The Use of Oil-Degrading Bacteria in Crude Oil Tankers for Spill Mitigation

Iulia Sircu

Specialization: Environmental Engineering, BSc

Institute: Institute of Aquaculture and Environmental Safety, Department of Ecotoxicology

Insider subject leader: István Szabó, habil. PhD. (DET), head of department

This thesis examines the biodegradation potential of hydrocarbon-degrading bacteria acquired from hydrocarbon contaminated sites, with the goal of assessing the efficiency of biodegradative strains to be used for bioremediation assessments and responses for cleaning up spills. The evaluation of biodegradative capacity was conducted with the use of a two-stage experiment. The first stage of the experiment involved screening for bacterial activity using the OIR III mineral medium, with resazurin as a redox indicator. Bacterial hydrocarbon degradation was tracked based on colorimetric change and spectrophotometric reading at 550nm and 620 nm in 72 hours, in 96 hours and finally within 168 hours. The results were used to infer the hydrocarbon-degrading ability of each strain. The top six most active strains included *Rhodococcus qingshengii* BA4.9, *Rhodococcus qingshengii* PT2/14B, *Rhodococcus pyridinivorans* K404, *Rhodococcus erythropolis* GP2b, *Pseudomonas aromaticivorans* MAP12, and *Mycobacterium trichoteceniticum* R17. The second stage of the experiment included gravimetric analysis conducted using crude oil and diesel fuel as the sole carbon source. Bacterial stains, suspended in OIR III medium with hydrocarbons, were incubated on a shaker for 168 h at room temperature 22-24 °C. After that remaining oil was separated from the media using petroleum ether and chloroform wash. Heidolph rotary evaporator was used to remove petroleum ether and chloroform with boiling(petroleum ether 50–60 °C; chloroform 60–65 °C). Once the evaporation was complete, flasks were placed in a drying oven at 65 °C for 45 min. Only after these processes residual oil weight was measured and compared with the initial one. The strains belonging to the genus *Rhodococcus*, especially *R. qingshengii* BA4.9, demonstrated the greatest degradation rates for hydrocarbons, with volumetric removal of >70% of the initial oil mass in 7 days at 22-24 °C under aerobic conditions. Strains *Rhodococcus qingshengii* PT2/14B and *Rhodococcus erythropolis* GP2b exhibited promising activity in utilizing hydrocarbons(degradation rate of 67.05% and 69.34%, respectively.), while *Mycobacterium trichoteceniticum* R17 degraded half of the crude oil mixture. *Pseudomonas aromaticivorans* MAP12 degraded less than others, just 31.61%, whereas the lowest degradation efficiency was found for *Rhodococcus pyridinivorans* K404 at 23.80%.

The study provides evidence that bacteria derived from contaminated sites can utilize hydrocarbons as a source of carbon. The combined use of plate screening and gravimetry test

results in more efficient determination of bacterial strains. With the degrading abilities such as *Rhodococcus qingshenigii* BA4.9 has, it could be developed as a tool for bioremediation in oil impacted environments.