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Isolation of microorganisms for the biodesulfurization of organosulfur compounds

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Abstract

Sulfur compounds in liquid fuel are undesirable and their level in diesel fuel is strictly regulated in the last 15 years from the European Union. These stringent regulations are imposing an urgent requirement for fuel terminals to produce fuels with ultra-low sulfur content [1]. One of the technologies commonly used to tackle this problem is the hydrodesulfurization that requires high temperatures and pressures and the exposure of crude oil fractions under severe conditions; this decreases the value of the fuel [2]. A promising alternative, is to employ biodesulfurization (BDS), a process where the bacteria (liquid phase) are mixed with oil at ambient temperature and pressure to remove selectively the organosulfur components from oil fractions without degrading the carbon skeleton of the compounds [3]. The latter approach is an "Eco Technology" method, as microorganisms are employed to remove sulfur specifically from hydrocarbon fractions without altering the carbon skeleton.

In the present study, microbial enrichment and DBT-guided (Dibenzothiophene) isolation of bacteria was studied. Various oil-contaminated soil and liquid samples were randomly collected from different sites (Cyprus). Towards this, forty milliliter (40 mL) of mineral salt medium (MSM) (2 g/L NH_4Cl, 5 g/L KH_2PO_4, 4 g/L Na_2HPO_4, 1 g/L NaCl)/100mL flask were inoculated with 2 g of homogenized soil sample (or 2 mL liquid), incubated at 30 °C and shaked for 1 week under 150 rpm. Enrichment of samples took place by exposing them individually to 200 mg/L DBT as a sole sulfur and carbon source in 40 ml (the same experiments were contacted for 2-phenylphenol (2-HBP)). After a week of the first exposure, 10 % of the samples was transformed to a higher concentration of DBT (300 mg/L). This took place several times, until enriched cultures were developed. The DBT reduction was monitored using HPLC-UV and GC-FID. The same experiments were contacted for 2-HBP.

Furthermore, the growth of isolates was investigated. Samples (0.1 ml) of appropriate dilutions were spread onto agar plates (with 300 mg DBT/L or 2-HBP), and incubated at 30 °C for 24 hrs. A single colony was picked with a sterile loop, to prepare a pure subculture in a fresh agar (15 g/L) plates by streaking. Pure isolates were growed in medium containing DBT

(200-500 ppm) to ensure their ability to desulfurize the DBT. In order to identify the respective species, DNA extraction was followed by PCR amplification (Macherey Nagel™ NucleoSpin™).

The enrichment experiments on various polluted environmental samples with oil resulted in the identification of two microorganisms, capable of decreasing the DBT content up to 90 %. *Serratia sp. and Burkholderia sp.* were isolated from both DBT and 2-HBP experiments. HPLC-UV and GC-FID methods can be used for the quantification of DBT and 2-HBP.

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