Study the role of rhizospheric bacterial community during the phytoremediation of distillery waste

SUMMARY OF THE THESIS

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SUMMARY

Rhizospheric bacterial community has been reported primarily for the enhancement of plant growth and maintenance of soil fertility. The roles of plant growth promoting rhizobacteria (PGPR) are particularly revealed in agriculture sector for the crop production. Generally PGPR can be divided into two basic groups based upon their relation with plants. It may be symbiotic or free living bacteria. The PGPR influences to associated plant by three mode of action: (a) Releasing specific compounds required for plant growth (b) fascilitating the uptake of certain nutrients from the complex environment (c) inhibiting to pathogenic bacteria and preventing to the plant from diseases. Simultaneously, plant also releases the various root exudates i.e. organic acids, amino acids, amides, carbohydrates, enzymes and, phenolics, which work as a basic nutrient to the associated microbial communities. In response to the root exudates the associated microbial community contributes for solubilization of various micro and macronutrient for the bioavailability to the plant. Besides, the growing microbial community within the vicinity of the rhizosphere also releases out some specific chemicals which act as inhibitory effect upon pathogenic population in the rhizospheric zone. This mechanism of the PGPR is to protect the plant from the disease in field condition. Thus, this properties of PGPR in association to the plant root is a basic character for the adaptation and sustainable growth of plant communities and bacteria both in variable environment. However, the soil characteristic and geographical altitude also determines the growth of plant communities place to place.

But, the global rapid industrialization and deforestation created adverse effect on environment. This caused the environmental imbalance and immerged out as climate change and global warming. In India various agrobased industries i.e. distilleries, pulp paper industries, tanneries, textile etc. are major source of industrial waste discharge for environmental pollution. The conventional methods of wastewater treatment do not meet out the required standard of environment for waste disposal. Therefore, researcher has attempted from past few decades to develop the novel technologies for removal of hazardous and complex pollutants from discharge of industrial waste. But, yet the suitable technologies are still warranted. However, In order to adapt the microbial population and plant communities in the environment,

some potential plant species have been reported for phytoremediation of the polluted environment for the eco-restoration due to their specialized cellular structure and physiological adaptation. Keeping in view these pollutant characteristics phytoremediation has been reported as a green economical, feasible and novel technology for the eco-restoration of polluted site. Since the distillery waste is a complex and cumbersome waste with a mixture of organo-metallic compounds. Due to these properties, organo-metallic complex compounds of distillery waste persist for several years in environment and causes toxicity to flora and fauna. Moreover, the recent findings have revealed that the pollutants present in distillery waste have androgenic and mutagenic properties also (Chandra and Kumar, 2017). Since the phytoremediation potential plant is regulated by the specific bacterial community present in the rhizospheric zone. But, the detail nature of bacterial community growing around the root zone of plant at polluted site is not much known so far.

Hence, the present study has focused on the role of rhizospheric bacterial community during the phytoremediation of distillery waste. In this study, the potential non-edible plants have been evaluated for their phytoextraction potential of heavy metals from the complex sludge and effluent. These properties of bacterial communities with specific plants may be utilized for removal and detoxification of distillery waste for eco-restoration of polluted environment. The present thesis is divided into eleven chapters. The first chapter is Introduction of the topic. This chapter has imbibed the background information and rational of title of thesis, this information has been described in detail in this chapter. In the second chapter global and national states of art of the problems have been reviewed to understand the problem for development of new technologies. The chapter has also highlighted to understand the problem of distillery waste i.e. organic pollutants, heavy metals, Post Methanated distillery effluent (PMDE), and their environmental impact. Some bacterial species and consortium also have been reported for their capability to degrade the various pollutants present in the distillery waste. The dark brown colourant in the distillery wastewater is basically caramelized sugar which is known as melanoidins. The decolourizsation abilities of various bacteria have been reported in this chapter. The roles of some wetland plant for phytoremediation potential in different conditions are also mentioned. Though, the various attempts have been reported for decolourization and degradation of distillery wastewater at laboratory

stages by using different bacterial consortium but all these studies have been reported at very low concentration of effluent. Therefore, this restrict for pilot-scale up-gradation of technology. Therefore, in the present study, the study has been focused for decolourisation and degradation of distillery wastewater at high concentration for feasibility of technology. Moreover, the hyperaccumulator herbaceous plants are also screened out for heavy metal phytoextraction potential from complex organo-metallic distillery sludge for eco-restoration of polluted site.

