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Title:Reclamation of red mud (RM) through microbial and vermi-technology- A lead towards sustainability

ABSTRACT

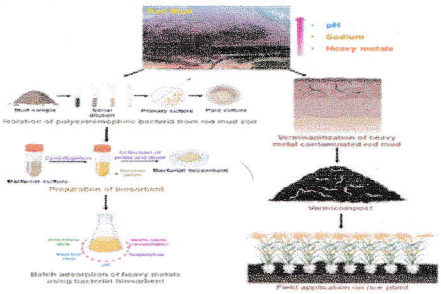

Fig. Graphical representation of the work.

This thesis illustrateshow the hazardous aluminum industry waste known as red mud (RM) is sustainably being managed. In this work waste (RM) was explored from two different perspectives in terms of their utilization: firstly, used as a vermicompost for agricultural applications, and secondly,as a portfor harbouringnumerous extremophilic bacteria that hasa wide range of utility.In present study we investigated the metal remediation potential of one polyextremophilic bacteria isolated from RM, which may help in wastewater treatment. Keeping an eye on the generation of RM on a global scale>100 million tons of RM are produced annuallywith which India contributes approximately10 million tons every year. Consequently, the global inventory of disposal is risingsharply, however, by 2015, it was past 4 billion tones. Effective post-production treatment and subsequent disposal are burning issuesworldwide in regard to their environmental impact.High alkalinity, disproportionate Na concentration, and elevated presence of heavy metal coupled with radioactive elements are alarming if not adequately managed. Their large volume of land-based stockpiling has added another feather to the directory of environmental distress.Due to the high population density, deficiency of land, and lack of environmentally appropriate dumping facilities, managing RM is a rather more fatal concern in developing countries like India. Management of this lethal waste following international standards in developing countries requires experts with sound knowledge and experience. Moreover, the aluminum industries should follow international waste management standards and environmental protection policies.Regrettably, India failed not to sustain its pollution control measures, and as a result, it is ranked as the 7th most environmentally hazardouscountry.

Therefore,the repository of alkaline sodic red mud is in dire need of clean sustainable technology to alleviate the risk from its disposal and storage. Revegetation with increment in soil faunal population is however an efficacious rehabilitation method though little is known about earthworm intervention strategy popularly vermitechnology.One of the most trustworthy bioprocessing techniques, vermitechnology involves the quick stabilization and conversion of stubborn waste materials into friable and nutrient-rich organic fertilizer through the mutually beneficial actions of earthworms and microbes. The technology is widely popular for recycling solid waste that can further be used in agriculture or horticulture as compost.Earthworms digest food (substrate) into tiny particles that are then quickly mineralized by their gut-associated bacteria. The worms then discharge these nutrient-rich breakdown products through their excretions (also known as vermicast) along with the gut microbiota. Although a wide category of solid waste (brick kiln ash, municipal solid waste, tannery sludge, oil industry sludge, etc.) is managed through vermitechnology, however, use of vermitechnology in managing RM is not getting momentum due to its threatening Na concentration. Under this context, we opted for a pretreatment technique that includes the use of calcium sulfate ($CaSO_4.2H_2O$) and calcium chloride ($CaCl_2.2H_2O$)in a 6 g/kg dose, followed by leaching (1:10 ratio) using a siphonic approach. The water-soluble Na leaches out due to the substitution of Na by Ca from its exchangeable site, thereby the water-soluble Na is lowered, and the addition of bulking agent (cow dung) to that of washed RM made it favourable for earthworm dwelling.The study's ultimate goal was to offer a vermistabilized RM with an appropriate field-scale agricultural application. The study was commenced by thoroughly analysing the RM and its effects on the environment and human health. Such in-depth research produced some crucial data and information needed to standardize vermitechnology. As harmful metals including Cr, Cd, Pb, and Ni are known to be abundant in RM, the earthworm species *Eisenia fetida* was chosen as candidate species due to their extensive popularity in managing noxious waste. This evaluation offered a solid scientific foundation for the widely applicable vermitechnology. In the end, the vermicomposted RM was used as a source of crucial nutrients and a plant growth stimulant for the production of rice in red-lateritic soil.

