# Restoration and Detoxification of Petroleum Contaminated Soil by Biosurfactant producing Plant Growth Promotory Bacteria for Growing Medicinal Plants and Studying the Stress Responses Based on Metabolomics

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With the advent of industrialization during last few decades, demand for fossil fuel (especially Petro-products) as an energy source has increased dramatically which lead to massive anthropological activities including increased rate of oil extraction/refinement. This has simultaneously resulted in contamination of important agricultural soils and thereby has become a prime concern for environmentalists and policymakers. The soil pollution by petroleum hydrocarbons is primarily responsible for the changes in physicochemical properties of soils and drop in the oxygen level that made the soil unfit for agricultural purposes. Hence, for sustainability and hoping for a better tomorrow for our successors, scientific remediation approaches must be of pressing need. In this regard, many technologies have been planned and tested; however, in general, most of them are either very expensive or result only in incomplete removal of contaminants. Notably, substantial attention has paid to the event and application of the latest biologically techniques, that is effective in remedy and cost, not harming the prevailing surroundings. Hence, use of biosurfactant producing bacterial strains is one of the recent eco-friendly techniques.

We have propelled this idea in the present study by working on 4 bacterial strains (selected potent strains) that were isolated from petroleum contaminated sites of Lucknow (26°55' N latitude and 80° 59' E longitude), Uttar Pradesh and Guwahati (26° 11'N latitude and 91° 44'E longitude) Assam, India. Based on morphological, biochemicaland on 16S rRNA studies, isolated bacterial strains were found similar to *Pseudomonas azotoformans* (AJ15 and N23), *Bacillus licheniformis* (J2) and *Bacillus safensis* (J2). Accession numbers allotted are KU671026, KT951266,

KU680817, KX865089 respectively. The selected strains produced biosurfactant on mineral saltmedium (MSM). Each test strains produced foam with surfactants. All the strains show positive results for drop collapse test and hemolytic assay indicates that the strains produced biosurfactants. In addition, all the strain posses plant growth promoting traits. Thes strains AJ15 and N23 were found positive for phosphate solubilization. In a qualitative analysis of phosphate reddish zone around the colonies indicates that the bacterial strains has the ability to solubilize phosphate, whereas quantitative test for solubilization of tricalcium phosphate in liquid medium by the test strain showed that strain solubilize tricalcium phosphate. Further, the qualitative, as well as quantitative test for siderophore production on Chrome Azurol-S media, were found to be positive for the strains AJ15, J2 and N23 as indicated by the change in color of the media around the colonies. Quantitative estimation of Indole acetic acid (IAA) in the presence of 100 µg/ml concentrations of tryptophan indicates that the strains were able to produce a good amount of IAA. All the 5 strains show good ACC deaminase activity which demonstrates their ability to survive in abiotic stress condition.

Biosurfactants extracted from the selected bacterial are subjected to various experiments such as cell surface hydrophobicity, emulsification index, surface tension reduction, and oil displacement assay. The cell hydrophobicity of the strains showed an increase in adherence in the presence of extracted biosurfactants. Emulsification index of biosurfactant produced by the strain AJ15 is 62.7%, J1 is 73.36%, J2 is 68.66% and N23 is 77% respectively. The surface tension of non-inoculated broth was 65.8 mN/m. After 72 h, the surface tension of the broth was 30.5 mN/m for AJ15, 29.5 mN/m for J1, 34 mN/m for J2 and 29 mN/m for N23. These indicate that biosurfactant produced by the strains has the a good surface

tension reduction ability. Toxicity assessment of the extracted biosurfactant from the strains exhibited no inhibitory effect on seed germination and earthworm survival rate. The effect of pH, temperatures and salt concentration on biosurfactant was also studied. The temperature effect on biosurfactant showed that the extracted biosurfactants were quite stable at 30 °C. Heating of the crude biosurfactant up to 90 °C caused no significant effect on the emulsification and surface tension. Studies on the effect of pH suggest that biosurfactants were stable at pH 7. Further, the obtained results have confirmed that extracted biosurfactants remains stable in basic pH as compared to acidic pH. The effect of NaCl was investigated by increasing salt the concentrations from 1% to 10%. Very low effect of salinity was observed on biosurfactant stability till 5% concentration of NaCl, but increasing the concentration of the salt above 5% reduced activities of the all extracted biosurfactants except the biosurfactant from strain J2 shows tendency to persist its activity at high salt concentration. FTIR spectroscopy and Liquid chromatography–mass spectrometry (LC-MS) characterization of the extracted biosurfactant reveal that the biosurfactant extracted from AJ15 is monorhamnolipid and from strain N23 is both mono-rhamnolipid and di-rhamnolipid nature, whereas, biosurfactant extracted from J1 is lichenysin and from J2 is surfactin. Agroindustrial waste (bagasse) was employed for biosurfactant production and was optimized through Box-Behnken Design (BBD) model with 4 variable factors (Bagasse concentration, temperature, agitation rate and pH).

- The strain AJ15 produce maximum 0.92 g of biosurfactant at 30º C temperature, pH 7, 160 rpm rate by utilizing 10 g bagasse for 72 hrs.
- The strain J1 produce maximum 0.91 g of biosurfactant at 35º C temperature, pH 7, 100 rpm rate by utilizing 10 g bagasse for 72 hrs.

