ABSTRACT

Major international concern is growing over the wide-scale contamination of soil and groundwater with high energy materials. The manufacture, use and disposal of high energy materials over the last hundred years have resulted in serious widespread contamination of the environment. High Energy Materials (HEM's) encompass different class of compounds and compositions containing fuel and oxidizer components that react rapidly upon initiation and release large quantities of energy in the form of heat and light. Energetic materials are typically classified as explosives, propellants, pyrotechnics or incendiaries. Explosives are broadly classified based on their chemical structures such as nitrate esters, nitramines, nitroaromatics etc. Propellants include both rocket and gun applications. Rocket propellant formulations are also based on nitrate esters usually nitroglycerine (NG), nitrocellulose (NC), nitroguanidine (NQ) or nitramine such as RDX and HMX. Gun propellants usually are single base (NC), double base (NC and NG) or triple base (NC, NG, and NQ) formulations.

The large-scale industrial production and processing of energetic materials such as NC, NG, trinitrotoluene (TNT) and triple base propellant (TBP) over the past 100 years led to the disposal of wastes containing explosives and nitrated organic by-products into the environment. Unexploded ordnances (UXO's) occurred during war military training pose serious environmental problems and other potential hazards. The UXO's on prolonged storage contaminate the soil & water due to leaching of explosive materials present in it. Present and historical methods for the disposal of munitions include open burning, open detonation, burial and incineration. These methods, however, are becoming increasingly unpopular due to environmental concerns. Current incineration of explosive contaminated soil results in the production of thousands of tons of unusable ash. This raises concerns regarding further disposal and also presents a threat to the quality of the atmosphere through the release of fine particulate matter. Furthermore, the disposal by open detonation can result in the release of further contaminants, including dioxins and polychlorinated biphenyls. Open detonation can also result in major habitat destruction, ecosystem disturbance and extensive structural damage to any property in the vicinity. Therefore, alternative technologies must be developed to minimize air, water and soil pollution and to comply with present and anticipated environmental legislation.

Very limited technological information is available in the open literature on disposal of HEMs. Therefore, a systematic study was undertaken on eco-friendly disposal of high energy and hazardous materials like nitrocellulose (NC), nitroglycerine (NG), triple base propellant (TBP) formulation and trinitrotoluene (TNT).

The aim of the present work is to carry out studies on development of eco-friendly and cost effective bioremediation process for the safe disposal of high energy materials and assessment of end products of remedial process as plant nutrient. The major objectives of the present study are divided into three parts. First, standardization and optimization of laboratory scale composting process for the bioremediation of explosive contaminated soil and testing efficacy of end products as manure for crop followed by detailed study of explosive incubated compost microflora. Second, comparative study on phytotoxicity of hydrolysates, prepared by alkaline hydrolysis of explosives and their propellant formulation and of composted hydrolysates and third, phytoremediation of explosive contaminated water. Exhaustive data have been generated on these aspects and findings have been reported accordingly.

The scope of present research work includes:

Studies on **compost bioremediation** of nitrocellulose (NC), nitroglycerine (NG), triple base propellant (TBP) and trinitrotoluene (TNT) using sugarcane byproducts as amendments to the compost matrix followed by seed germination and plant growth studies on compost matrices incubated with NC, NG, TBP and TNT. Complete isolation, characterization and identification of microflora from compost matrices incubated with high concentration of NC, NG, TBP and TNT have been carried out systematically.

Alkaline hydrolysis of NC, TBP and TNT coupled with phytotoxicity assessment of hydrolysates of NC, TBP and TNT using seed germination assay on wheat seeds followed by composting of hydrolysates. Phytotoxicity data of composted hydrolysates has been compared with unhydrolysed NC, TBP and TNT and uncomposted NC, TBP and TNT hydrolysates.

Phytoremediation of NG by using two commonly available aquatic macrophytes *Eichhornia* and *Pistia* and two common wetland plants *Typha* and

Phragmites in hydroponic culture. Study of biochemical parameters of NG exposed plants is one of the important parts of the present thesis.

Explosive grade NC containing 12.6% nitrogen, NG, TBP formulation comprise of NC, NG, NQ and additive like carbamite as stabilizer and TNT has been used under the present research work.

Composting of NC, NG, TBP and TNT

Based on high C:N ratio, rich microflora, ease in handling and availability, five different compost fillers i.e. soil, cow dung, vermicompost, sugarcane pressmud and sugarcane bagasse, were selected for the preparation of compost matrix. Parameters like pH, electrical conductivity (EC), total nitrogen (N)%, organic carbon (OC)%, C/N ratio, total P_2O_5(%), total K_2O(%), micronutrients like Mg, Cu, Fe, Mn, CFU counts for bacterial and fungal population were determined in all compost fillers by using standard methods. Concentrations used under the study for NC and TNT ranges from 1000 to 10,000 ppm and for NG and TBP, 100 to 1500 ppm. Estimation of NC, NG, TBP and TNT and the characterization of degraded products in compost matrix was carried out by spectrophotometric method using NC and TNT assay, FTIR, HPLC and GCMS analysis.

