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Anaerobic biodesulfurization in different inoculums

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Abstract. Sulfur compounds in liquid fuel are undesirable and the level of these compounds in diesel fuel is strictly regulated in the last 15 years by the European Union. These stringent regulations are imposing an urgent requirement for fuel terminals to produce fuels having ultra-low sulfur content. 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 to selectively remove organosulfur components from oil fractions without degrading the carbon skeleton of the compounds. Most of the studies have examined the BDS under aerobic conditions using pure cultures or mix inoculum, and the main byproducts remain in the liquid phase. However, the present study explored a new proof of concept; BDS under anaerobic conditions using inoculums from various anaerobic sources. The enrichment in each inoculum took place several months and electron donors were used. In addition, the microbial profile over time was examined at the end of the BDS using next generation sequencing. The BDS of oil under anaerobic conditions profits of aeration cost, as well as the advantage of releasing H_2S in the gas phase, which can be easily treated using the existing H_2S technologies.

Keywords: Fuels; oil; sulfur; microorganisms; dibenzothiophene.

1. Introduction

Liquid fuel contains sulfur compounds that are undesirable, and their content is regulated. These compounds consist of different forms such as thiols, sulfides, disulfides, and thiophenic compounds. The latter, being the most recalcitrant in treating processes are used as target model compounds (*e.g.* Dibenzothiophene, DBT) in both aerobic and anaerobic biodesulfurization (BDS) studies. In aerobic BDS experiments, the bacteria are mixed with oil at ambient temperature and pressure to remove selectively organosulfur from oil fractions without degrading the carbon skeleton of the compounds through the 4S pathway, converting DBT to 2-phenylphenol (2-HBP) (Martínez et al., 2016).

In contrast to aerobic BDS, the evidence for the anaerobic conversion of organic sulfur compounds is unclear (Mohebali and Ball 2016). Up to date, very few works have been performed on anaerobic BDS (Armstrong et al., 1995; Armstrong et al., 1997; Kareem et al., 2016; Marcelis et al., 2003). These studies showed that desulfurization of DBT via aerobic microorganisms results in 2-HBP as a product through the 4S pathway, whereas the anaerobic BDS uses the sulfate-reducing bacteria to desulfurize DBT to biphenyl (BP), as depicted in Fig.1.

The present study aims at identifying BDS under anaerobic conditions using inoculums from various anaerobic sources. DBT is used as a model compound; analytically, it can be measured by an array of methods, including the gas chromatography-flame ionization detector (GC-FID) (Stylianou et al., 2021).

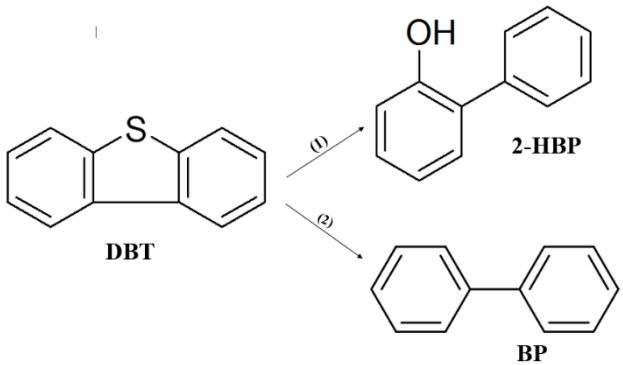

Figure 1. Desulfurization pathways products of (1) aerobic microorganisms, and (2) anaerobic bacteria.

2. Materials and Methods

Three solid samples were used in the current study: (i) a polluted soil sample collected from Larnaca district (Cyprus), (ii) a sludge sample collected from a wastewater treatment facility, and (iii) a compost sample derived from

a compost facility. The latter samples (sludge, compost, polluted soil) were cultured at 30 °C in 120 ml serum vials closed with viton stoppers and aluminium crimp seals (Figure 2) in triplicates. The vials contained 50 ml mineral salt medium (MSM) and 1% organic phase (ethanol). Different concentrations of DBT were applied ranging from 50 to 200 mg/L. The gas-phase consisted of N_2/CO_2 (80:20 %v/v).

The chemically defined MSM contained the following S-free chemicals per liter: 2 g/L NH_4Cl, 5 g/L KH_2PO_4, 4 g/L Na_2HPO_4, 1 g/L NaCl (Table 1).

The DBT reduction in the liquid culture was monitored through GC-FID (Agilent 8890) after 15 days of incubation.

Figure 2. Anaerobic samples

Table 1. Chemicals used in the present study

Chemical	CAS number
DBT	132-65-0
Sodium chloride	7647-14-5
Ammonium chloride	12125-02-9
Sodium carbonate (anhydrous)	497-19-8
di-Sodium hydrogen phosphate	7558-79-4
Potassium dihydrogen phosphate	7778-77-0

3. Results

DBT has been used as a model compound to identify the presence of microorganisms in the examined samples. The developed GC-FID method was able to quantify the concentration of DBT; its removal could be used as an indicator of the transformation of DBT to BP (Figure 3).

Enriched samples showed promising % removal of DBT from the initial samples, highlighting the presence of specific anaerobic bacteria that transform DBT to BP. Figure 4, shows that DBT was removed from 30 to 60% in the examined samples. The obtained results are promising for further future research, due to the limited studies reported in the literature. For example, Armstrong et al., (1995) reported that the isolated sulfate-reducing bacteria could desulfurize DBT to BP, to a limited extent.

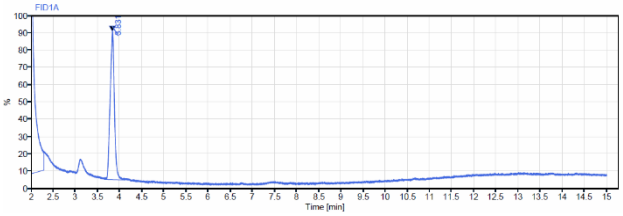

Figure 3. GC-FID chromatogram of DBT

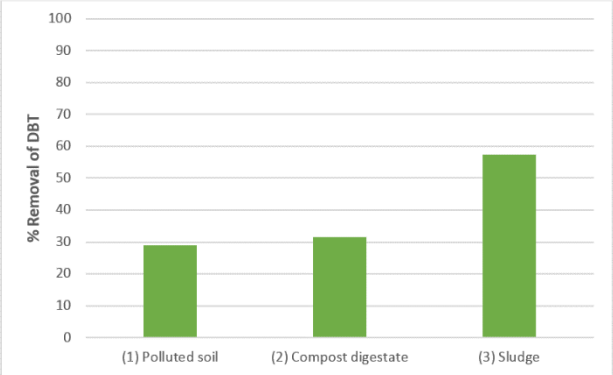

Figure 4. % Removal of DBT from the examined samples (mean values, n = 3)

4. Conclusions

A new approach for removing sulfur compounds in liquid fuel is presented and discussed. The enriched cultures from the 3 environmental samples (sludge, compost, polluted soil with hydrocarbons), enabled to decrease the DBT concentration up to 60% after 15 days of incubation, showing that the specific sulfate-reducing bacteria could be successfully used in the anaerobic BDS process. More research is needed in this direction to fully understand the process.

5. Acknowledgments

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