycling via active and passive transfer of elements into the food-chain. To overcome this problem composting has been found to be one of the most economical ways of treatment because it combines materials recycling and waste disposal at the same times (Huang et al., 2001; Khan and Joergensen, 2009). However, biology and understanding of microbial ecology can be useful to optimize the process (Xi et al., 2005). Additionally, standardized microbial analysis may be potentially used for judging the quality and maturity of compost and it has been recognized that temperature and its interaction with chemicals are the primary driving forces in the succession of microbial communities during the process (Zheng et al., 2007).

Besides, key factors for a successful composting such as, temperature, aeration, moisture and nutrients should be appropriately controlled. C/N and C/P ratio is one of the important factors effecting composting process and compost quality (Huang et al., 2004; Zhou et al., 2004). The resilience of compost system to perturbation is usually attributed to the highly active and diverse microbial population (Zorpus et al., 2003; Khan and Joergensen, 2009). The microbial succession, involvement of microbial communities and their activities are little known during the specific phases of composting process (Said-Pullicino et al., 2007; Adams and Frostick, 2008). Although, application of compost to agricultural soils has many advantages (Wei and Liu, 2005), land application of compost has been limited due to higher heavy metal contents (Karvelas et al., 2003; Wei and Liu, 2005). However, fractionation and chemical speciation of the heavy metal may allow the prediction of its bioavailability and is related to its different nature including the bonding strength, either in free ionic form or complexed by organic matter, or incorporated in the mineral fraction of the compost (Kunito et al., 2001; Amir et al., 2005; Gupta and Sinha, 2007a).

In India, although composting of bio-degradable wastes has been made compulsory by imposing solid waste handling rules

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(CPCB, 2002), improper disposal of chromium contaminated hazardous waste from tanneries has become a major environmental problem. Tannery effluent contains high amount of Cr, pathogens, many organic and inorganic toxic compounds, which poses serious threat to the local environment (Fang and Wong, 2000; Contreras-Ramos et al., 2004). In this context, several aquatic macrophytes such as; *Spirodela polyrrhiza, Ceratophyllum demersum, Bacopa monnieri, Alternanthera sessilis, Hygrorrhiza aristata* and *Hydrilla verticilata,* have been reported for phytoremediation of metals (Rai et al., 1995; Vajpayee et al., 1995; Chandra et al., 1997). However, uptake of metal largely depends on chemical speciation and life form of macrophytes (Gupta and Sinha, 2007b). Amongst these submerged plants *Vallisneria spiralis* exhibited high phytoremediation potential for the treatment of Cr loaded tannery effluent (Vajpayee et al., 2001; Gupta and Sinha, 2007a; Shukla et al., 2007b). Besides, microbial reduction of toxic hexavalent chromium *vis a vis* accumulation by the plants has practical importance (Shukla and Rai, 2006; Shukla et al., 2007a) in developing an integrated bioremediation system (Rai et al., 2006).

Therefore, in the present investigation we have selected *V. spiralis* for remediation of Cr from tannery effluent and for composting of treated biomass a laboratory scale composter was designed to study the involvement of microbial communities in stabilization and bioavailability of Cr and physico-chemical changes during the process. Result of the study is being reported in this communication.

2. Methods

2.1. Experimental set-up

2.1.1. Treatability studies

Young acclimatized plants of *V. spiralis* (100 g fw) were treated with raw tannery effluent in a 2 L beaker containing 1000 ml of tannery effluent. The effluent level in the containers was maintained throughout the experiment. It was carried out at room temperature (25 ± 2 °C) in the laboratory and the treated plants were harvested after 0, 2, 4, 7 and 14 d for the accumulation of Cr in the plant tissues. The harvested plants were thoroughly washed and oven dried at 80 °C. Plants were digested in HNO_3:$HClO_4$ (3:1, v/v) and Cr was estimated using GBC Avanta atomic absorption spectrophotometer.

2.1.2. Composter and composting process

A bench scale composter was developed to standardize the conditions for optimal composting which was a square shaped structure made of reinforced plastic. The composting reactor included the composting chamber (45, 25 and 38 cm in length, width and height) with thermometric sensors for temperature and pH and outlet/inlet point for loading phytomass and taking out compost.