For phytotoxicity assessment various feedstock concentrations of explosive contaminants (NC and TNT 10,000 ppm, NG and TBP 1500 ppm) were selected for the study. Seed germination studies were carried out on wheat seeds in an environmental chamber at 27°C & 80% humidity as per EPA protocol (OPPTS 850.4200, EPA 1996). Seeds were set to germinate on the NC NG TBP and TNT composted matrix (after 15 days of incubation). All test and control groups were kept for germination in 3 replicates. Delay in germination and percentage seed germination was recorded in comparison with the control. After a maximum vegetative growth was attained, root and shoot length of the test groups and controls were recorded. Shoots were processed for the estimation of their chlorophyll, carbohydrate and protein content as well as for their biomass weight.

All the compost matrices used in the compost bioremediation study were subjected to isolation, characterization and identification of microflora. Bacterial and fungal colonies were isolated by employing the dilution plate method. Potato dextrose agar (PDA) was used for the isolation and estimation of fungal numbers. Ten Petri dishes were inoculated with 1ml portion of the ultimate dilution in a parallel way. The dishes were incubated at 27°C for 6-7 days. Following the calculation of the number of micro fungi per gram of dry compost, the micro fungi were transferred into potato dextrose agar (PDA) and malt extract agar (MEA) media for identification. Slide culture technique was used for the isolation of single fungi.

Desiccated nutrient broth powder at a concentration of 0.08 g/L was used to prepare dilute nutrient broth (DNB) and used for growth of all viable bacterial population present in the compost samples. Gram staining, motility, cell shape, density, elevation, configuration and pigments were observed under the microscope. Biochemical characterization of bacterial isolates was done by performing biochemical tests using API20NE system. Identification of bacterial isolates was carried out by using 16-S ribosomal technique.

Since most of the processes occurring in soils and compost are microbially mediated and are metabolized by enzymes, it is reasonable to suggest that the determination of enzyme activities in soil and compost may be used as a research tool to assess / study microbial functional diversity, biochemical process, microbial ecology and ultimately to provide indicators of soil and compost quality. Selected compost samples with high concentration of test compounds (compost mixtures incubated with NC, 10,000 ppm; NG, 1500 ppm; TBP, 1500 ppm and TNT, 10,000 ppm) along with control compost i.e without any test compounds were assayed on moist samples for enzyme activities particularly of urease, phosphatase, dehydrogense and L-glutaminase at '0' hrs and after 15 days of incubation.

The compost matrix selected and optimized in the present study showed significantly faster and larger extent of degradation towards NC in comparison to NG. In case of NC (feedstock 10,000 ppm) the residual NC was found below detectable limit i.e less than 1 ppm on 14^{th} day of incubation, whereas in case of NG (feedstock 1500 ppm) it took 15 days to degrade the energetic material to undetectable limit. The degradation of NC, NG & TBP of similar feedstock concentrations prolonged to 40-50 days to degrade up to below detectable limit as reported by other researchers. The incubation of energetic materials in compost matrix also generates an enriched product that can sustain vegetation as shown by the performance of seed germination parameters on the matured compost as compare to control unincubated compost. Significant increase in root / shoot length, biomass and metabolic products of wheat plant was observed in case of seeds germinated on matured composts which were having initial feedstock concentration of NC and TNT as high as 10,000 ppm and NG and TBP as high as 1500 ppm. Present compost bioremediation studies results in significant cost savings by way of eliminating costly chemical fertilizers.

16-S ribosomal identification revealed the identification of first isolate (VRI-1) as *Micrococcus sp.,* second isolate (VRI-2) as *Exiguobacterium sp.,* third isolate (VRI-3) as *Cellulomonas hominis* and the fourth isolate (VRI-4) as *Bacillus cereus.* These isolates have not been reported for the biodegradation of nitrate ester and other explosives. Fungal isolates identified under the present study has also not been reported for the biodegradation of explosives and hence the findings of this study will be useful in optimization of the conditions for effective biodegradation of explosives and particularly nitrate esters using these bacterial and fungal isolates.

