IMPROVED BIODESULFURIZATION OF PERSISTENT ORGANOSULFUR COMPOUNDS

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by

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CERTIFICATE

This is to certify that the thesis entitled "**Improved biodesulfurization of persistent organosulfur compounds**" being submitted by **Ms. Pooja Singh** to the Indian Institute of Technology, Delhi, for the award of Degree of **Doctor of Philosophy**, is a record bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi.

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

Fossil fuels are the major source of energy on earth. Sulfur emission due to combustion of these fossil fuels is a global problem. These emissions cause various environmental and health hazards. Reduction in sulfur emissions is highly desirable and many countries have, therefore, established stringent sulfur emission standards. Conventionally sulfur is removed from petroleum fractions by hydrodesulfurization (HDS) which is capable of removing most of the inorganic as well as some organic sulfur compounds. Some organosulfur compounds such as dibenzothiophene, benzothiophenes and their derivatives, however, are not removed completely by this process. For their removal, a modified HDS method, referred as deep HDS, is needed, which requires a higher temperature i.e. 400-450 ^{o}C and presence of some specialized catalysts. Due to this the process becomes more expensive. While dibenzothiophenes and benzothiophenes are removed by this process, their alkylated derivatives such as 4,6- dimethyl dibenzothiophene, 4- methyl dibenzothiophene etc. are not removed, they are termed as persistent/ recalcitrant organosulfur compounds. For removal of such type of compounds biodesulfurization is an attractive alternative. Biodesulfurization, where in microorganisms are used to break the target compounds to release sulfur from petroleum fractions, offers the advantage of removing the sulfur selectively without affecting the calorific value of fuel. It is energy efficient, and is therefore environmentally and economically favorable. In the past several bacterial strains have been isolated that have the capability to remove sulfur from such persistent organosulfur compounds. The bacteria isolated so far have limited substrate range, low activity and less solvent tolerance. For commercialization, the desired rate of biodesulfurization should be 3 mM/gDCW/h. whereas the maximum rate achieved till date is 320 μM/gDCW/h. A bacterial strain *Gordonia* sp. IITR100 was isolated in our lab from petroleum contaminated soil by enrichment culture using 4,6-dimethyl dibenzothiophene as the sulfur source. The biodesulfurization genes were also identified. The present work involves a) determination of the efficacy of the strain towards biodesulfurization of persistent organosulfurs such as Benzonaphthothiophene and 4,6 dimethyl dibenzothiophene, b) elucidation of the biodesulfurization pathway of benzonaphthothiophene c) development of molecular tools for gene expression in *Gordonia* and d) construction of a recombinant *Gordonia* with improved biodesulfurization activity.

The bacterium was found to desulfurize benzonaphthothiophene and 4,6 dimethyl dibenzothiophene efficiently. A novel method was developed for elucidation of the biodesulfurization pathway of benzonaphthothiophene. The set of constructed plasmids can be used for the determination of biodesulfurization pathway of any organosulfur compound.

For constructing a recombinant strain, an improved protocol for electroporation in *Gordonia* was developed. A new promoter was isolated from *Gordonia* and characterized in detail. To the best of our knowledge this is the first report on the detailed characterization of a stationary phase promoter from *Gordonia*.

An expression vector was constructed using the isolated promoter. Expression of biodesulfurization genes was achieved. A recombinant *Gordonia* strain was constructed which had many useful features. The constructed recombinant strain has high potential for applications in biodesulfurization of diesel and crude oil.

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ABBREVIATIONS

2-HBP	2-Hydroxybiphenyl
2-HBPS	2(2’-hydroxybiphenyl)-benzene sulfinate
2-MBP	2 Methoxy biphenyl
3MDBT	3-Methyl dibenzothiophene
Amp	Ampicillin
ATCC	American Type Culture Collection
BDS	Biodesulfurisation
BLAST	Basic local alignment search tool
BNT	Benzonaphthothiophene
BSM	Basal salt media
Cm	Chloramphenicol
CSPD	3-(4-methoxyspiro(1,2-dioxetane-3,2'-(5'-chloro)tricyclo(3.3.1.1(3,7))decan)-4-yl) phenyl phosphate
DBT	Dibenzothiophene
DBTS	Dibenzothiophene sulfone
DIG	Dioxigenin
DMDBT	4,6Dimethyldibenzothiophene
dNTP	Deoxy ribose nucleotide triphosphate
DszA	DBTS-monooxygenase
DszB	2'-Hydroxybiphenyl-2-sulfinate desulfinase
DszC	DBT- monooxygenase
DszD	Flavin-reductase
EDTA	Ethylene diamine tetra acetic acid
Fig.	Figure
GC-MS	Gas Chromatography/Mass Spectroscopy
Gibbs Reagent	4,6dichloroquinone4chlorimide
HCl	Hydrochloric acid
HDS	Hydrodesulfurisation
HPLC	High Pressure Liquid Chromatography

INH	Iso nicotinic acid hydrazide
IPTG	Isopropyl β-D thio galactopyranoside
Kan	Kanamycin
LB	Luria Broth
MCC	Microbial culture collection
MTCC	Microbial type culture collection
ODS	Oxidative desulfurization
ONPG	O-nitrophenyl β-D galactopyranoside
PASHs	Poly aromatic sulphur hetro cycles
PCR	Polymerase Chain Reaction
RACE	Rapid amplification of c-DNA ends
RNase	Ribonuclease
SDS	Sodium dodecyl sulphate
Tet	Tetracycline
TLC	Thin Layer Chromatography
Tris	Tris (hydroxymethyl) amino methane
UV	Ultra violet
X-gal	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside