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Exploring the Potential of Microbial Consortia for Biodesulfurization in Petroleum and Fuel Applications

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Abstract

The growing environmental concerns regarding sulfur emissions from petroleum and fuel products have driven significant interest in alternative desulfurization methods. Microbial consortia, consisting of diverse microorganisms, present a promising solution for biodesulfurization (BDS) processes in petroleum and fuel applications. This research explores the potential of microbial consortiums for the breakdown of containing sulfur substances, particularly dibenzothiophene (DBT), and a major sulfur component in petroleum and fuel products. Oil-contaminated soil samples were gathered from several petroleum extraction locations. Microbial consortia were isolated using serial dilution techniques on nutrient agar and basal salt media (BSM) supplemented using DBT as the only carbon and sulfur source. The consortia were then tested for DBT degradation using growth monitoring by optical density (OD) and sulfur removal efficiency via Gas Chromatography (GC) and Atomic Absorption Spectroscopy (AAS). The Most Probable Number (MPN) method was employed to estimate the concentration of live bacteria in the samples, based on serial dilutions and incubation. The microbial consortia exhibited improved sulfur removal compared to individual strains, although these strains displayed varying levels of DBT degradation. Various bacterial genera were identified, including Thiobacillus, Bacillus, Sulfobacillus, Rhodococcus, Sphingomonas, Klebsiella, and Geobacillus. Sulfur removal was confirmed through GC and AAS analysis, showing a significant decrease in DBT concentration over time. Growth monitoring using OD620 revealed that the consortium reached an OD of 1.2 after 48 hours, while individual isolates average Thiobacillus, Bacillus, Sulfobacillus, Rhodococcus, Sphingomonas, Klebsiella, and Geobacillus. Sulfur removal was confirmed through GC and AAS analysis, showing a significant decrease of 0.71. This research highlights the effectiveness of

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microbial consortia in BDS processes, offering substantial implications for improving fuel quality and promoting environmental sustainability.

Keywords:

Microbial consortia, biodesulfurization (BDS), petroleum and fuel, dibenzothiophene (DBT), basal salt media (BSM).

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Introduction

The detrimental consequences of sulfur oxide emissions on the environment and human health have made transportation fuels with almost minimal sulfur content necessary (Dinesh et al., 2024). Oil refineries frequently utilize hydro-desulfurization to eliminate sulfur from fuels obtained from petroleum. There are significant medical, financial, and ecological concerns. High expenses, inefficiency for the most common organ sulfur compounds in diesel (DBT and alkylated variants), toxic wasted catalysts, greenhouse gas emissions, and severe treatment conditions constitute several disadvantages of hydro-desulfurization (Awadh et al., 2020). Petroleum and its fractions account for about 50% of world energy consumption and provide key components for the pharmaceutical sector (Almolhis, 2024). Sulfur is the most common hexagonal atoms in natural oils, which can limit catalyst action during secondary oil processing (Thanh et al., 2024). Crude oil contains sulfur in both pure and complex forms, including mercaptans, sulfides, disulfides, and heterocyclic molecules including condensed polynuclear aromatic hydrocarbons, TH, BT, and DBT (Maslova et al., 2021). Natural sulfur can be found in hydrocarbons such as thiol sulfones, disulfides, thiophenes, thioether, and mercaptans, whereas inorganic sulfur can be found in hydrogen sulfide, elemental sulfur, and pyrites. Petroleum products, such as crude oil, liquids, and distillate fractions, contain significant amounts of complex sulfur compounds. Crude oil is a complicated blend of more than 200 distinct hydrocarbon chemical components. Crude oil's quality is determined by its API gravity, specific gravity, and sulfur content (Ahmad et al., 2023). BTFs efficiently purify biogas and remove hydrogen sulfide. This approach uses SOB to transform H_2S into sulfate and elemental sulfur via an aerobic mechanism. In biological conversion, O_2 functions as an electron acceptor in aerobic and NO_3 in anoxic situations (Sutarut et al., 2023). Significant amounts of sulfur dioxide are emitted into the environment when sulfur-containing oil flames directly, destroying car catalysts and having a detrimental effect on human and animal wellness (John et al., 2024). Acid rain occurs when sulfur oxides in the atmosphere combine with water vapor. Acid rain may damage ecosystems, increase corrosion in buildings and metallic objects, and reduce photosynthetic effectiveness, resulting in crop failures (Mamuad & Choi, 2023). Sulfur is the third most common component of crude oil, and when sulfur-containing oil flames directly, massive volumes of sulfur oxides are emitted into the atmosphere, generating significant pollution; BDS is detrimental to both human and animal health. Global associations for environmental protection have suggested stronger regulations to control the quantity of sulfur in oil to resolve the issue. Current rules require crude oil to have a sulfur level of less than 10 mg/kg. Future restrictions are expected to be more rigorous, increasing the demand for oil desulfurization (Chen et al., 2021). Sulfur emissions from fossil fuel burning are a global concern. Several techniques are utilized to decrease sulfur content, including oxidative, ionic, adsorptive, hydro, and BDS. Bacteria are used in BDS to eliminate sulfur (Alkhalili et al., 2020). Advantages of g-C_3N_4 include Longevity, minimal density, and high chemical stability. BDS performs well in a wide range of applications, including catalysts, membranes, and carriers and has become a prominent chemical due to its low cost and simplicity of manufacturing. Graphitic carbon nitride is made up of either triazine C_3N_3 or tri-S-triazine (C_6N_7) molecules with Van der Waals interactions operating between the layers (Saeed et al., 2022).

