Bioremediation of distillery effluents through bio-adsorbent column and constructed wetland and assessment for fertigation of wheat

Summary of Thesis

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Summary

Rapid globalization and development and increasing demand in last few decades resulted in industrialization on one hand and pollution of the environment on the other. Distillery is one such industry which has grown rapidly in the country as well as in the world owing to its use in bioethanol/gasoline. Brazil is one such nation which is dependent on ethanol to meet its energy requirement. Government of India is also giving emphasis on blending of ethanol with petroleum hydrocarbons and has targeted to achieve a target of 20% from its present 5-10% by 2030. So, the production of ethanol is set to enhance in coming years. So, the distillery industry is going to occupy a major role in industrialization and development of the country. But, distilleries operate at the cost of environmental contamination as well as they generate high organic load containing dark brown colored wastewater. Therefore, distillery effluent needs to be treated before its final discharge into the environment and as the production is set to increase in coming years, more focus is to be given on this aspect. Various physic-chemical methods are available capable of both color and organic load reduction, but these methods are costly and generate a large amount of sludge as secondary pollutants. Hence, biological methods including use of constructed wetlands and macrophytes (use of plants for phytoremediation) are gaining momentum and a number of researchers are working in this arena of wastewater treatment methods due to their cost effectiveness and eco-friendly nature, but these methods are time-consuming and need more effective ways of treatment. Therefore, there is an urgent need to address the limitations in existing treatment methods and to develop the combined treatment processes that can provide a solution to distillery effluents treatment and their management for agricultural purposes as water is a

scarce commodity that is depleting very fast and for irrigation purposes its necessary to manage this kind of wastewater which will not only irrigate the crop but also provide essential nutrients present in them required by the microbes and crops. With this background, present work was under taken with constructed wetlands fitted with bio-adsorbent columns and use of macrophytes (phytoremediation) and beneficial microbes (bioremediation) and fertigation of wheat with this treated wastewater. Present study and its findings are summarized below:

The first objectives of this study focused on physicochemical characterization, screening of microbial population dynamics of distillery effluents. Second objective focused on isolation and characterization of bacterial isolates morphologically, biochemically and at molecular level with plant growth promotory attributes and biosurfactant production. Third objective was development of constructed wetland equipped with bio-adsorbent column fitted for the management of the effluents. Fourth objective focused on testing the efficacy/ bioremediation potential of macrophyte *Typha anguistifolia* and pseudomonads and their metabolites. Fifth objective was to test the efficacy of treated effluent on wheat crop at different dilutions (1:2, 1:3, 1:4) under fertigation and monitoring of wheat for agronomic parameters. Last objective was to study the impact of diluted effluent on beneficial microbial population dynamics of wheat rhizosphere (metagenomic analysis).

Physico-chemical analysis revealed that distillery wastewater was dark brown coloured, pH (7.63-8.18), chemical oxygen demand (COD) was 26850-62852 mg/L, biochemical oxygen demand (BOD) 9706-34659 mg/L, Total solids (TS) 5661-15557 mg/L, total nitrogen (TN) 1726-14593 mg/L, nitrite 37-40 mg/L, phosphate 56-599 mg/L, Sulfate 3128-33168 mg/L, chromium (Cr) 1.24-1.81 mg/L, cadmium (Cd) 1.73-2.57 mg/L, nickel (Ni) 3.15-7.16 mg/L, Zinc (Zn) 4.46-11.55 mg/L, iron (Fe)

102.26-193.76 mg/L, lead (Pb) 1.63-2.74 mg/L etc. Gas chromatography-mass spectrometry (GC-MS) analysis of distillery wastewater depicted presence of various organic pollutants such as naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha., 7.alpha.,8a.beta.)]-; bicyclo[3.1.1]heptane-2-methanol, 6,6-dimethyl-, acetate; 9-octadecenoic acid (Z)-, methyl ester; coronarin E and 1,2-benzenedicarboxylic Acid, octadecane; thieno [2,3-b]thiophene; iron, tricarbonyl[N-(phenyl-2-pyridinylmethylene)benzenamine-N,N']; n-nonadecanol-1 and heneicosane also reported in distillery wastewater. Some of them such as octadecane; heneicosane; 1,2-benzenedicarboxylic acid, butyl 8-methylnonyl ester; oxirane, tetradecyl-; 1,4-dioxane-2,5-diol, 2TBDMS derivative; n-tetracosanol-1 are listed as endocrine disruptive chemicals (EDCs) in the United States Environmental Protection Agency (USEPA, 2012). Some of them reported to cause acute toxicity, mutagenicity, developmental abnormalities, behavioural excitement, reproductive toxicity, respiratory irritation, aquatic toxicity, eye irritation, allergy or asthma, allergic skin reaction and corrosion, depression etc. Distillery industry is one of the major sources of environmental pollution like water and soil pollution.