Further, chapter three has focused on five objectives as per topic of thesis. Chapter four has focused on characterization of organic pollutants and heavy metals from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. The study has focused on the characterization of organic pollutants by using GC-MS technique and heavy metals through Inductively Coupled Plasma (ICP) from Post-Methanated Distillery Effluent (PMDE) and their environmental effect. Various unknown complex recalcitrant organometallic complexes with EDC properties which are a major source of environmental pollutants and health hazards are identified. The absorption maxima of PMDE were noted between 200–350 nm in the UV–Vis spectral analysis also indicated polymerized-form of Maillard product. Hexadecanoic acid, butyl ester, octadecanoic acid, monopalmitin 2 TMS, effusanin E, 1-(benzyl) -2-fluoro-2-phenyl-3-(p-toluene's sulfonyl) propane, and 24-hydroxy-3,4-secolanost-4,(28),8- died-3-nitrile were prominent organic compounds in the category of mutagenic and androgenic compounds characteristics, detected in PMDE. In addition, the presence of various activity in germinating Zea mays seeds and gel imaging at different concentrations of PMDE heavy metals also indicated the contribution of toxic property of PMDE, as resulting in phototoxic effect on seed germination with Zea mays and *Heteropneustes fossilis* with the degradation of lamellae in gill. Thus, this affirmed the source of various unknown mutagenic, carcinogenic, and EDCs compounds with organometallic complexes.

The fifth chapter summarizes the characterization of rhizospheric bacterial communities from hyperaccumulator plant growing on organometallic sludge during phytoextraction for ecorestoration. The findings of study revealed that *P. hysterophorus* is as hyperaccumulator for various heavy metals from complex organometallic wastes during its growth on the disposed distillery sludge. The

analysis of organic compounds showed degradation of pollutants present in sludge after growth of *P. hysterophorus*. Further, the histological observations of root by transmission electron microscopy (TEM) confirmed the deposition of metal granules in their tissue after accumulation by plant from distillery sludge. Besides, identified rhizospheric bacteria i.e. *Alcaligenes faecalis* (ON024323), *Cytobacillus firmus* (ON024324), *Bacillus* subtilis (ON024325) and, *Niallia circulans* (ON024326), also showed potential for plant growth promoting rhizobacteria activities which has supported the bacterial assisted phytoextraction potential of heavy metals from complex organometallic sludge of distillery from polluted site. This plant may be used as biotechnological tools for eco-restoration of polluted site by industrial waste as a green technology.

The sixth chapter showed the comparative assessment of phosphate, zinc and potassium solubilization by rhizospheric bacterial communities in *Phragmites communis* and *Typha spp. Phragmites communis* and *Typha spp.* is a naturally robust and vigorous primary species in many wetland environments worldwide. This plant has characteristic capacity to grow in different environmental conditions and can uptake, translocate, and accumulate a wide range of pollutants in both belowground and aboveground tissue. The ability of the plant to develop and grow in the polluted ecosystems allowed for the use of *Phragmites communis* in many types of sewage treatment plants also. To increase the efficiency of phytoremediation of a polluted natural or artificial aquatic ecosystem and to estimate the required purification time and accelerate the rate of its reclamation, the interaction processes between common *Phragmites communis* and soil microbes, metal accumulation, and ionic homeostasis in the hydrophyte purification systems should be further tested. The research carried out by interdisciplinary teams (plant physiologist, biochemist, geochemist, microbiologist, and agriculture and genetic engineer) in a short time can advance the efficiency of removing both metals and organic impurities.

