
# Isolation of microorganisms for the biodesulfurization of organosulfur compounds

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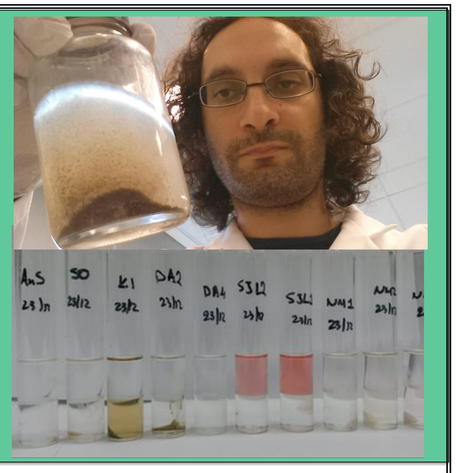

## Introduction:

- Sulfur compounds in liquid fuel are undesirable and the level of these compounds 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 "Eco Technology" is to employ Biodesulfurization (BDS), a process where the bacteria (liquid phase) are mixed with oil at ambient temperature and pressure and remove selectively, the organosulfur components from oil fractions without degrading the carbon skeleton of the compounds [3].
- The use of microorganisms might offer an alternative way to remove sulfur specifically from hydrocarbon fractions without altering the carbon skeleton.
- BDS is considered as an environmentally friendly process, because of the mild conditions (low pressure and temperature) and the no addition of chemicals [1].

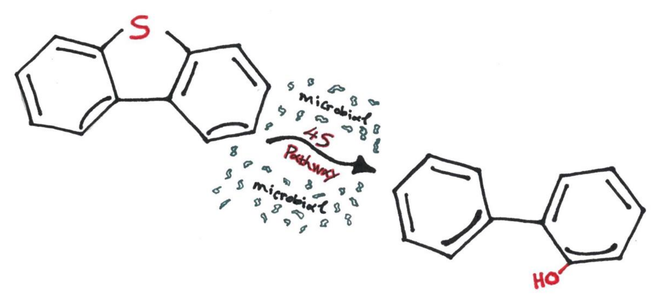


**Figure 1**. Dibenzothiophene (DBT) desulfurization.

## Materials and methods:

**(A) Microbial enrichment and DBT-guided isolation of bacteria:**

- Varius oil-contaminated soil and liquid samples were randomly collected from different sites (Cyprus).
- 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.
- For the isolation of sulfur oxidizing species, enrichment of samples (5 g) 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-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 enrich cultures were developed.
- The DBT reduction was monitored using HPLC-UV and GC-FID. The same experiments were contacted for 2-HBP.

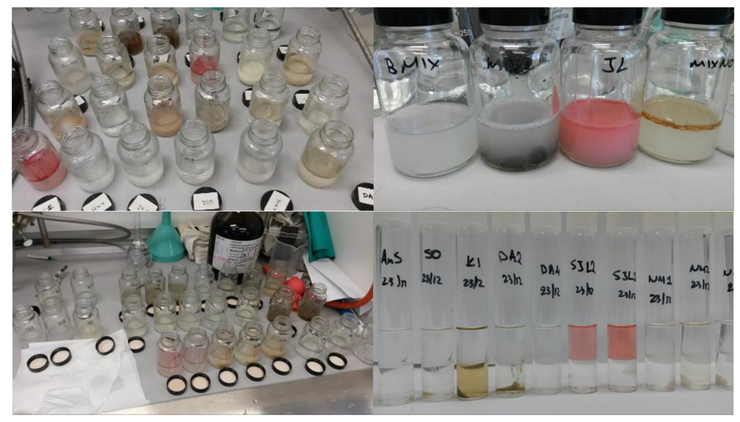

**Figure 2**. Soil and waste samples for enrichment experiments.

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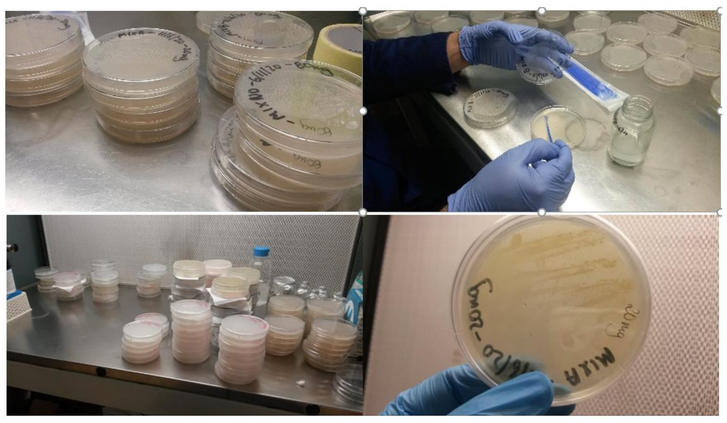

**Figure 3.** Bacteria observed after cultivation in petri dishes.

**(B) Growth of isolates:**

- 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 (Figure 3).
- A single colony was picked with a sterile loop to prepare a pure subculture in a fresh agar (15 g/L) plates by streaking.
- The purity of the isolated colonies was checked by microscopic examination.
- Pure isolates were growed in medium containing DBT (200-500 ppm) to ensure their ability to desulfurize the DBT.

**(C) Genomic DNA extraction from soil:**

- In order to identify the species, DNA extraction was followed by PCR amplification (Macherey Nagel™ NucleoSpin™). Then, the PCR products were send to Macrogen (Netherlands).

