ASPHALTENE BIOTRANSFORMATION FOR HEAVY OIL UPGRADATION BY A DEFINED MICROBIAL CONSORTIUM

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by

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Submitted

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CERTIFICATE

This is to certify that the thesis titled "**Asphaltene biotransformation for heavy oil upgradation by a defined microbial consortium**" being submitted by **Mr. Arif Nissar Zargar** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him under our supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi, New Delhi and Indian Oil Corporation R&D, Faridabad.

The results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

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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.

सार

दुनिया भर में भारी कच्चे तेल के भंडार हल्के कच्चे तेल के भंडार की तुलना में सात गुना प्रचुर मात्रा में हैं, लेकिन उनकी उच्च चिपचिपाहट और घनत्व के कारण उनका कम उपयोग किया जाता है, जो मुख्य रूप से बड़ी मात्रा में डामर की उपस्थिति के कारण होता है। भारी तेल की चिपचिपाहट को उसके डामर को छोटे मेटाबोलाइट्स में बायोट्रांसफॉर्म करके कम करना संभव है। हालांकि डामर को बायोडिग्रेडिंग करने में सक्षम कई बैक्टीरिया की पहचान की गई है, केवल कुछ को ही बायोट्रांसफॉर्म के लिए चिह्नित किया गया है। इस अध्ययन में, हमारी प्रयोगशाला में पहले से अलग किए गए 9 सदस्यीय जीवाणु संघ की विशेषता थी और 16 एस आरआरएनए अनुक्रमण का उपयोग करके माइक्रोबियल संघ के सदस्यों की पहचान की गई थी।

बैक्टीरियल कंसोर्टियम को बायोडिग्रेड और बायोट्रांसफॉर्म डामर की क्षमता के लिए आगे की विशेषता थी। संघ 7 सप्ताह के भीतर केवल 35% डामर को नष्ट करने में सक्षम था। हालांकि, कंसोर्टियम ने बायोट्रांसफॉर्मिंग डामर में उच्च दक्षता का प्रदर्शन किया और परिणामस्वरूप 7 सप्ताह के भीतर 78% परिवर्तन हुआ। उच्च इनोकुलम आकार का उपयोग करते हुए, बायोट्रांसफॉर्म का समय 7 सप्ताह से घटाकर 3 सप्ताह कर दिया गया और 73.5% डामर बायोट्रांसफॉर्म प्राप्त किया गया। माइक्रोबियल कंसोर्टियम की डामर बायोट्रांसफॉर्म क्षमता की पुष्टि करने के लिए कई नियंत्रण प्रयोग किए गए थे।

इसके बायोट्रांसफॉर्म के कारण डामर में संरचनात्मक परिवर्तन एनएमआर, एफटी-आईआर, सीएचएनएस विश्लेषण का उपयोग करके निर्धारित किया गया था और यह पाया गया कि बायोट्रांसफॉर्म के परिणामस्वरूप, एस्फाल्टीन की एन और एस सामग्री में लगभग 80% की कमी प्राप्त हुई थी। इसके अलावा, ऑक्सीजन को डामर की संरचना में पेश किया गया था, यह सुझाव देते हुए कि माइक्रोबियल कंसोर्टियम के उपभेदों द्वारा ऑक्सीजन का उत्पादन किया जा सकता है जो डामर के बायोट्रांसफॉर्म को उत्प्रेरित करता है।

तेल के उच्च प्रतिशत का उपयोग करके उच्च तापमान पर एस्फाल्टीन बायोट्रांसफॉर्म का पता लगाया गया था और यह पाया गया कि इन परिस्थितियों में डामर बायोट्रांसफॉर्म की मात्रा 35% तक सीमित थी। इन शर्तों के तहत बायोट्रांसफॉर्म की सीमा बढ़ाने के लिए, संस्कृति माध्यम और प्रक्रिया की स्थिति का सांख्यिकीय अनुकूलन किया गया था। डामर बायोट्रांसफॉर्म को शेक फ्लास्क से लैब स्केल रिएक्टरों तक बढ़ाया गया था और रिएक्टरों को विभिन्न तरीकों से संचालित किया गया था जिसमें बैच, फेड-बैच और निरंतर शामिल थे। बैच रिएक्टरों में, रिएक्टर चलाने के 2 सप्ताह के भीतर 80-81% डामर बायोट्रांसफॉर्म प्राप्त किया गया था। फेड-बैच मोड (दोहराया दावत और अकाल रणनीति) में संचालित 1.5 एल हलचल टैंक रिएक्टर में उच्चतम और सबसे तेज़ बायोट्रांसफॉर्म (7 दिनों में 93%) हासिल किया गया था। इस माइक्रोबियल कंसोर्टियम का उपयोग करते हुए एस्फाल्टीन के बायोट्रांसफॉर्मेशन के लिए निरंतर स्टिरड टैंक रिएक्टर ऑपरेशन को अक्षम पाया गया क्योंकि इसके परिणामस्वरूप अधिकांश माइक्रोबियल सदस्यों की धुलाई हुई और नगण्य डामर बायोट्रांसफॉर्म देखा गया।