Furthermore, the seventh chapter correlates bacterial community and organometallic pollutants during in-situ phytoremediation by *Parthenium hysterophorus* and *Cannabis sativa* growing on organometallic pollutants-rich hazardous distillery sludge. The sequence analysis of 16S rRNA V3–V4 hypervariable region with Illumina MiSeq platform showed 230166 and 277515

OTUs derived from rhizospheric distillery sludge samples out of 305005 sequences read, respectively. The major genus detected in rhizospheric sludge sample were uncultured- *chloroflexi* (0.8%), *Candidatus-solibacter* (1.4%), *Bacillus* (1.4%), *Flavobacterium* (1.4%), others (26.2%), *Bdellovibrio (*0.6%) and unknown (42.0%). Our results suggested that rhizospheric bacterial communities associated with *Parthenium hysterophorus* and *Cannabis sativa* were substantially different in richness, diversity, and relative abundance of taxa compared to rhizospheric sludge. Further, the comparative organic pollutant analysis from non-rhizospheric and rhizospheric sludge samples through GC–MS analysis revealed the disappearance of several compounds and generation of some compounds as new metabolic products by the activity of rhizospheric bacterial communities. The results of this study will be helpful in understanding the role of rhizospheric bacterial communities responsible for degradation and detoxification of complex organometallic waste and, thus, can help in designing appropriate phytoremediation studies for eco-restoration of polluted sites.

The eighth chapter has focused on characterization of rhizospheric bacterial communities of *Typha aungustifolia* during rhizofilteration of distillery effluent in constructed wetland treatment system by integrating bacterial degraded PMDE with constructed wetland treatment at pilot scale to evaluate the feasibility of technology based on the promising result. Post-methanated distillery effluent (PMDE) was found dark brown with very high BOD, COD, TDS and metallic content beyond its permissible limit. Hence, due to high TDS bacterial growth was not possible. Therefore, the TDS reduction was optimised for bacterial growth at variable range of pH and concentration. The optimum reduction of TDS was obtained with application of ferric chloride (0.34%) within 6-8hrs of incubation. The detail value of TDS and other pollution parameters are shown in table (Table 8.1 and 8.3). 71.70% decolorization was observed. The efficiency of decolorization and TDS reduction of PMDE was evaluated with rapid mixing along with the variable pH. The optimum TDS reduction resulted 80% supernatant and 20% precipitation of colloidal material. Further, the PMDE supernatant is assessed for bacterial growth in presence of variable nitrogen (0.5%, 1.0%, 1.5% & 2.0%) and carbon (0.5%, 1.0%, 1.5% & 2.0%) source to evaluate the bacterial growth for reduction of color from supernatant of PMDE. 79.45% decolorization was further observed. Subsequently, the biomass was

separated and supernatant was passed through the constructed wetland treatment system with plantation of *Typha angustata*. The reduction of TDS was observed 87.46%, BOD 96.38% and COD 99.37%. While the color was reduced up to 90.49%, and the others physicochemical parameters also reduced up to 95%. Therefore, the current chapter showed Biological decolourisation of post-methanated distillery effluent (PMDE) in biphasic bacterial and wetland plant treatment system for environmental safety and identification of potential bacteria.

The chapter nine has summarized the whole thesis work and findings with research output in a systematic manner. The chapter ten has compiled the all cited references of thesis which are relevant to the topic and they are cited in each chapter. Each chapter has cited the recent references based on methodology and results. The available references are cited on synthesis of melanoidin, physico-chemical properties of distillery wastewater, impact of post methanated distillery wastewater and plant growth promoting rhizobacteria reported at polluted sites. The complete thesis has 200 cited references.

The last chapter has listed all the scientific output based on the thesis work. There is two original research paper published in peer reviewed journal of international repute with high impact factor. Two review paper are also published based on topic of thesis have been published in the international journal and three book chapter as invited chapter published CRC press. A certificate of participation and best oral presentation award has been provided in international conferences. One international patent also has been granted based on the work of thesis in credit of candidate.