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A Novel Method for Conversion of Valuable BDS Intermediates HPBS/Cx-HPBS from DBT/Cx-DBT

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Abstract: A simple recombinant PCR method was used to delete the *dsz*B gene responsible for the slowest step of the Dsz pathway and allow the accumulation of hydroxyphenyl benzene sulfinate (HPBS) in the recombinant *E. coli*. Using GC/MS, HPBS accumulation was confirmed. The recombinant *E. coli* could also desulfurize Cx-DBT to corresponding Cx-HPBS. The result gave a new insight to BDS process and explored a new method to obtain valuable surfactants from cheap raw materials.

Keywords: Biodesulfurization, biosurfactant, recombinant PCR.

Sulfur oxide generated by combustion of sulfur-containing fossil fuel causes environmental pollution. Biodesulfurization was operated in ambient temperature and pressure and endowed with a high selectivity, resulting in decreased energetic costs, low emission and no generation of undesirable by-products. It is thought to be an interesting alternative for the development of new petroleum refining process[1,2]. Dibenzothiophene (DBT) and its alkylated derivatives are known to constitute the majority of organic sulfur compounds refractory to conventional HDS process[3]. *Rhodococcus erythropolis* XP was isolated from soil sample as an effective and stable bacterium that can desulfurize DBT, alkyl DBTs, and some of the alkyl benzothiophenes, through the specific cleavage of C-S bonds by GC/MS detection. DBT is stepwise S-oxidized by DszC, first to DBT-5-oxide (DBTO) and then to DBT-5, 5′-dioxide ($DBTO_2$). DszA catalyzes the conversion of $DBTO_2$ to 2-(2′-hydroxyphenyl) benzene sulfinate (HPBS). HPBS is then desulfurized by DszB to produce 2-hydroxybiphenyl (2-HBP). The biodesulfuri- zation genes were also characterized and the *dsz*ABC genes (Genbank Accession No. AY278323) were in ABC linear arrangement. Each gene coding the correlative protein in the metabolic pathway of DBT was shown in **Figure 1**.

Traditional biodesulfurization process allowed the recuperation of the corresponding 2-HBPs that could be reintroduced to the fuel. Now BDS process was less efficient and more costly that it could not compete with HDS. The *dsz*B gene eliminated the slowest step of the Dsz pathway[4]. Deletion of the *dsz*B gene could allow the accumulation of

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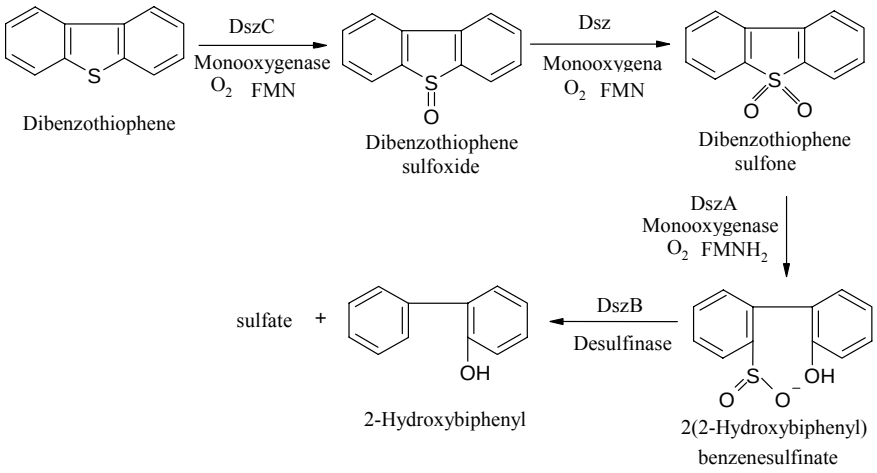
Figure 1 Metabolic pathway of DBT desulfurized by *Rhodococcus erythropolis* XP



hydroxyphenyl benzene sulfinate (HPBS), which is a more valuable product than sulfate because it can be recovered easily from the aqueous phase and used as surfactant. Because the feedstock for this material is a low value high-sulfur refinery stream and the conversion occurs at low temperatures and pressures, the process for producing this material look quite economical.

Genetic manipulation consisted in increasing *dsz* copy number[5, 6], eliminating the sulfate repression by promoter change[7] and co-expression of DBT uptake gene[8], were used to increase biodesulfurization activity. In this paper, we described the simple recombinant PCR method to delete the limited step gene to accumulate valuable HPBS. Primers were synthesized as followed: 5′-aacgggaattcatgactcaacaacgacaaatg-3′, 5′-cagtgtcatgcggatgttcctatgaaggttgtccttgcag-3′, 5′-ctgcaaggacaaccttcataggaacatccgcatgacactg-3′ and 5′-gatcaaagcttcagatcctcaggaggtgaa-3′. The *dsz*A and *dsz*C were amplified by PCR respectively and then used recombinant PCR methods[9] to ligate the *dsz*A and *dsz*C. No native promoter and RBS site of *dsz*A was included in the amplification product. The recombinant *E. coli* cells were cultivated in LB medium. BSM medium[10] was also used in biodesulfurization of dibenzothiophenes.

