ABSTRACT

The ever-increasing demand of crude oil is leading to its rapid consumption, causing a scarcity in the conventional fuels available. Hence, newer and alternative energy resources are needed. Even with the emerging renewable energy resources, yet today, the maximum demand of energy resource is met with coal and crude oil. Thus, before we are completely able to shift to green and clean energy, efficient utilisation of the existing non-renewable energy resource is necessary. Most of the fuel products such as petrol, diesel, gasoline, etc are derived from light crude oils. It is because, the high density and viscosity of heavy crude oils makes them unsuitable to be used for commercial production using the available conventional methods. The number of heavy crude oil reservoirs in the world are about seven times more in number than the rapidly utilised light crude oil reservoirs. This makes the heavy crude oil reserves a large untapped source of energy. The reason for such heaviness of these oils is the presence of asphaltenes in them. Biotransformation of asphaltene can lead to a breakdown of its structure resulting in a decrease in viscosity and density of heavy oil. By targeting asphaltene breakdown, the trapped energy from heavy oil reservoirs could be harnessed to meet with the world's energy demand. A nine membered microbial consortium has been reported to biotransform about 75% asphaltene in 21 days. The members have been reported to secrete enzymes which can act on asphaltene (Zargar 2021). The study carried out in this thesis works on the focused biotransformation of asphaltene using those enzymes. This provides a biological, ecofriendly and cost-effective method of asphaltene biotransformation.

In the present study, the genome of all members of the consortium were assessed to find the presence of genes acting on aromatic hydrocarbons and alkane chains. The various enzymes secreted extracellularly by the consortium during biotransformation were analysed. Based on the genome and secretome data, certain enzymes known to act on sulphur and nitrogen were

investigated to check for their role in asphaltene biotransformation. The genes were cloned, and the protein was overexpressed in a heterologous host. The action of the enzymes on asphaltene was assessed. An enzyme thiol peroxidase from four different strains of the consortium was tested for its asphaltene biotransformation efficiency. The biotransformation achieved by the several thiol peroxidases overexpressed from a heterologous host, ranged between 45 to 64.5%, the highest being from the thiol peroxidase of *Micrococcus* sp. IITD107. The enzyme was purified, and its enzyme activity and kinetics were studied. The action of the enzyme was studied on asphaltene and characterization of transformed asphaltene was done. The asphaltene fraction was analysed by GC-MS and it was found that during enzymatic biotransformation, several peaks corresponding to PAHs were found to reduce in size as respect to control. The FTIR and NMR spectra indicated changes in the functional group and chemical bonds of treated asphaltene. Change in elemental composition was checked and a reduction in sulphur and nitrogen content was observed whereas the carbon and hydrogen remained largely unaffected. The change in aromaticity levels of asphaltene and model polyaromatic hydrocarbon (PAH) compounds due to the action of the enzyme was studied to find a reduction in their levels. Change in surface morphology was assessed by Scanning Electron Microscopy (SEM). The role of the enzyme on asphaltene biotransformation was confirmed by overexpressing the gene in its native host. The recombinant *Micrococcus* showed increased rate of biotransformation as compared to wild type. The gene was also successfully deleted by homologous recombination in the native host. The deletion of gene led to a major drop in the asphaltene biotransformation capability of the host strain as compared to wild type. The purified enzyme was immobilised for creation of a packed bed column which could be applied for asphaltene biotransformation. Model oil when passed through this column was found to have a 34 % reduction in weight of asphaltene in just 6 hours of treatment whereas asphaltene from crude oil reduced by 23.5% in weight in 48 hours. Due to action of the enzyme on

asphaltene, the smooth surface of asphaltene was converted into a porous structure. This led to the development of a novel and rapid enzymatic process for development of porous carbon from asphaltene. The process can lead to successful valorization of asphaltene into useful porous carbons.