डामर के सफल बायोट्रांसफॉर्मेशन के बाद, भारी माया कच्चे तेल को अपग्रेड करने के लिए माइक्रोबियल कंसोर्टियम का उपयोग किया गया था। माइक्रोबियल कंसोर्टियम द्वारा तेल उन्नयन के परिणामस्वरूप इसकी डामर सामग्री में 60% की कमी आई और इसलिए भारी तेल की प्रवाह विशेषताओं में इसकी चिपचिपाहट को 91% तक कम कर दिया। इसके परिणामस्वरूप भारी तेल से हेटेरोएटम (एस और एन) को हटा दिया गया, जिसके परिणामस्वरूप एक क्लीनर तेल बन गया।

डामर बायोट्रांसफॉर्म में बायोसर्फैक्टेंट्स की भूमिका का अध्ययन करने के लिए, कंसोर्टियम के अलग-अलग उपभेदों की जांच की गई और नौ में से चार सदस्यों को बायोसर्फैक्टेंट बनाने में सक्षम पाया गया। इन उपभेदों से बायोसर्फैक्टेंट्स निकाले गए, उनकी जैव रासायनिक प्रकृति का अध्ययन किया गया और उन्हें रासायनिक रूप से चित्रित किया गया। सभी चार उपभेदों को ग्लाइकोलिपिड्स का उत्पादन करने के लिए पाया गया। बेसिलस सपा। आईआईटीडी 106 को एक उपन्यास बायोसर्फैक्टेंट का उत्पादन करने के लिए पाया गया था

जिसे कोलोसाइड सी नामक एक सैपोनिन के रूप में पहचाना गया था - एक कार्बोक्सिल युक्त ट्राइटरपीन ग्लाइकोसाइड जिसका द्रव्यमान 766.45 दा के बराबर होता है और सी 41 एच 66 ओ 13 का आणविक सूत्र होता है। अन्य उपभेदों को रमनोलिपिड्स का उत्पादन करने के लिए पाया गया। माइक्रोबियल सदस्यों द्वारा बायोसर्फैक्टेंट उत्पादन को डामर बायोट्रांसफॉर्मेशन के दौरान देखा गया था, हालांकि यह पाया गया कि बायोसर्फैक्टेंट्स ने केवल बड़े पैमाने पर स्थानांतरण सीमाओं को कम किया और इसलिए डीग्रेडिंग सूक्ष्मजीवों के लिए डामर की पहुंच में वृद्धि हुई जिसके परिणामस्वरूप बायोट्रांसफॉर्म में वृद्धि हुई।

उत्पादित सैपोनिन का विस्तृत लक्षण वर्णन किया गया और बैसिलस एसपी द्वारा सैपोनिन के उत्पादन के लिए बायोसिंथेटिक मार्ग का प्रदर्शन किया गया। IITD106 प्रस्तावित किया गया था। सैपोनिन उत्पादन के लिए संस्कृति माध्यम के सांख्यिकीय अनुकूलन के परिणामस्वरूप बायोसर्फैक्टेंट की एकाग्रता में 9.3 गुना वृद्धि हुई। मध्यम अनुकूलन के बाद, सैपोनिन उत्पादन को शेक फ्लास्क स्तर से 2.5 लीटर स्टिरड टैंक बायोरिएक्टर तक सफलतापूर्वक बढ़ाया गया। रिएक्टरों से प्राप्त सेल मुक्त शोरबा युक्त बायोसर्फैक्टेंट का उपयोग विभिन्न पॉलीएरोमैटिक हाइड्रोकार्बन के घुलनशीलता और अवशिष्ट तेल की वसूली के लिए किया गया था। यह सभी फेनेंथ्रीन और पाइरीन (क्रमशः 95% और 100%) और नेफ़थलीन (52%) और बेंज़ोपाइरीन (59.9%) के आधे हिस्से को घोलने में सफल रहा, जब वे पानी में इन पीएएच की घुलनशीलता से 10 गुना अधिक सांद्रता में मौजूद थे। . सेल फ्री बायोसर्फैक्टेंट रेत पैक कॉलम प्रयोगों में 63% अवशिष्ट तेल को पुनर्प्राप्त करने में सफल रहा। माइक्रोबियल कंसोर्टियम को एक अन्य बायोडेसल्फराइजिंग जीवाणु गॉर्डोनिया एसपी के साथ पूरक करना। IITR100 के परिणामस्वरूप डामर बायोट्रांसफॉर्म में कोई परिवर्तन नहीं हुआ। गॉर्डोनिया एसपी द्वारा निर्मित बायोसर्फैक्टेंट। IITR100 को ग्लाइकोलिपिड के रूप में पहचाना गया और विस्तार से इसकी विशेषता बताई गई।