Rhizospheric soil from naturally growing plant in distillery waste dumping site, sludge and distillery wastewater sample were collected for isolation of bacterial isolates. Further, on the basis of colonial differences, a total of twenty two isolates named SRRBL1, SRRBL2, SRRBL3, SRRBL4, SRRBL5, SRRBL6, SRRBL7, WRBL1, WRBL2, WRBL3, WRBL4, WRBL5, WRBL6, WRBL7, S1, S2, S3, S4, S5, S6, S7 and S8 isolates were isolated from rhizospheric soil, distillery wastewater and sludge. On the basis of plant growth promoting and biosurfactant producing properties screening only two isolates SRRBL1 and WRBL2 were selected. Morphological, biochemical and molecular characterization confirmed that SRRBL1

and WRBL2 isolates have 98.08% and 99.76% similarity to *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. Selected isolates were optimized for distillery wastewater degradation with simultaneous biosurfactant production. SRRBL1 reduced the COD, BOD, TS, TN, nitrite, phosphate and sulfate were 61.32%, 53.53%, 56.61%, 44.72%, 67.35%, 53.31% and 95.40% respectively from distillery wastewater . WRBL2 also reported reduction in pH (12.10%), TN (51.57%), COD (83.46%), BOD (68.13%), TS (83.49%), chloride (84.27%), potassium (73.81%), sulphate (70.36%), phosphate (90.04%), nitrate (87.27%), Na (43.52%), Ca (64.62%), Mg (35.60%), Fe (87.54%), Zn (78.84%), Cu (76.33%), Ni (48.10%), Pb (84.56%), Cr (65.82%) and Cd (41.02%) etc. from distillery effluents. FTIR, GC-MS analysis comparison of untreated and *Pseudomonas aeruginosa* SRRBL1 and *Bacillus subtilis* WRBL2 treated distillery effluent clearly indicated biodegradation. Further phytotoxicity assay of untreated and *P. aeruginosa* SRRBL1 and *B. subtilis* WRBL2 also confirmed toxicity reduction in distillery wastewater after treatment. At optimum conditions biosurfactant production was up to 2.9 g/L 1.02 g/L respectively by *Pseudomonas aeruginosa* SRRBL1 and *Bacillus subtilis* WRBL2. Further Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H NMR) and Liquid chromatography-mass spectrometry (LCMS) characterized confirmed that SRRBL1 and WRBL2 produced biosurfactant were di-rhamnolipid (glycolipids) and surfactin (lipopeptides) type, respectively. So, these observations suggested that *P. aeruginosa* SRRBL1 may be a potential candidate for di-rhamnolipid production by using Distillery Waste Water (DWW) as a substrate. Furthermore, multiple PGP properties with di-rhamnolipids production play an important role for societies and industrial sectors by reducing the cost by using DWW as a low cost substrate and can be applied in fields as a biological alternative to

chemical fertilizers. So production of di-rhamnolipid as a clean product during DWW treatment is an environmentally friendly and innovative approach. Further study is needed to explore the application of SRRBL1 augmented phytoremediation of DWW. Assessment of treated DWW depicted improvement in growth and productivity of crops through fertigation practices. SRRBL1 treatment reduced toxic waste generation and diminished the deleterious environmental impact of DWW with simultaneous value-added products production. Consequently, *P. aeruginosa* SRRBL1 treatment might be an eco-friendly economically efficient approach for DWW management. While in case of *Bacillus subtilis* WRBL2, producing surfactin type of surfactant further study would be needed to explore other industrial wastes for reduction of cost as use od DWW as a substrate is not very economical due to its low production.

Bio-adsorbent synthesis form *Typha* could be a cheaper and ecofriendly alternate for chemically synthesized activated carbon. Therefore, *Typha* activated bio-adsorbent was synthesized and characterized through FTIR, SEM, XRD and TGA analysis. Characterization confirmed that synthesized bio-adsorbent are amorphous, small sized with less impurity and thermally stable at high temperature.

Constructed wetland has mainly three components such as vegetation (macrophytes), supporting media, and microorganisms. In the current study *Typha anguistifolia* (cattail) were selected as a macrophytes in vertically flow constructed wetland (VFCW). The treatment efficiency of constructed wetlands depends on the metabolism of macrophytes Filter bed consisted of five successive layers from top to bottom, sa height of 7 cm autoclaved soil, fine sand, sand, fine gravel and coarse gravel. The media provided a path through which wastewater can flow and surfaces on which microorganisms can live and remove pollutants. They also help in pollutant reduction via adsorption, precipitation and filtration. Selected potential *Pseudomonas*

aeruginosa SRRBL1 and its metabolites inoculated as bacterial culture and it's producing metabolites as a crude biosurfactant used in VFCW. Besides distillery wastewater treatment, constructed wetlands also provide benefits to the environment by providing wildlife habitat, carbon sequestration, recreational uses, and add aesthetic value to the surroundings.