Hasanbeik et al., (2022) evaluated the effects of a novel hybrid nanostructure, montmorillonite/ graphitic carbon nitride, on Rhodococcus erythropolis IGTS8's bio-desulfurization activity using Fourier-transform infrared spectrum using X-ray diffraction. Nanoparticles have been examined using field-emitted imaging and transmitted electron microscopy techniques. The beneficial variables in the procedure were identified. The Construct Expert program was used to construct optimal settings for microorganisms. Bhavyaet al., (2024) investigated the BDS capability of mixed microbial consortia for removing sulfur compounds from synthetic wastewater (Hafezieh et al., 2024). Experiments with sulfide loadings (3000-6000 mg/L) and ORP $(-300 \pm 20\,mV, -360 \pm 20\,mV)$ revealed dominant genera (Pseudomonas 73%, Alishewanella 24%, Zobellella 3%). Maximum sulfide conversion occurred at 6000 mg/L sulfide and -360 ± 20 mV ORP. Controlled lab settings limit real-world application.

Khan et al., (2023) used an indigenously isolated microbial community for desulfurization, isolating DBT-Enrichment-based desulfurization of hydrocarbon-contaminated soil. The most effective strain, I5, was found using Illumina sequencing, with 77% desulfurization. 16S rRNA sequencing identified five bacterial phyla. Byproduct inhibition and scalability were two major challenges for industrial applications. El-Sheshtawy et al., (2022) described the natural decomposition of oil contamination with petroleum-degrading bacteria. Nineteen bacterial strains were identified, with Flavobacterium johnsoniae BS1 and Shewanella baltica BS2 demonstrating the most destruction. Goethite-chitosan nanocomposite improved bioremediation efficiency. However, more research was required to improve immobilization techniques and evaluate large-scale environmental applications.

Li et al., (2024) evaluated the O_2 resistance system of sulfate-reducing bacteria (SRB) and its impact on As^+ immobilization. The performance was evaluated using bio-precipitate identification and microbial-metabolic studies in both aerobic and anaerobic conditions. Sulfate reduction effectiveness was decreased in aerobic circumstances (47.37% vs. 66.72%). Limited environmental conditions were investigated; wider applicability requires additional research. Glekas et al., (2022) examined sustainable BDS by examining microbial biocatalysts with high activity against heterocyclic sulfur compounds and durability in petroleum settings. Two Rhodococcus sp. strains were obtained using a 4,6-dimethyl-dibenzothiophene enrichment procedure and found to have improved BDS activity and stability. However, biocatalyst stability loss under biphasic circumstances remains a problem.

Akram et al., (2024) identified Tsukamurella sp. 3OW as a novel desulfurising bacterium capable of digesting dibenzothiophene using the 4S pathway. The genome research identified 57 sulfur metabolism genes, including dszABC. The strain reduced sulfur in crude and diesel oil by 19% and 37%, respectively, although real-world BDS applications require more tuning. Khan et al., (2022) examined how BDS decreases sulfur in resistant molecules such as dibenzothiophene. The bacterial consortia IQMJ-5 were investigated at various temperatures, pH levels, and DBT concentrations to optimize carbon and sulfur supplies. Optimal conditions were 25°C, pH 7.6, and 0.3 mM DBT. Glycerol and Na_2SO_4 promote growth, however, precise parameter management was necessary for optimal results. Parveen et al., (2024) explored Gordonia rubripertincta W3S5 for dibenzothiophene desulfurization. Metabolic and genetic studies verified the 4S pathway and trehalose biosurfactant synthesis. The genome annotation revealed otsA, otsB, trey (Another type of otsA), treZ, and associated genes, validating trehalose production pathways. Further research was required to optimize strain W3S5 for large-scale BDS applications.