Alkaline hydrolysis of NC, TBP & TNT and Phytotoxicity studies of their hydrolysates

Nitrocellulose (NC) containing 12.6% Nitrogen, TBP and TNT was subjected to alkaline hydrolysis at $70^{\circ}C$ in a glass reactor under continuous stirring. The temperature of the reaction mass was controlled to $70\pm1^{\circ}C$ with the help of temperature controller. Reactor assembly was equipped with vertical reflux condenser with the provision of chilled water circulation. Potassium Hydroxide (KOH) in desired concentration was taken in the reactor and heated to $70^{\circ}C$. NC was fed subsequently and the reaction was terminated, when the total suspended solid (TSS) was found nil. The reaction mass temperature was brought down to $35\pm 1^{\circ}C$ in a span of 3-4 minutes by circulating chilled water in the water bath. The reaction product was neutralized with phosphoric acid (H_3PO_4) and the final pH was brought to 7.5-7.8. The product obtained after neutralization was termed as hydrolysates of representative energetic materials i.e NC, TBP and TNT. The hydrolysates were subjected to seed germination studies on wheat seeds using stanadard EPA protocol. Hydrolysates (prepared with 1%, 2% and 5% of alkali with feedstock conc. of 1000 to 5000 ppm) showed no phytotoxic concern as per EPA protocol (% seed germination > 50%). Hydrolysates prepared with higher concentration of alkali (5% and 10%) were subjected to composting. Seeds grown on composted hydrolysate matrix showed significant increase in root length, biomass and metabolic products of plants like chlorophyll and protein content as compare to control.

Phytoremediation of NG

Two commonly available aquatic macrophytes (*Eichhornia* and *Pistia*) and two common wetland plants (*Phragmites* and *Typha*) were selected for the phytoremediation studies of explosive grade nitroglycerine. No report is available in open literature on the phytoremediation studies of explosives and explosive contaminated waste using these common aquatic macrophytes. All the plants were hydroponically cultivated in Hoagland's solutions. Two sets of control experiments were conducted parallels with the treatments sets. In order to check the loss of NG from experimental systems due to evaporation and adsorption on the walls of the containers, a control experiment without plant was performed side by side with the experimental run. The other set contained the plant roots that had been frozen overnight in a solution with sodium azide (1g/L) added to inhibit bacterial activity. All treatments were prepared in triplicate. Four different concentrations (25, 50, 75, 100 mg/L) of NG were added to the hydroponic solution in the container set for each treatment. The concentration of NG, which inhibits growth of plant in 50%, was provided from growing factors for each species. The plants were analyzed initially and after 8 days of retention in NG for various biochemical parameters. Metabolic response of all the plants to NG was assessed by estimating chlorophyll a, b and total chlorophyll, total proteins, free amino acids, total carbohydrates, free reducing sugar, carotenoids and oxidative enzymes like catalase and peroxidase.

The presence of NG in the leaves was clear evidence that NG was taken up and translocated to the leaves by the macrophytes. In case of *Typha* plants, there was significant loss of NG from solution, yet no accumulation in the tissues was observed. It is likely that enzymes within the *Typha* tissues were especially effective at transforming NG. Trials with killed plant roots showed high concentration of NG absorbing to the dead roots. Based on the observation that NG concentrations in the dead roots were significantly higher than NG concentrations in the live roots, further indicates that enzymes within all of the live macrophytes were actively transforming the NG. The values of peroxidase activity indicate a high antioxidant capacity of *Phragmatis australis* in the presence of oxidative stress produced in condition of NG retention. There was a significant ($p<0.05$) reduction in chlorpphyll a, chlorpphyll b, total chlorophyll, chlorophyll a:b , carotenoides, total proteins, total carbohydrates and free reducing sugars content as a result of the growth of the *Eichhornia* plant in the

NG and a significant increase ($p < 0.05$) was noticed in the free amino acids level. This indicates that the NG has phytotoxic effects on the *Eichhornia* plant.

Based on the observations of biochemical profile and the NG retention in the root tissues of plants and its translocation to leaves, a conclusion was drawn that among the four plants under the study, *Typha* is having better phytoremediating potential towards NG than *Eichhornia* and *Phragmitis*. *Eichhornia* can be useful as a bioindicator for NG contaminants while *Pistia* if left for longer period in the NG it may ultimately result in the death of the hydrophyte.

In the present research work, NC, NG, TBP and TNT were successfully degraded into non- hazardous and non-explosive products by compost bioremediation and the end products of compost bioremediation showed application as plant nutrient as well. The data generated on plant performance parameters like % seed germination, root-shoot length, biomass, chlorophyll, carbohydrate and protein content under seed germination study on explosive incubated compost and hydrolysate incubated compost are not reported in open literature. Therefore, phytotoxicity studies, in conjunction with the appropriate controls and analytical evaluations were carried out to establish composting and alkaline hydrolysis as an alternative technology for explosive contaminants. Detailed study carried out on compost microflora is of vital importance as Bacterial isolates reported in this study has not been reported for the biodegradation of nitrate esters as well for the other group of explosives and therefore the findings of this study opens the scope for the further assessment of biodegradation potential of these isolates towards such explosives. No report is available on phytoremediation of NG by aquatic macrophytes used under this study.

Studies carried out under the present work are of vital importance to scale-up the process for conversion of waste HEMs into the products, which have application as plant nutrient. Findings of the present studies have high utility potential in terms of safe and ecofriendly disposal process for explosives and also useful end products as plant nutrient.