ABSTRACT

Global population rise and industrializations have led to the emergence of three major issues such as water scarcity, environmental pollution and energy crisis. The best feasible solution to augment potable water availability is desalination of brackish water and sea water. But the current desalination technologies are energy-intensive. The control of environmental pollution demands proper disposal of industrial effluents and other contaminants, which also requires large amount of energy. The use of fossil fuels as energy sources releases more CO$_2$ to the atmosphere and causes global climate change. Therefore, it has become a need of the hour to find out a novel method to address these problems in an energy efficient way. Microbial Desalination Cell (MDC) technology has been emerged as a carbon-neutral, sustainable method which can simultaneously alleviate energy crisis, desalinate salt water and biodegrade toxic pollutants.

In the present investigation, the optimized MDC reactors were found to have higher efficiency in power generation and COD removal than a Microbial Fuel Cell (MFC), when operated under same conditions. It was observed that MDC produced maximum power density of 7.01±0.46mW/m$^2$ (6.86±0.62mW/m$^2$ for MFC), COD removal of 85±3% (79±6% for MFC) and 72.34±0.35% desalination with *Shewanella putrefaciens* as the anodic inoculum. Two elite hydrocarbon degrading bacteria isolated from oil contaminated sludge were found to have good degradation ability towards waste engine oil within a short incubation period. The isolates were identified as *Bacillus subtilis moh3* (NCBI accession no. KF021537) and *Pseudomonas stutzeri* by morphological, biochemical and molecular characterization. *B. subtilis* and *P. stutzeri* degraded the oil by 63.17± 0.61% and 59.86 ± 0.21
% respectively within 21 days. Different parameters such as temperature, pH, carbon source and nitrogen source were optimized for maximum growth and biodegradation. Both the isolates showed positive results for DCPIP assay (colourless reaction mixture indicated positive result) and dehydrogenase assay (blue coloured colonies indicated positive result). This confirmed the hydrocarbon degradation ability of the isolates.

*Bacillus subtilis* moh3 was found to exhibit good electrochemical activity when used as inoculums in MDC with maximum power density of 6.81±0.36mW/m², 70% desalination and 81.2% COD removal. The anodophilic nature of *B.subtilis* was proved by scanning electron microscopic examination of the anode biofilm and by cyclic voltammetric analysis. Hence, the isolated bacterium was identified as a good candidate for bioremediation applications in MDC.

The waste engine oil treated in MDC with *B.subtilis* cultures yielded degradation of 74.77±0.69% and desalination of 68.36 ± 0.6% within 14 days. This indicated an enhanced and accelerated biodegradation in MDC when compared to the batch biodegradation in shake flasks. Maximum power density of 3.1 ± 0.3mW/m² and current density of 142.9±0.6mA/m² were observed by polarization and power density curves with varying external resistances. The treated oil was analyzed by FT-IR and GC-MS and it was observed that the long chain, branched and cyclic aliphatic hydrocarbons were degraded into simpler and harmless byproducts.

*B.subtilis* and *P.stutzeri* were found to be potent biosurfactant producers and this property could be attributable to their hydrocarbon degradation capacity. Biosurfactant produced by *B.subtilis* exhibited more reduced surface tension value than that produced by *P.stutzeri*. The crude biosurfactant from *B.subtilis* exhibited surface tension value of 32.28±0.42
mN/M and *P. stutzeri* showed a surface tension value of 36.44±0.26 mN/M. *B. subtilis* produced maximum biosurfactants during late log phase of growth and production was completed within 72 hours. Similarly, the maximum biosurfactant production was observed during the stationary phase of growth and the production was completed within 96 hours of growth for *P. stutzeri*. It was seen that both biomass and biosurfactant yield were higher in *P. stutzeri* than *B. subtilis*, but the surface tension values were lower in *B. subtilis* supernatants indicating its potential as an efficient biosurfactant producer.

The biosurfactants extracted from culture broth by foam fractionation method were found to recover more biosurfactants with surface tension values of 30.86±0.34mN/M and 36.84±0.36mN/M for *B. subtilis* and *P. stutzeri* respectively. Biochemical characterization revealed the chemical nature of extracted biosurfactants tentatively as lipopeptides from *B. subtilis* and rhamnolipids from *P. stutzeri*. This was further confirmed by FTIR analysis after purification by TLC. These purified biosurfactants exhibited even lower surface tension values as 26.28mN/M for lipopetide biosurfactant and 31.6mN/M for rhamnolipid biosurfactant.

A lower CMC value of 60mg/L was observed for *Bacillus* biosurfactant than *Pseudomonas* with a value of 80mg/L. Stability of biosurfactants was checked under wide ranges of temperature, pH and salinity conditions. Both the biosurfactants showed significant stability over the range of conditions where lipopetide biosurfactant was found to be highly stable than the rhamnolipid biosurfactant. Both the biosurfactants were found to have antimicrobial activity against a number of pathogenic bacteria. Both biosurfactants showed inhibition to the other isolate as well. The antimicrobial property is of high significance when these biosurfactants are used for diverse application such as in medical, food or cosmetic industries.
B. subtilis was able to decolourize two model dyes malachite green and sunset yellow. Both the dyes were decolourized completely within 48 hours of treatment in MDC. HPLC analysis of treated effluents confirmed the biodegradation of the dye structure in both the dyes. Higher power production and desalination were observed for MG-MDC with maximum power densities of 3.01±0.04mW/m$^2$ for MG-MDC and 2.86±0.25mW/m$^2$ for SY-MDC.

The biodegradation kinetics of engine oil degradation was found to follow second order kinetic model. The parameters obtained from experimental data for biodegradation in both batch experiment as well as MDC, fitted well to Quiroga’s second order kinetic model with R$^2$ values >0.91 at all the initial concentrations, with the maximum of 0.99 for MDC data. The process parameters such as maximum growth rate of bacteria, maximum substrate concentrations and minimum substrate concentrations were predicted by the model. Theoretical degradation percentage was calculated using these parameters and found that there was a good agreement between the theoretical and experimental biodegradation pattern especially for MDC assisted biodegradation.