## Results:

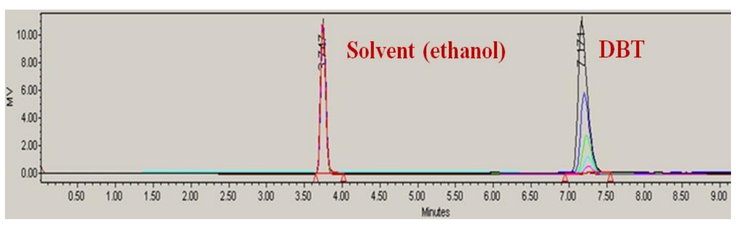


**Figure 4.** Identification of DBT via HPLC analysis with ethanol as mobile phase (calibration of 5-400 ppm).

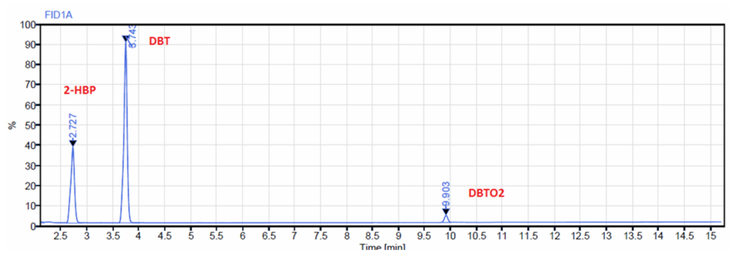


**Figure 5.** GC-FID spectrum of DBT, $DBTO_2$ and 2-HBP, respectively.

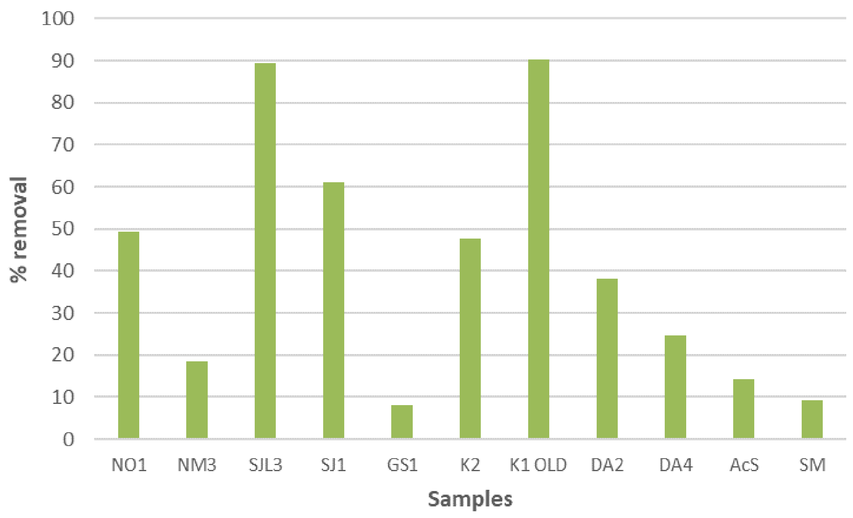


**Figure 6.** % removal of DBT from various samples.

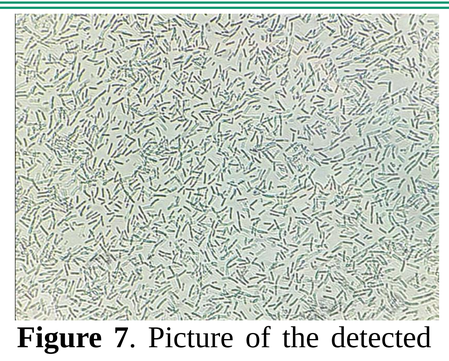

**Figure 7**. Picture of the detected microorganism *Serratia sp.*

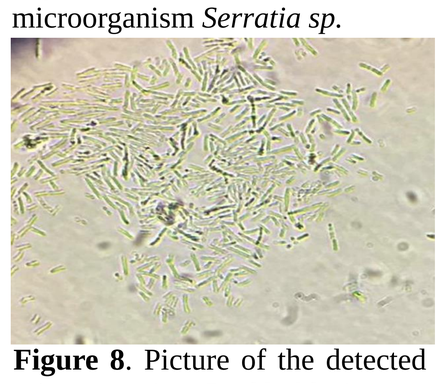

**Figure 8**. Picture of the detected microorganism *Burkholderia sp.*

## Conclusions:

- Enrichment experiments on various polluted environmental samples with oil, resulted in 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.

## References:

[1] Kilbane, J.J., 2017. Biodesulfurization: How to Make it Work? Arab. J. Sci. Eng. 42, 1–9.
[2] Martínez, I., Mohamed, M.E.S., Rozas, D., García, J.L., Díaz, E., 2016. Engineering synthetic bacterial consortia for enhanced desulfurization and revalorization of oil sulfur compounds. Metab. Eng. 35, 46–54.
[3] Stylianou M., Vyrides I., Agapiou A., 2021. Oil biodesulfurization: A review of applied analytical techniques. J Chromatogr B. 1171, 122602.

**Project Funding**
This work was co-funded by the European Regional Development Fund and the Republic of Cyprus through the Research and Innovation Foundation (OilEcoDesulfur: POST-DOC/0916/0121)
"RESTART 2016 – 2020".
RPF PROJECT NUMBER: POST-DOC/0916/0121
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