The treated biomass of *V. spiralis* (water content: 75%) was initially air - dried at room temperature (20 ± 2 °C) until the water content reached about 55–60%; then the treated biomass was mixed with cow dung manure (1:1, w/w) as bulking agent and loaded into the composting reactor. At the desired time, 10 g of compost sample was taken from the sampling port, and processed for further determination.

2.2. Physico-chemical analysis of tannery effluent

Effluent samples were collected in a glass container from common effluent treatment plant (CETP), Unnao and physico-chemical analysis was started in the laboratory within four hour of collection. All the parameters were determined in triplicate in each case by following the Standard Methods of Examination of Water and Wastewater (APHA, 2005) before and after treatment by *V. spiralis.* Some parameters like; temperature, dissolved oxygen, pH, total dissolved solids etc were determined on the spot with the help of Portable Water Analysis Kit (Century, India).

2.3. Analysis of compost

2.3.1. Physico-chemical analysis

Compost slurries (10 g in 100 ml) were prepared by agitation into stirrer in distilled water. Moisture content of the samples was determined as weight loss upon drying at 105 °C in an oven for 24 h (Tiquia and Tam, 1998). Electrical conductivity and pH were determined from 1:10 (w/v) compost: water extract ratio. Temperature of compost samples were monitored with the help of a thermometer fixed inside the composter. Bulk density, water holding capacity and organic matter (OM) were analyzed in air dried samples, and was calculated as the difference between ash and dry weight (Tiquia and Tam, 1998). Total organic carbon (TOC) was estimated by the method of Kalra and Maynard (1991). As a measure of bacterial biomass, direct counts of bacterial numbers (AO count) were performed using formaldehyde-fixed (final concentration of 0.2% (w/v)) compost samples, stained with acridine orange (final concentration of 0.001% (w/v)) filtered through 0.2 μM polycarbonate filters and examined. Most provable numbers (MPN) counts and were performed using compost slurries (APHA, 2005). For each samples, the MPN count was divided by the direct bacterial count to obtain the culturability of microorganisms present in compost.

2.3.2. Microbial population

The colony forming units of cultivable bacteria, actinomycetes and fungi were determined using a standard dilution-plating procedure, and replicated three times. Five grams of compost was suspended in 45 ml of sterile water and shaken for 20 min. Ten fold dilutions were made. Bacteria were quantified on yeast peptone glucose agar (yeast extract 5 g l^{-1}, peptone 5 g l^{-1}, glucose 10 g l^{-1}, agar 15 g l^{-1}), actinomycetes were quantified on Gause's synthetic agar (agar 18 g l^{-1}, starch 20 g l^{-1}), and fungi were quantified in melting malt extract agar (malt 15 g l^{-1}, agar 10 g l^{-1}).

2.3.3. Microbial diversity

Microbial diversity was assessed by Shannon–Weaver index as described below (Strom, 1985). Soil extract agar was used as incubation media for enumerating total microorganisms. The temperature for incubation was the same as the temperature of composting pile when sampled. The compost were sampled at 0 d (the initial stage of composting, 37.7 °C), 7 d (temperature just rose to 41 °C) 14 and 21 d when temperature was maintained between 47 and 49 °C. The species diversity index was calculated by using Shannon–Weaver index (Shannon and Weaver, 1949).

2.3.4. Fractionation and transformation of chromium

The method of sequential extraction used in compost samples was developed by Tessier et al. (1979), which is widely applied in various studies of composting (Ciba et al., 2003; Amir et al., 2005). The method yields five different solutions: exchangeable (1 mol L^{-1} Mg Cl_2, pH 7); bounds to carbonates (1 mol L^{-1}NaOAc/HOAc, pH, 5); bound to Fe–Mn oxide (0.04 mol L^{-1} $NH_2OH \cdot HCl$ in 25% HOAc); bound to organic matter (0.02 mol L^{-1} HNO_3 in 30% H_2O_2, pH 2; 3.2 mol $L^{-1}$$NH_4OAc$ in 20% HNO_3); residual (digested with concentrated HNO_3 + $HClO_4$). The compost samples were extracted with purified water at a solid: water ratio (w/v) of 1:10 by shaking in a 25 °C both for 1 h. The suspensions were centrifuged at 10,000 rpm and supernatants were filtered through 0.45 μm membrane filter papers.