Materials and Methods

In this section, the researchers isolate and test microbial consortia from oil-contaminated soil to evaluate their ability to degrade DBT, a sulfur-containing compound found in petroleum.

Isolation of Microbial Consortia

Source of Microorganisms: The research used soil that had been polluted by oil during the petroleum extraction process. These samples are rich in petroleum products and microorganisms adapted to break down these contaminants.

Serial Dilution Technique: The soil sample was exposed to a serial dilution process, which involves gradually diluting the sample and plating it on a solid medium. Serial dilution helps to isolate individual microorganisms.

Nutrient Agar: Nutrient agar medium that promotes the development of a wide range of microorganisms.

- Dilute $28g$ of nourishing agar powder (CM0003B) with 1L of water that has been distilled.
- Mix until fully disintegrated.
- To disinfect, autoclave at $121°C\ for\ 15\ minutes$.
- Pour the liquid into a Petri dish and let it solidify. To avoid contamination, ensure that the agar is prepared in a clean location. When the agar has hardened, it is ready for use.

Basal Salt Media (BSM): BSM is a nutrient-poor medium that stimulates microorganisms to degrade pollutants. To prepare BSM $MgCl_2{\cdot}6H_2O$ (0.0245 g/L), $K_2HPO_4{\cdot}3H_2O$ (3 g/L), $CaCl{\cdot}2H_2O$ (0.001 g/L), $NaH_2PO_4{\cdot}H_2O$ (4 g/L), and $FeCl_3{\cdot}6H_2O$ (0.001 g/L) and $CaCl{\cdot}2H_2O$ (0.001 g/L) were used. Glassware was autoclaved at $121\ °C\ for\ 20\ minutes$. The compounds were dispersed in distilled water until the compounds were completely dissolved. To maintain the medium's pH of 7.0, 0.5 M NaOH was inserted drop wise. The solution was autoclaved using the same circumstances as the previously stated glassware. The created BSM media was kept at normal temperature, away from sunlight.

Dibenzothiophene (DBT): DBT is employed as the only sulfur and carbon source in the medium. DBT implies that the microbes had to depend on DBT for their development, causing them to break down the molecules as part of their metabolic process. Instead of isolating individual strains, a microbial consortium was developed. The community of various microorganisms can work together to break down DBT. Samples of sludge, wastewater (Ghara et al., 2024) and soil from a petroleum refinery were used to identify and improve DBT-desulfurizing bacteria. Ten milliliters of sterilized distilled water were combined with one gram of dirt and one milliliter of water to make suspensions. DBT-assimilating bacteria were isolated and cultivated in SFM. After dissolving DBT (100 ppm) in 10% hexane, 0.5 ml of soil and water concentrations were added to 10 ml of sterilized SFM medium. Samples were shaken at 200 rpm for five days at 30°C.To 10 milliliters of fresh SFM media containing DBT, turbid bacteria (0.1 milliliters) were transferred. The tissue culture was sub-cultivated, placed on LB agar plates, diluted, and then incubated at 30°C for the entire night. The zone of clearance on DBT-coated SFM agar plates, which indicates the isolates' ability to proliferate, was examined. DBT was used to evaluate the isolates for induction.

Testing for DBT Degradation

To evaluate the ability of microbial consortia to degrade DBT, a sulfur-containing compound in petroleum products. The consortia were grown in a controlled environment where DBT was provided as the sole source of sulfur and carbon. To monitor microbial activity and measure DBT concentration over time to assess their ability to break down the compound. DBT testing is crucial as BDS requires efficient degradation of sulfur compounds like DBT. If microbial consortia can effectively degrade DBT, it could potentially improve fuel quality and reduce harmful sulfur emissions making them a promising alternative to traditional chemical desulfurization methods.