RM was characterized by highly sodic (Na^+6.5%±0.5), elevated electrical conductivity (12.2 ±2.1 mS/cm) with high pH (11.5 ±1.9) and elevated toxic metal concentration which ranged as follows: 392 ±46.66 mg/kg (Cr), 9.71 ±1.37 mg/kg (Cd), 139.68 ±12.74 mg/kg (Ni) and 228.64 ±16.66 mg/kg (Pb). Concurrently, the labile metal concentration (0-15 mg/kg range; Ni >Pb >Cr >Cd) of those toxic metals was alarming, andtheir large amount of DTPA- extractability (0.78-4.76 mg/kg; Pb >Cr >Ni >Cd)was a concern froman environmental standpoint. FESEM-EDX study revealed the aggregated structure with an elemental abundanceofFe_2O_3, TiO_2, Al_2O_3, SiO_2, and Na_2O.According to FTIR resultsFe-O, Ti-O, Al-O, and Mg-O type bonds were largely present, while the XRD- study described their partial crystalline structure enriched with Fe_2O_3, Al_2O_3, quartz, and cristobalite. Solubility dynamics were pretty much shocking where a high amount of Na^+, Ca^{2+}, and K^+ released into leachate along with increased concentrations of Cr (23 ±2.4 mg/kg), Pb (22.1 ±1,9 mg/kg) and Ni (12.11 ±1.88 mg

/kg).Environmental impact indices analysedthat RM is posing a high pollution load to the environment (Pollution Index= 2.68) with severe ecological risk (Ecological Risk Index = 587.86). Enduring exposure to Cr, Pb, and Ni from RM poses a serious risk to human health. Since RM is aluminum industry-generated waste, so, in our study, we also compared their characteristics with native bauxite ore (BO; chief commercial ore of Al^{3+}) to evaluate the degree of deviation after industrial Bayer extraction of aluminum. The study revealed an exclusive result thatimplied a drastic increase in Na and Ca content, DTPA- available metal including the enhancement of leaching characteristics of metals (TCLP study) in RM. Furthermore, microbial presence was largely reducedin RM compared to native BO indicating a dramatic fall of microbial load, confirmed by some microbial indicator enzymes viz., dehydrogenase (DHG), fluorescein diacetate hydrolysing activity (FDA), and microbial biomass C.

Following this thorough investigation, we conducted an RM-vermicomposting experiment with four distinct waste-to-bulking agent ratios (RM: CD), namely 1:1 (RM: CD), 1:2 (RM: CD), 1:3, and 1:4. In order to investigate the role of earthworms in altering the bioavailability of metal concentration, plant nutrients, organic matter content, and enhancement of microbial activity, a parallel traditional aerobic composting was carried out to evaluate the efficacy of vermicompost. Each ratio for vermicomposting inoculated with *Eisenia fetida* @ 10 worms per kg of substrate and incubated under a shaded vermicompost unit for 90 days, maintaining 50% - 60% moisture content with adequate aeration.Simultaneously, another series of aerobic composting was maintained using the same treatment combinations without earthworm inoculation.Samples were taken periodically at intervals of 0 days, 30 days, 60, and 90 days, and they were analysed for changes in microbial characteristics such as compost respiration, microbial count, and enzyme activity, while the physicochemical properties and compost quality characteristics like pH, EC, total organic carbon (TOC), available N, P, K were analysedbefore and after the incubation. Changes in the metal concentration were evaluated through a fractionation study which also demonstrated their distribution and shifting of state after vermicomposting. The result disclosed that exorbitant alkalinity was pacified to neutrality in all *E. fetida*-based feedstock with a considerable reduction in TOC, Na^{+}, and Ca^{2+} and increased NPK availability. Greater improvement in C_{mic}, respiration, and enzymatic (FDA, sulphatase, phosphatase, glucosidase, dehydrogenase) activity was evidenced in vermireactors. Water soluble and exchangeable fractions of metal reduced by >55% (Cu, Cr, Pb, Ni, and Cd) in vermibeds. Fatty acid profiling (PLFA) and species diversity analysis (alpha diversity, E-var, E-PLFA) outlined that community distribution was significantly impacted by RM proportion in feedstock however it was proficiently compensated in VC2 (1RM:2CD) and VC3 (1RM:3CD). A switch from P to N limitation to microbial requisite in the reactors is an important scientific observation from vector analysis. Pearson's correlation statistics signify that metal availability was efficiently compressed by microbial activity at the end of incubation. Lastly, the field trial with monsoon rice (*Oryza sativa L.*)showed the most prolific vermicompost mixture was 1:2 and 1:3 which produced the highest yield of 4.2 tons/ha, when substituted with 50% NPK (50% RM-VC+ 50% NPK). A concurrent soil health analysis (available NPK, TOC, and microbial activity) revealed an improvement in RM-VC treated plots. In terms of the metal uptake results, rice roots accumulated metal less than untreated plots on average, however, shoot and grain did not accumulate any metal.