- The strain J2 produce maximum 0.92 g of biosurfactant at 35º C temperature, pH 7, 160 rpm rate by utilizing 15 g bagasse for 72 hrs.
- The strain N23 produce maximum 0.98 g of biosurfactant at 30º C temperature, pH 7, 160 rpm rate by utilizing 10 g bagasse for 72 hrs.

Further, the study involves the collection of contaminated soil from contaminated site of Lucknow, Uttar Pradesh, India. Gas chromatography-mass spectrometry (GC-MS) analysis of the contaminated soil reveals the presence of hydrocarbon in the soil. The detected hydrocarbon chromatogram is identified using (The National Institute of Standards and Technology) mass spectral library which reveals that the contaminated soil comprises 121 organic compounds and hydrocarbon that were toxic in nature, as demonstrated by seed germination inhibitory test and earthworm survival test. Then, after the contaminated soil was subjected to bioremediation treatment within lab scale using the selected bacterial strains. HPLC and GC-MS study reveal that the bacterial strains have the ability to remediate the petroleum contaminated soil and also focuses that the bacterial strain partially degrades the compounds in 90 days. But, for complete degradation of compound the degradation time period needs to be increase as most of the petroleum hydrocarbon are recalcitrant in nature.

Through mixing contaminated soil with garden soil, sand, bagasse, cow urine and bacterial strains, various treatments were also developed.

**Treatment 1 :(** Both contaminated and garden soil were autoclaved soil and used in the treatment)

**T11:** 1kg Petroleum contaminated soil + 1kg garden soil + Consortium
**T12:** 1kg Petroleum contaminated soil + 1kg garden soil + Strain AJ15
**T13:** 1kg Petroleum contaminated soil + 1kg garden soil + Strain J1
**T14:** 1kg Petroleum contaminated soil + 1kg garden soil + Strain J2

**T15:** 1kg Petroleum contaminated soil + 1kg garden soil + Strain N23

**T16:** 1kg Petroleum contaminated soil + 1kg garden soil (control)

**Treatment 2: (** Both contaminated and garden soil were autoclaved soil and used in the treatment)

**T21:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Consortium

**T22:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain AJ15

**T23:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain J1

**T24:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain J2

**T25:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain N23

**T26:** 1kg Petroleum contaminated soil + 250 g sand + 750 g garden soil (control)

**Treatment 3: (Both** contaminated and garden soil were autoclaved soil and used in the treatment)

**T31:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Consortium + 15 ml cow urine

**T32:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain AJ15+ 15 ml cow urine

**T33:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain J1+ 15 ml cow urine

**T34:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain J2+ 15 ml cow urine

**T35:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain N23+ 15 ml cow urine

**T36:** 1kg Petroleum contaminated soil + 250 g sand + 750 g garden soil + 15 ml cow urine (control)

**Treatment 4: (**Both contaminated and garden soil were autoclaved soil and used in the treatment)

**T41:** 1kg Petroleum contaminated soil + 750 g garden soil + 235g sand + Consortium + 15 ml cow urine + 15 g sugarcane bagasse

**T42:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain AJ15+ 15 ml cow urine + 15 g sugarcane bagasse

**T43:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain J1+ 15 ml cow urine + 15 g sugarcane bagasse

**T44:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain J2+ 15 ml cow urine + 15 g sugarcane bagasse

**T45:** 1kg Petroleum contaminated soil + 750 g garden soil + 250 g sand + Strain N23+ 15 ml cow urine + 15 g sugarcane bagasse

**T46:** 1kg Petroleum contaminated soil + 250 g sand + 750 g garden soil + 15 ml cow urine + 15 g sugarcane bagasse (control)

**Treatment 5: :(** Both contaminated and garden soil were not autoclaved soil and used in the treatment)

**T51:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Consortium + 15 ml cow urine + 15 g sugarcane bagasse

**T52:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain AJ15+ 15 ml cow urine + 15 g sugarcane bagasse

**T53:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain J1+ 15 ml cow urine + 15 g sugarcane bagasse

**T54**: 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain J2+ 15 ml cow urine + 15 g sugarcane bagasse

**T55:** 1kg Petroleum contaminated soil + 750 g garden soil + 235 g sand + Strain N23+ 15 ml cow urine + 15 g sugarcane bagasse

**T56:** 1kg Petroleum contaminated soil + 235g sand + 750 g garden soil + 15 ml cow urine + 15 g sugarcane bagasse (control)

The developed treatment pots were allowed to stabilize for 90 days. The seeds of *Withania somnifera* were sown in the pots after 90 days. The plant arose from a bacterial treated pot in various treatment expressed high values for all the parameters studied *viz.*germination, shoot length, root length, fresh and dry weight as compared

to the treatment (T16, T26, T36, T46 and T56) without bacterial strains.Further, metabolomics modeling based on the phytochemicals and antioxidant activity infers the variation in the stress effect of petroleum contaminated soil on the treatment with and without bacterial strains.