ABSTRACT

Worldwide reservoirs of heavy crude oils are seven times more abundant than light crude oil reserves, but they are underutilized due to their high viscosity and density, which is primarily due to the presence of large amounts of asphaltenes. It is possible to reduce the viscosity of heavy oil by biotransforming its asphaltene into smaller metabolites. Although several bacteria capable of biodegrading asphaltene have been identified, only a few have been characterized for biotransformation. The study conducted in this thesis aimed at development of an efficient and optimized process for biotransformation of heavy oil asphaltenes using an isolated microbial consortium. The work also aimed to develop a process for biological upgradation of heavy crude oil to reduce their viscosity and enhance their flow characteristics such that the upgraded oil can be used in place of conventional crude oil.

In this study, a 9 membered bacterial consortium previously isolated in our lab was characterized and the members of the microbial consortium were identified using 16 S rRNA sequencing. The bacterial consortium was further characterized for its ability to biodegrade and biotransform asphaltene. The consortium was capable of degrading only 35 % of asphaltene within 7 weeks. However, the consortium exhibited higher efficiency in biotransforming asphaltene and resulted in 78 % transformation within 7 weeks. Using higher inoculum size, the biotransformation time was reduced from 7 weeks to 3 weeks and 73.5 % asphaltene biotransformation was obtained. Several control experiments were performed to confirm the asphaltene biotransformation potential of the microbial consortium.

Structural changes in asphaltene due to its biotransformation were determined using NMR, FT-IR, CHNS analysis and it was found that as a result of biotransformation, around 80% decrease in N and S content of asphaltene was obtained. Also, oxygen was found to be introduced into the structure of asphaltene suggesting that oxygenases may be produced by the strains of the microbial consortium which catalyze biotransformation of the asphaltene.

Asphaltene biotransformation was further explored at higher temperature using higher percentage of oil was and it was found that under these conditions the amount of asphaltene biotransformed was limited to 35 %. To increase the extent of biotransformation under these conditions, statistical optimization of the culture medium and the process conditions was performed. Asphaltene biotransformation was scaled up from shake flasks to lab scale reactors and the reactors were operated in various modes which included batch, fed-batch and continuous. In batch reactors, 80 - 81 % asphaltene biotransformation was obtained within 2 weeks of reactor run. Highest and fastest biotransformation (93 % in 7 days) was achieved in a 1.5 L stirred tank reactor operated in a fed-batch mode (repeated feast and famine strategy). Continuous stirred tank reactor operation was found to be inefficient method for biotransformation of asphaltene using this microbial consortium as it resulted in washing out of most of the microbial members and negligible asphaltene biotransformation was observed.

After successful biotransformation of asphaltene, the microbial consortium was used to upgrade heavy Maya crude oil. Oil upgradation by the microbial consortium resulted in 60 % decrease in its asphaltene content and therefore increased the flow characteristics of the heavy oil by reducing its viscosity by 91 %. It also resulted in removal of heteroatoms (S and N) from heavy oil, resulting in a cleaner oil.

To study the role of biosurfactants in asphaltene biotransformation, individual strains of the consortium were screened and four out of nine members were found to be capable of producing biosurfactants. The biosurfactants from these strains were extracted, their biochemical nature was studied and they were characterized chemically. All four strains were found to produce glycolipids. *Bacillus* sp. IITD 106 was found to produce a novel biosurfactant which was identified as a saponin called cauloside C - a carboxyl-containing triterpene glycoside with mass equal to 766.45 Da and molecular formula of $C_{41}H_{66}O_{13}$. The other strains were found to produce rhamnolipids. Biosurfactant production by the microbial members was observed

during asphaltene biotransformation, however it was found that the biosurfactants only decreased the mass transfer limitations and therefore increased the access of the asphaltene to the degrading microorganisms resulting in enhanced biotransformation.

Detailed characterization of the produced saponin was performed and the biosynthetic pathway for production of saponin by *Bacillus* sp. IITD106 was proposed. Statistical optimization of the culture medium for saponin production resulted in 9.3-fold increase in the concentration of the biosurfactant. After medium optimization, saponin production was successfully scaled up from shake flask level to a 2.5 L stirred tank bioreactor. The biosurfactant containing cell free broth obtained from the reactors was used for solubilization of various polyaromatic hydrocarbons and for recovery of residual oil. It was successful in solubilizing all the phenanthrene and pyrene (95% and 100% respectively) and half of naphthalene (52%) and benzopyrene (59.9%) when they were present at a concentration 10 times higher than the solubility of these PAHs in water. The cell free biosurfactant was successful in recovering 63 % of the residual oil in sand pack column experiments. Supplementing the microbial consortium with another biodesulfurizing bacterium *Gordonia* sp. IITR100 did not result in any change in asphaltene biotransformation. The biosurfactant produced by *Gordonia* sp. IITR100 was identified as glycolipid and characterized in detail.

LCMS, FT-IR, NMR, CHNS of the purified asphaltene was performed to construct a representative structure of asphaltene used in the study. Various enzymes such as catechol 2,3 dioxygenase and catechol 1,2 dioxygenases, laccases and peroxidases like lignin peroxidase and manganese peroxidase were found to be present in the aqueous medium suggesting that the enzymes produced by the consortium are responsible for the biotransformation of asphaltene. SDS PAGE analysis of the cell free supernatants of test and control showed differential banding pattern. The presence of asphaltene in the flasks induces specific production of certain enzymes that are responsible for its biotransformation.

Thus, this study has led to development of a process for biotransformation of asphaltene which can be used for upgradation of heavy oils. Detailed understanding of the process will require elucidation of the pathway of asphaltene biotransformation which can be used to design a faster and efficient process of biotransformation.