Focusing on the second direction of work, initially the RM was explored for its biological characteristics through enzymatic analysis (FDA, dehydrogenase, β-glucosidase, phosphatase etc.), which showed incredibly poor activity. Nevertheless, a 16S V3-V4 Metagenome study revealed the presence of microbes that mostly belonged to the genera *Bacillus*, *Halomonas*, *Alcanovorax*, *Indibacter*, *KSA 1*, etc.*Proteobacteria*, *Bacteroidetes*, *Planctomycetes*,and*Actinobacteria* were the predominant phylum. The total heterotrophic bacterial count showed the fresh residue was completely barren, however, long-term deposited RM was obtained by 1.4 X 10^{2}- 2.9 X 10^{2}cfu/g. A total of 20 alkaliphilic isolates were obtained and biochemically characterized. Focusing on objective of the study of finding metal-tolerant alkaliphilic bacteria, five isolates were selected based on their minimum inhibitory concentration(MIC). Out of these five isolate *Bacillus xiamenensis* ISIGRM16, accession no. OP243450 showed their wide range of pH, temperature, and NaCl concentration, thus selected as polyextremophilic bacteria, which subsequently explored for their metal adsorption ability for three predominant metal (Cd^{2+}, Ni^{2+}, Cr^{6+}) species of the wastewater stream. The dried biomass biosorbent was prepared by centrifugation of the culture broth, following cell mass was collected and washed with phosphate buffer saline solution (PBS) and finally dried in a hot air oven at 45^{0}C to 50^{0}C.The bacterial-biosorbent was able to remove Cd^{2+}(>99%), Ni^{2+}(>85%), and Cr^{6+}(>40%) from the aqueous solution under different temperatures, pH, ion concentration, salinity, and time exposure. The interaction mechanism followed both Freundlich (Cd^{2+}) and Langmuir (Cr^{6+}, Ni^{2+}) isotherm models in accordance with pseudo-2nd-order kinetics. The reaction was both exothermic (Cd^{2+}, Ni^{2+}) and endothermic (Cr^{6+}) based on the choice of metal. Maximum loading capacities were 31.99 mg/g, 29.30 mg/g, and 13.68 mg/g for Cd^{2+}, Ni^{2+}, and Cr^{6+} respectively. Since wastewater is loaded with multiple metal species and different ions (cations and anions), the biomass-biosorbent was assessed for its efficacy of adsorption in the multimetal system as well the presence of cations and anions. In multi-component systems, adsorption capacity remained most consistent for Ni^{2+} followed by Cd^{2+} and Cr^{6+} confirmed by their relative adsorption capacity (Ri). FT-IR reveals that the carboxyl, amide, and hydroxyl are accountable for host-guest interaction. FESEM-EDAX analysis closely divulged the morphological alteration along with the adsorbed metal. A sequential adsorption-desorption study confirmed significant removal efficacy conservation up to 3rd cycle ($p<0.05$) making it gainful for commercial scale.

Keywords: *Eisenia fetida*, red mud, toxic metal, PLFA, soil health, crop growth, extremophilic, relative adsorption, isotherm, kinetics, thermodynamics, heavy metals.

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