Monitoring Growth and Sulfur Removal

Optical Density (OD) Measurement: OD was measured at OD620 to monitor the bacteria' development. OD quantifies how much light the microbial culture absorbs or scatters. Higher OD levels imply more microbial growth. The researchers measured OD to determine how effectively the microbial consortia grew over time, which indicates how aggressively degraded DBT. Bacterial optical density was measured at 620 nm using the Asys Microplate Reader Expert Plus. To determine ATP, samples were reduced in 10% trichloroacetic acid and analyzed using the conventional luciferin-luciferase technique on an LKB Wallac 1251 Luminometer. To count CFUs, bacterial cultures were serially diluted and distributed on Tryptone Glucose Yeast Extract Agar. CFUs were counted after 48 hours of incubation at 28°C.

Gas Chromatography (GC): This method separates and analyses chemicals in a mixture. In this situation, GC was utilized to determine the DBT concentration in the culture medium. A reduction in DBT levels suggests that the bacteria are degrading. To analyze DBT deterioration and evaluate sulfur elimination, GC was used. The microbial consortia were cultured with DBT as the only sulfur source, and samples were collected at various time points. To extract sulfur-containing compounds from the samples, the GC system was set up to operate at temperatures ranging from 100^0 c to 250^0c. The DBT concentration was obtained by comparing the peak areas of DBT in the chromatograms before and during microbial degradation. The decrease in DBT peak area over time demonstrated the capacity of the microbial consortia to degrade DBT and the drop in sulfur content was used to calculate sulfur removal efficiency. An FID was employed for detection with the oven temperature set at 1800 c and the carrier gas being helium at a flow rate of 1mL/min.

Atomic Absorption Spectroscopy (AAS): The AAS approach was utilized to measure the sample's sulfur concentration, which helped to validate the removal of sulfur from the DBT molecule. A decrease in sulfur levels would indicate effective BDS. The AAS Thermo Fisher Logical SOL. The AAR 3300 model was used to analyze metal content in previously treated soil samples. The nitrous oxide, acetylene gas, and blower were immovable. The blower was switched on, and the fluid snare was blown to remove any trapped fluid. The extractor and AAS control were switched on. The chamber and nebulizer were cleaned with sterilizing wire, while the burner opening was cleansed with a course of action card. Open the AAS programming worksheet on the PC and insert the unfilled cathode light into the light holder. The light was switched on, and the cathode bar was aimed at the desired zone on the sequence of motion cards for maximum light output. The fine was put in a 10 ml graduated container containing deionized water, and the yearning rate was measured. The diagnostic clear was generated using a series of changes to known analyze component norms. The clear guidelines were atomized, and their responses were calculated. Adjustment charts were created for each arrangement, followed by atomization and estimation of the examples. The metal fixations in the example arrangement were identified using the absorbance obtained for the puzzling example.

Estimation Viable Microorganisms

Most Probable Number (MPN): The MPN technique was used to determine the amount of viable microorganisms in soil samples. The method includes growing microorganisms by repeated dilution and incubation before determining their concentration based on patterns of growth observed in the dilutions. Using the approach, how many microbes were capable of living and developing in the polluted environment as well as how many actively participated in DBT degradation was determined. The sedimentary medium for tracer MPN (T MPN) identification using soil samples was prepared as follows. The dirt was diluted 1:1 (vol/vol) with the water from the sample and homogenized in a blender. The filtered solution was passed through sieves with mesh sizes of 1-, 0.5-, and 0.25 mm before being autoclaved at 121°C for 20 minutes. After autoclaving, the soil sample solution was cooled by vigorous magnetic whirling and purged with oxygen-free nitrogen. The soil suspension (8.9mL) was aerobically transferred into culture tubes, which were sealed with butyl rubber stoppers in an N2 environment. Tubes underwent incubation for 24 hours at the outside temperature before undergoing two rounds of autoclaving and storage at 50°C to reduce the sediment medium; before inoculation, 0.1 mL of freshly prepared sodium dithionite ($Na_2S_20_4$) solution was aseptically added, achieving a final concentration of 200 μm.

Data Analysis

Data was analyzed statistically using the SPSS program package. ANOVA tests were used to determine significance.