The contents of dibenzothiophenes were analyzed by gas chromatography (GC, CP3380, Varian Associates) using a SPB-5 column (0.32 mm i.d.U30 m length; Supelco) after extracting the reaction solution with ethyl acetate at a pH<2. The molecular structures of metabolites were analyzed using GC-mass spectrometry (GC-MS, GCD 1800C, Hewlett-Packard) equipped with a 50-m DB-5MS column (J&W Scientific Folsom, CA, USA). The recombinant proteins expression was detected by SDS-PAGE (12% acrylamide).

*dsz*A and *dsz*C were 1362 bp and 1254 bp respectively. The recombinant *dsz*AC will be about 2.6 kb. **Figure 2 (A)** gave the profile of the gene amplification results. The recombinant *dsz*AC was ligated to expression vector of pKK223-3 to construct plasmid of pKKAC and then transformed to *E. coli* JM109. **Figure 2 (B)** gave the DszAC proteins expression in *E. coli* JM109 (pKKAC). Lane 1 is protein of *E. coli*

JM109 (pKKAC) before induction; lane 2 is the protein after induction. The DszA is a dimmer in its native state (Mr of 100 KDa); the DszC is a tetramer (Mr of 180 KDa) [4]. The subunits of DszAC are 50 KDa and 45 KDa, respectively. As shown in the **Figure**, after induced by IPTG, two bands of about 50 KDa and 45 KDa appear. These results indicated the *dsz*AC were expressed successfully in *E. coli* JM109.

Figure 2 Profiles of amplification of *dsz*A、*dsz*C and *dsz*AC by recombinant PCR (A) & SDS-PAGE detection of DszAC proteins expression in *E. coli* JM109 (pKKAC) (B)

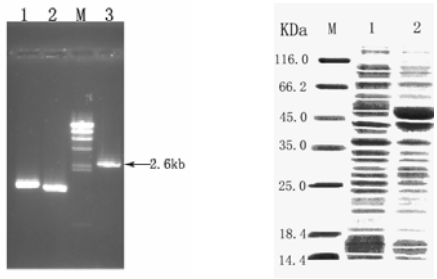


Figure 3 Acid catalyzed cyclization of 2-(2′-hydroxyphenyl) benzene sulfinate to sultine

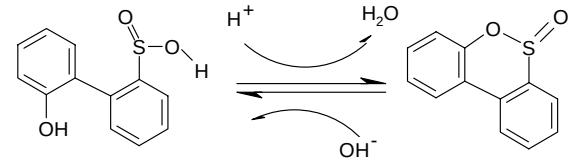
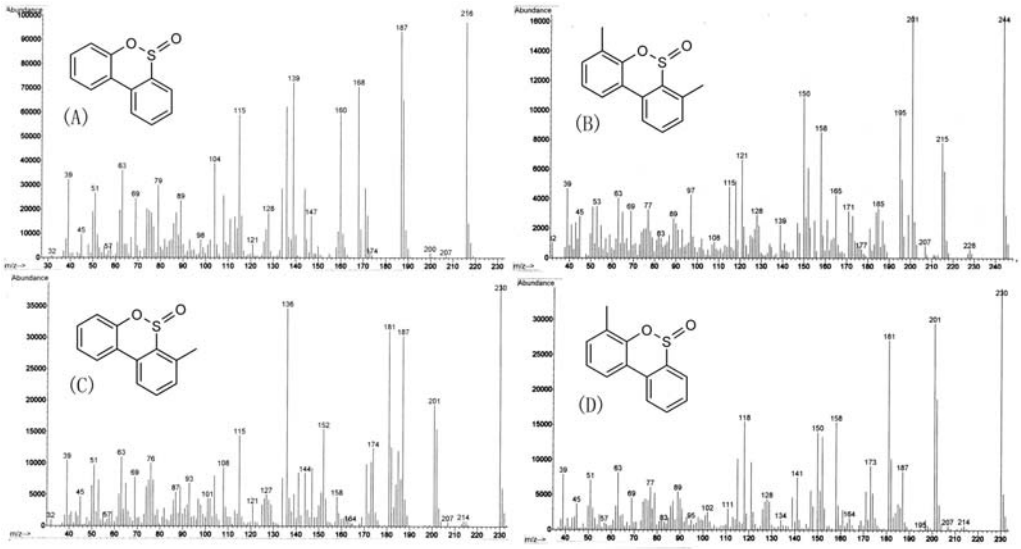


Figure 4 GC/MS chromatography of the conversion product from DBT and Cx-DBT



GC-MS analysis of DBTs metabolites were shown in **Figure 4**. Recombinant *E. coli* JM109 (pKKAC) could degrade DBT to dibenz[c,e][1,2]oxathiin-6-oxide (**Figure 4A**), which was an acid product of HPBS[11] (**Figure 3**). In 24 hours, about 80% DBT was transformed into HBPS by GC quantification. These results indicate that the *dsz*AC genes were expressed with an activity form. In the same way, metabolites of 4,6-DM-DBT gave a signal at *m/z* 244 (as shown in **Figure 4B**), corresponding to molecular mass of dimethyl-dibenz[c,e][1,2]oxathiin-6-oxide.

Two peaks of product were found in 4-M-DBT degradation, as showed in **Figure 4 (C) & (D)**, mass ions at *m/z* = 230 were accordance with the molecular mass of methylated dibenz[c,e][1,2]oxathiin-6-oxide respectively. This indicated that the enzymatic system could recognize either of the two C-S bonds in DBTs. The recombinant could also desulfurize DBT and Cx-DBT to corresponding Cx-HPBS.

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