INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title	:	Treatment of Hydrocarbon-rich and Complex Refinery Wastewater in Aerobic Granular Reactors (AGR) with Polyhydroxyalkanoates (PHA) Biopolymer Production
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SHORT ABSTRACT

The modern era of bioremediation has been practicing aerobic granulation technology since last three decades in various recalcitrant industrial wastewater treatments. Compact granular structure, excellent settling property, presence of numerous pollutant degrading microbes and high tolerance to toxicity made granulation a promising biotechnology. Petroleum industries are the producers of toxic and poorly-biodegradable hydrocarbon polluted wastewater. Our first study provides extensive information about oily wastewater treatment in aerobic granular reactors (AGR) using three different inocula from sewage, refinery and brewery. In emulsified diesel exposure, previous oil adaptation helped the refinery sludge granules to achieve maximum granule size of 3.49±0.01 mm and extracellular polymeric substances (EPS: 404 mg/g volatile suspended solids (VSS)) with maximum 67.39±0.15% oil removal efficiency. *Brevibacterium paucivorans* strain SG001, *Micrococcus aloeverae* strain SG002 and *Staphylococcus hominis* strain SG003 were identified as the major pollutant degraders isolated from sewage, refinery and brewery granules. *Micrococcus aloeverae* exhibited maximum hydrocarbon removal efficiencies (oil: 61.34±0.85%) among the three species. Re-addition of sodium acetate reconstructed broken granules.

We further checked the impacts of hydraulic retention time (HRT) on granule characteristics, hydrocarbon removal efficiency and removal mechanism while treating emulsified diesel wastewater in AGRs. AGR with shortest HRT (12 h) achieved maximum 4.72±0.05 mm granule size, 400.31±0.01 mg/g VSS of EPS among the AGRs. But longest HRT (48 h) played major role providing longer reaction time for 90.31±0.26% hydrocarbon removal. Degradation of short and long chain alkanes (C_6-C_7, C_9-C_{10}, C_{11}-C_{13}, C_{15}-C_{18}, C_{27}) were observed in the AGRs which further produced fatty acids as metabolites. About 24-48 h HRT with 0.125-0.25 kg/m^3.day hydrocarbon loadings providing below 77 mg/L effluent hydrocarbon was recommended for AGRs to avoid phytotoxicity after effluent disposal.

Rapid granulation of oil degrader *Micrococcus aloeverae* strain SG002 was conducted with investigation of its most efficient approach of bioaugmentation in AGRs to reduce granule rupture and enhance pollutant removal in multiple hydrocarbon laden refinery wastewater treatment. Highly settleable *Micrococcus* granules with 0.5±0.02 mm size formed in 15 days achieving 80% organics removal. In control AGR, recalcitrant hydrocarbons deteriorated size and stability of aerobic granules. About 2 doses of 10% (w/w) granular augmentation of *Micrococcus* facilitated cocci

abundant granule microstructure with 404.65±1.45 mg/g VSS of EPS content ensuring simultaneous 99% phenolics removal and 19% nitrification in AGR. Degradation of C_8-C_{15} *n*-alkanes followed partial fatty acid conversion in control reactor and C_6-C_{36} removal was observed indicating β-oxidation of fatty acids in bioaugmented AGRs.

Thereafter, accumulation of polyhydroxyalkanoates (PHA) biopolymers was observed in aerobic granules having mixed sludge and pure strain inoculum. Small sized (0.71±0.04 mm) *Micrococcus* granules achieved 81.40±0.2% hydrocarbon removal efficiency accumulating 0.47±0.01 mg PHA/mg cell dry weight (CDW) due to cocci populated strong microbial structure. Changing organic loading (0.6-1.8 kg COD/m^3.day) and high C/N (8-24) ratio stimulated 0.71±0.04 mg PHA/mg CDW yield in the refinery granules with 90% hydrocarbon removal. Long and short chain *n*-alkanes (C_{16}-C_{36}, C_6-C_{10}) were mostly biotransformed into PHA. Granule extracted PHA was characterized as copolymer P(3HB-*co*-3HV) having 3.5-4.5 of butyrates and valerates (PHB:PHV) ratios.

At last, a batch scale study was conducted to produce salt tolerant aerobic granules of *Brevibacterium paucivorans*, *Staphylococcus hominis* and *Micrococcus aloeverae* evaluating organics removal and nitrification efficiencies in refinery wastewater treatment. Each strain separately formed granules in 20-25 days. In *Brevibacterium*, granule rupture took place at 20 g/L NaCl influence. Refinery sludge origin and thick layer of 226 mg/g VSS EPS prevented 35 g/L NaCl saline inhibition in *Micrococcus* granules improving nitrification up to 28% with 93% hydrocarbon removal in 12 h of batch operation.