Thesis Title: ANAEROBIC DIGESTION OF PETROLEUM REFINERY SLUDGE: EFFECT OF PRETREATMENT AND CO-DIGESTION

ABSTRACT

Rapid industrialization in conjunction with urbanization is devouring natural resources worldwide and their processing or refining into useful products are leading to mounting global waste generation. Petroleum refinery sludge (PRS), an egregious solid residue generated from the effluent treatment plants (ETPs) of petrochemical refineries, poses an environmental hazard owing to its intricate hydrocarbon composition, necessitating competent treatment for secure disposal. The PRS generated from the biological treatment units of the ETPs has enormous potential for biogas production through anaerobic digestion. Selection of appropriate inoculum is crucial to foster a well-balanced environment with favourable microbial communities for initiating the anaerobic digestion process. In order to select an appropriate inoculum, the biodegradation of PRS was compared between undigested residue of animal manure, and anaerobically acclimated or digested sludge from an operational anaerobic reactor. Upon performing 1L batch anaerobic biodegradation assays, digested sludge provided a better seeding environment exhibiting maximum biogas yield with higher organic fraction removal resulting in improved biodegradability. 16S metagenome sequencing of digested sludge revealed significant predominance of *Proteobacteria*, *Bacteroidetes* and *Firmicutes* at phylum level possessing hydrocarbon-degrading properties.

However, the hydrolysis phase of the complex PRS substrate was lengthy due to its recalcitrant nature. To overcome this, different pretreatment techniques were applied (thermal, electrokinetic, and microbial). Thermal pretreatment involved the optimization of different modes of heat application (dry heat, pressurized moist heat, agitated open moist heat and microwave irradiation). Dry heat at 140 °C for 60 min proved most effective, leading to a 40% increase in biogas production and 48.7% organic matter removal. Electrokinetic pretreatment was optimized using central composite design- response surface methodology (CCD-RSM) to ascertain a combined variation of applied voltage (40-80 V), exposure duration (20-120 min) and distance between graphite electrodes (8-16 cm). Electrokinetic pretreatment at 60 V, 83.5 min, and 11.6 cm electrode spacing resulted in maximum solubilization, leading to 61% enhancement in biogas production. Furthermore, microbial pretreatment was optimized utilizing two bacterial strains, Laccase enzyme-producing *Pseudomonas putida* 7525, and novel lignin peroxidase (LiP) enzyme-producing *Kosakonia oryziphila* IH3 (MZ605201) to optimize the accelerated solubilization of PRS. Pretreatment utilizing *P. putida* at a dosage of 10^8 colony forming units per mL (CFU/mL) resulted in maximum solubilization within 6 d, leading to 54.6% biogas augmentation. Consequently, pretreatment with *K. oryziphila* at a dosage of 10^8 CFU/mL maximized solubilization within 4 d of pretreatment, leading to 50.2% biogas enhancement. Scaled up (20 L) anaerobic biodegradability batch studies revealed maximum removals of total petroleum hydrocarbon (57.3%), and oil and grease (71.5%) for electrokinetically pretreated PRS whereas, utmost removal of total phenol (91.7%) was observed for *P. putida* pretreated PRS. The phytotoxicity assay conducted on batch digestates using *Vigna radiata* L. showed concentration-dependent decrease in seed germination, shoot length, root length and biomass compared to control (untreated PRS), thereby, suggesting dilution of toxicant concentrations.

Initial characterization of PRS expressed the restriction of biodegradability due to lack of nutrient balance and substrate recalcitrance. Anaerobic co-digestion of nitrogen-rich PRS was performed with carbon-rich yard waste (YW), balancing the nutrients and moisture content for efficient microbial proliferation. Using CCD-RSM, anaerobic biodegradability batch experiments were conducted with varying carbon/nitrogen (C/N) ratios and pH to achieve maximum biogas at C/N= 32.5 and pH = 7.0. However, the sluggish biogas recovery (40%) indicated a slow rate-limiting hydrolysis in absence of pretreatment. Electrokinetically-assisted co-digestion process, when optimized at an applied voltage of 53.5 V for 53 min maximized solubilization of PRS mixed with YW at optimum co-digestion condition. Upscaled batch studies resulted in 84.2% enhancement in biogas production compared to monodigestion of PRS with significant total petroleum hydrocarbons, emulsions, and lignocellulosic degradation. Consequently, when microbially-assisted pretreatment was conducted utilizing two bacterial strains isolated from PRS (LiP enzyme-producing *Bacillus subtilis* IH1, MZ618640 and Lac enzyme-producing *Bacillus velezensis* IH2, MZ605121), *B. subtilis* IH1 strain led to maximum improvement in solubilization, resulting in 76% enhancement in biogas against monodigestion of PRS. Further identification of major organic pollutants in the batch digestate revealed significant degradation of the toxic organic hydrocarbon pollutants apotheosizing the efficacy of the synergistic sustainable technique for the management of PRS.

In order to ascertain the feasibility of anaerobic biodegradation of PRS, a lab-scale 20 L anaerobic biphased baffled reactor (ABBR) was operated in semi-continuous mode for 200 d. The ABBR was operated with untreated PRS for 100 d resulting in average methane content of 47.5%, at an OLR of 1.6 kg COD m^{-3} d^{-1} along with a mere 19.5% COD removal. Operation of electrokinetically pretreated PRS at optimum condition obtained from the electrokinetic pretreatment (60V, 83.5 min, 11.6 cm electrode spacing) resulted in average methane content of 61% at optimum OLR of 1.5 kg COD m^{-3} d^{-1}, along with 70.6% COD removal. Electrokinetic pretreatment of PRS resulted in enhanced solubilization with average methane enhancement by 1.3-folds suggesting enhanced degradability and improved process stability.