Constructed wetland equipped with bio-adsorbent is a combination of physico-chemical and biological treatment methods. Out of six combinations, vertically flow constructed wetland (VFCW) as a control, (C) VFCW with SRRBL1 producing crude biosurfactant (CB), VFCW with *Typha* plant (P), VFCW with plant and crude biosurfactant (P+CB) and VFCW with plant and SRRBL1 bacteria and VFCW with plant crude biosurfactant and bacteria (P+CB+B) equipped with bio-adsorbent column; treatment Vertical Flow Constructed Wetland (VFCW) equipped with bio-adsorbent column (P+CB+B) shows optimum reduction in colour, (98.38%), pH (25.95%), COD (90.25%), BOD (85.95%), total nitrogen (87.70%), Cr (98.09%), Cd (98.75%) and Fe (92.33%). *Pseudomonas aeruginosa* SRRBL1 transformed complex and toxic pollutants into less toxic compounds. Further degraded pollutants were utilized by microbes and phytoremediation by *Typha*. Various layers of constructed wetland work as a filter bed for precipitation and adsorption of pollutants. Further FTIR and GCMS analysis of constructed wetland equipped with bio-adsorbent column treated distillery effluents shows reduction/degradation of pollutants.

A broad range of stretching (FTIR) at 3554 cm^{-1} (–OH) group 2961 cm^{-1} C-H bonds in control reduced with different successive treatment combination with generations of new C=C conjugate and CH out of plane aromatic bond new small peak range from 2501-2499 cm^{-1} and 700-619, cm^{-1} generated during plant, crude biosurfactant and bacterial (P+CB+B) culture SRRBL1 treatment with bio-adsorbent column. Alcoholic

and nitrogenous compounds were formed at the final stage of the Maillard reaction as various high and low molecular weight melanoidin polymers. Moreover, organic acids and fatty acids also generated during the fermentation of sugars and the digestion or breakdown of complex melanoidin compounds. GCMS analysis of organic compounds extracted with ethyl acetate depicted the presence of docosyloctyl ether, oxalic acid, 6-ethyloct-3-yl heptyl ester, Phenol, 2,4-bis(1,1-dimethylethyl)-, phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) 1-Dodecanol, 2-hexyl-, 1-tridecanol Squalene Hexatriacontane 1-decanol, 2-hexyl. These compounds cause aquatic chronic neurotoxicity, genotoxicity, cytotoxicity, are irritant (skin, eye and throat) cause dizziness, headache, chronic multisymptom illness, gastric adenocarcinoma, haemorrhage toxicity and were found to be present in control (C) treatment while treatment P+CB+B resulted in remediation of these compounds. In the reports of USEPA, some of these compounds were persistent and some of them are listed under endocrine-disrupting chemicals (EDCs) as per the USEPA screening program list (USEPA 2012). However, the EDCs properties of various pollutants discharged in distillery effluents are still unknown and needs to be explored further. *Typha* based synthesized bio-adsorbent material could be applied on industrial and domestic wastewater adsorption. Synthetized bio-adsorbent material was ecofriendly and cost effective as compared to chemically synthesized activated carbon but further studies on its reusability and recycling needs to be taken up. More studies are needed to explore treatment efficiency of synthesized adsorbent with other biological materials (algae/fungi/microbial fuel cell) and their physico-chemical treatment methods. Further fertigation efficiency of constructed wetland equipped with adsorbent column treated distillery wastewater indicated that a particular dilution (1:2 tap water: treated distillery wastewater) enhanced the agronomical parameters such as length (root and

shoot) and biomass (fresh and dry) of wheat crop. Further, phytochemical analysis indicated that chlorophyll (a, b and total chlorophyll) and carotenoids were higher in treatment TA as compared to C, but it decreased with increasing concentration of treated distillery wastewater in TB and TC. Antioxidant activity in fertigated wheat plant indicated higher DPPH activity in 1:2 tap water: treated distillery wastewater (TB) as compared to control. But at higher concentration of DWW in 1:3 and 1:4 (tap water: Distiller effluent) gowth of the plant was reduced while ratio of tap water and treated distillery wastewater of DWW reduced plant growth and antioxidant activity in plants. Further microbial diversity based on metagenomcs studies revealed that major phylum, class, order, family and genus were Proteobacteria, Proteobacteria Desulfobacterales, Cellvibrionacceae, and Acidothermus, respectively in all rhizospheric soil of C, TA, TB, and TC treatment. Further, relative abundance of major phylum, class, order, family and genus is directly proportional to concentration of treated distillery wastewater. This confirms shift in relative abundance of bacterial diversity at different concentration of treated distillery wastewater.

So, constructed wetland equipped with bio-adsorbent column assisted treatment of distillery wastewater and its reuse as a fertigation could be sustainable solution to distillery wastewater management. This treatment approach could effectively reduce the environmental pollution caused by distillery wastewater with simultaneously reuse of treated wastewater as fertigation resulting in conserving huge amounts of freshwater (surface water and groundwater). Based on this work, three research papers have been published in high impact journal and one research paper is under consideration, while four conference papers have been presented in national and international conferences.