SCREENING OF MICROORGANISMS AND EXPRESSION OF GENE INVOLVED IN DEGRADATION OF DIBENZOTHIOPHENE

by
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Submitted
In fulfillment of the requirements of the degree of
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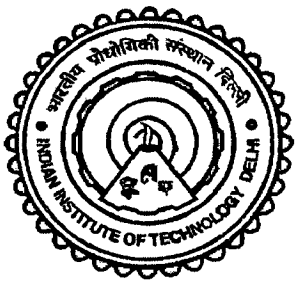
to the



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CERTIFICATE

This is to certify that the thesis entitled " **Screening of microorganisms and expression of gene involved in degradation of Dibenzothiophene**" *being submitted by* ***Nidhi Gupta*** *to the Indian Institute of Technology, Delhi, for the award of Degree of* ***Doctor of Philosophy****, is a bonafide research work carried out by her under our supervision and guidance. The results presented in this thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.*

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ABSTRACT

Organic sulfur compounds in fossil fuels have long been one of the major sources of environmental pollution. Sulfur oxides emissions from the combustion of diesel have been shown to be responsible for acid rain, which could dissolve buildings, kill forests, and poison lakes. To avoid the production of such pollutants, sulfur must be removed from the diesel. Currently, the refining industry applies the energy intensive physico-chemical hydrodesulfurization (HDS) process in order to reduce the sulfur content. Due to its high costs and inherent inability to act upon low levels of aromatic sulfur compounds, biodesulfurization of hydrocarbon streams might represent an attractive complementary method as it can act on low concentration of aromatic sulfur containing component. Bacteria require relatively mild process conditions (pressure and temperature) and bacterial enzymes are very selective in converting target molecules. Moreover, biodesulfurized diesel has better lubricating property as compared to hydrodesulfurized diesel and this process does not result in the production of green house gas. Further, it has been reported that biodesulfurization can be competitive with hydrodesulfurization technology only with the concomitant production of sulfones that can be further used as surfactants and hydrotopes.

Dibenzothiophene (DBT) is considered to be model compound that is difficult to desulfurize using hydrodesulfurization technology. Keeping this in mind, we collected various soil, water and oil sludge samples for the screening of microorganisms, which could utilize DBT as the sulfur source. We were able to screen two different microorganisms, which utilized this compound efficiently. They have the ability to degrade 4,6 Dimethyl-DBT (DM-DBT) in addition to DBT, the former being present in diesel and is difficult to desulfurize than the latter. The two bacteria identified are *Citrobacter freundii* and *Rhodococcus* sp.

It is for the first time that *Citrobacter* is reported to be responsible for the degradation of DBT and DM-DBT. We have identified the intermediate of DBT degradation but its pathway is yet to be worked out. The desulfurization activity of diesel using *Citrobacter* is comparable to those of other bacteria.

Rhodococcus was found to desulfurize DBT following 4S pathway and contains all the three genes. The first step of 4S pathway is catalyzed by DBT monooxygenase and is able to convert DBT to DBT sulfone. Removal of DBT sulfone not only leads to desulfurization of diesel but also the production of valuable sulfones. With this objective in view

cloning and expression of *dsz C* was done. DBT monooxygenese was further purified and its activity studied.

A single step purification procedure resulted in 39 fold purification of the DBT monooxygenese. The basic kinetic parameter K_m and V_{max} of 0.012 mM and .002 mM/min respectively suggest that this enzyme has got high affinity for DBT and can even be used to remove sulfur from low sulfur diesel.

Thus both these microorganisms have the potential to be developed into an efficient biocatalyst for biodesulfurization.

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