एलसीएमएस, एफटी-आईआर, एनएमआर, सीएचएनएस शुद्ध किए गए डामर का अध्ययन में प्रयुक्त डामर की एक प्रतिनिधि संरचना के निर्माण के लिए किया गया था। विभिन्न एंजाइम जैसे कैटेचोल 2,3 डाइअॉॉक्सिनेज

और कैटेचोल 1,2 डाइअॉॉक्सिनेज, लैकेसेस और पेरोक्सीडेस जैसे लिग्निन पेरोक्सीडेज और मैंगनीज पेरोक्सीडेज जलीय माध्यम में मौजूद थे, यह सुझाव देते हुए कि कंसोर्टियम द्वारा उत्पादित एंजाइम डामर के बायोट्रांसफॉर्म के लिए जिम्मेदार हैं। परीक्षण और नियंत्रण के सेल मुक्त सतह पर तैरनेवाला के एसडीएस पृष्ठ विश्लेषण ने अंतर बैंडिंग पैटर्न दिखाया। फ्लास्क में डामर की उपस्थिति कुछ एंजाइमों के विशिष्ट उत्पादन को प्रेरित करती है जो इसके बायोट्रांसफॉर्म के लिए जिम्मेदार होते हैं।

इस प्रकार, इस अध्ययन ने डामर के बायोट्रांसफॉर्मेशन के लिए एक प्रक्रिया का विकास किया है जिसका उपयोग भारी तेलों के उन्नयन के लिए किया जा सकता है। प्रक्रिया की विस्तृत समझ के लिए डामर बायोट्रांसफॉर्म के मार्ग को स्पष्ट करने की आवश्यकता होगी जिसका उपयोग बायोट्रांसफॉर्म की तेज और कुशल प्रक्रिया को डिजाइन करने के लिए किया जा सकता है।

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ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
µm	Micrometer
ACN	Acetonitrile
ANN	Artificial Neural Network
ANOVA	Analysis of Variance
AOR	Additional Oil Recovery
API	American Petroleum Institute
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
BDM	Biodemetallation
BDN	Biodenitrogenation
BDS	Biodesulfurization
BLAST	Basic Local Alignment Search Tool
CHNS	Carbon Hydrogen Nitrogen and Sulfur
CII	Colloidal Instability Index
CMC	Critical Micelle Concentration
Cp	Centipoise
CSS	Cyclic Steam Injection
CSTR	Continuous Stirred Tank Reactor
Cyt	Cytochrome
DBT	Dibenzothiophene
DMF	Dimethylformamide
DNA	Deoxyribose Nucleic Acid
E_{24}	Emulsion Index after 34 hours
EOR	Enhanced Oil Recovery
FID	Flame Ionization Detection
Fig	Figure
FT-IR	Fourier Transform Infrared Spectroscopy
GCMS	Gas Chromatography coupled to Mass Spectroscopy
HCl	Hydrochloric Acid
HMN	Heptamethyl nonane
HPLC	High Performance Liquid Chromatography
ICPMS	Inductively coupled Plasma Mass Spectroscopy
ICS	In-situ Combustion
IOR	Improved Oil Recovery
IOSV	Initial Oil Saturation Volume
K_La	Mass Transfer Coefficient
LA	Luria Agar
LB	Luria Broth
LCMS	Liquid Chromatography coupled to Mass Spectroscopy

m/z	Mass to charge ratio
MEOR	Microbial Enhanced Oil Recovery
Mg	Milligram
Ml	Microlitre
MSM	Minimal Salts Medium
MTCC	Microbial Type Culture Collection
NaOH	Sodium Hydroxide
NCBI	National Center of Biotechnology Information
NEB	New England Biolabs
Nm	Nanometre
NMR	Nuclear Magnetic Resonance
OD	Optical Density
OFAT	One Factor at a Time
PAH	Polyaromatic Hydrocarbon
Pas	Pascal Second
PCR	Polymerase Chain Reaction
Rpm	Revolutions Per Minute
RSM	Response Surface Methodology
SAGD	Steam Assisted Gravity Drainage
SARA	Saturates Aromatics Resins and Asphaltenes
SDS	Sodium Dodecyl Sulfate
SF	Steam Flooding
TGA	Thermogravimetric Analysis
TLC	Thin Layer Chromatography
TNT	Trinitrotoluene
UV	Ultraviolet
v/v	Volume per Volume
VFA	Volatile Fatty Acids
DTT	Dithiothreitol
TEMED	N N N’ N’ tetramethylene diamine
EDTA	Ethylene diamine tetra acetic acid