



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student : Rahul Kumar

Roll Number : 166107116

Programme of Study : Ph.D.

Thesis Title: Biodegradation of petroleum hydrocarbons by mixed bacterial cultures isolated from local contaminated sources

Name of Thesis Supervisor(s) : Prof. Mahuya De

Thesis Submitted to the Department/ Center : Chemical Engineering

Date of completion of Thesis Viva-Voce Exam : 28-01-2025

Key words for description of Thesis Work : Petroleum hydrocarbons, Pollution, Biodegradation, Bacteria, Mixed culture

SHORT ABSTRACT

Petroleum hydrocarbons, essential for industry, transportation, and daily life, often lead to environmental pollution due to spills during production, refining, and transportation of crude oil and its derivatives. To address hydrocarbon pollution, bacterial degradation offers a practical, economical, and environmentally friendly solution. This study aims to develop an effective bacterial mixed culture using newly isolated bacteria from locally contaminated sources for the degradation of petroleum hydrocarbons and remediation of diesel pollution in soil and water. Emphasis was given on isolating strains from local sources to develop more adaptable and effective strains for local use.

Bacterial strains were isolated from various contaminating sources, and most effective strains were selected based on degradation abilities of hydrocarbons. Optimal degradation conditions were identified. Strains showing the highest degradation were further evaluated in soil, marine water, and freshwater ecosystems. A simulated field study using the mixed culture of was conducted in diesel-contaminated water and soil. The treated soil and water were subsequently used for phytotoxicity test with black mustard plants to assess their usability.

Two strains, *Klebsiella michiganensis* RK and *Acinetobacter baumannii* IITG19 were isolated from a local petrol pump and repairing workshop respectively and showed highest overall degradation for diesel at 60 and 76 %, respectively after 15 days of incubation period. For the significant compounds the degradation reached up to 77 and 90%, respectively. *Pseudomonas aeruginosa* IITG21 and *Providencia vermicola* IITG20 were isolated from local refinery waste and showed highest diesel degradation of 70% and 76%, respectively, during same period. The *Acinetobacter baumannii* IITG19 showed higher

degradation than *Providencia vermicola* IITG20 in all ecosystems. Their mixed culture, showed degradation of 66%, 66%, and 81% in the soil, marine water, and freshwater ecosystems, respectively. The mixed culture containing all the four isolated bacteria, showed 90% degradation of 4% v/v diesel in 15 days in lab scale study. Subsequent field studies in soil and water resulted in 96 and 99%, respectively. Analysis of degradation kinetic of mixed culture confirmed first-order degradation with a rate constant (k) of 0.144 day⁻¹ and half-life of 4.7 days in lab scale study. The kinetics of the mixed culture was enhanced in field studies resulting in shortened half-lives of 3.5, and 2.4 days in soil and water, respectively. Phytotoxicity study on black mustard seed growth established the efficacy of the remediation by the mixed bacterial culture by showing positive growth with 68-70% germination rates.

The study highlights the higher efficiency of bacterial mixed culture over single-strain applications for diesel degradation. The mixed culture, consisting of *Acinetobacter baumannii* IITG19, *Providencia vermicola* IITG20, *Pseudomonas aeruginosa* IITG21, and *Klebsiella michiganensis* RK, showed almost complete degradation of hydrocarbons in field conditions. Notably, *Klebsiella michiganensis* RK is reported for the first time in hydrocarbon degradation studies. These findings suggest that mixed bacterial cultures offer a viable, environmentally sustainable solution for petroleum hydrocarbon remediation. Future research should focus on pilot-scale applications in industrial bioreactors or directly at contaminated sites to validate large-scale feasibility and long-term ecological impact.