Result and discussion

Desulfurization Results Using Bacterial Consortium (BC) Concentrations

Figures (1-3) show the BDS, showing that increasing reaction time enhances sulfur removal, with 2.5% BC at 30° leading to a rise from 85 mg/kg at 2 hours to 275 mg/kg at 8 hours. Higher biocatalyst concentration improves efficiency, as seen at 6 hours, where 2.5% BC removed 210 mg/kg (21%), while 5% BC removed 300 mg/kg (30%). The BDC rate declines over time, indicating reduced efficiency at later stages, with 2.5% BC at 30°C dropping from 42.5 mg sulfur. Kg-1.h-1 at 2 hours to 34.4 at 8 hours. Temperature increase from 30°C to 35°C at 5% BC enhances sulfur removal from 300 mg/kg to 400 mg/kg and efficiency from 30% to 40%, with the BDS rate rising from 50 to 66.7 mg sulfur Kg-1.h-1. These findings indicate that optimizing BC, reaction time and temperature significantly enhance BDC efficiency.

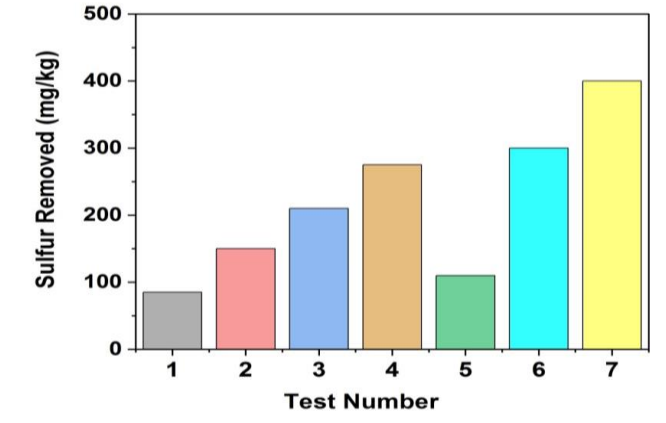

Figure 1. Comparison of sulfur removed (mg/kg) based on test number

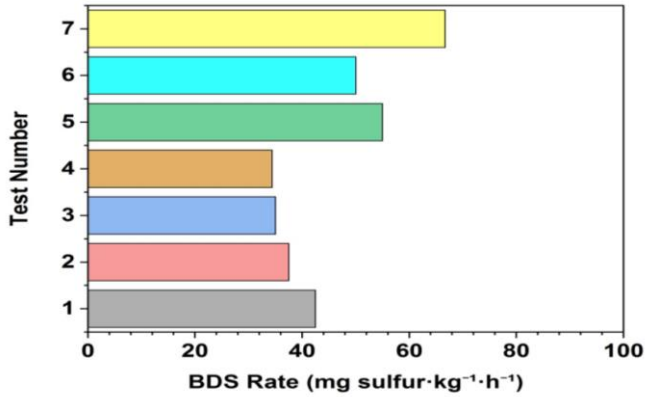

Figure 2. Comparison of BDS rate based on test number

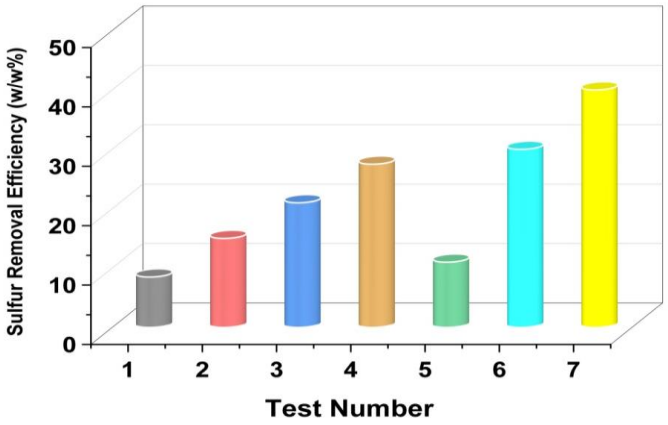

Figure 3. Comparison of sulfur removal efficiency (%) based on test number

Biodesulfurization Capacity Using Different Microorganisms

Table 1 compares the BDS performance of several microorganisms in terms of sulfur removal from DBT a sulfur-containing molecule. The microorganisms were evaluated for their ability to degrade DBT across various periods. *Rhodococcus* had the maximum sulfur removal of 110 mg/kg, which 85% sulfur removal after 24 hours of processing, followed by *sphingomonas* at 100 mg/kg and 80% removed in 20 hours. Other microbes, such as *sulfobacillius*, removed 90 mg/kg of sulfur (75%) in 24 hours, *bacillus* removed 85 mg/kg sulfur (70%) in 18hrs, *Geobacillus removed 95mg/kg sulfur (78%) in 20hrs*, and Thiobacillus removed 75 mg/kg (60%) of sulfur in 24 hours. *Klebsiella* had the lowest sulfur removal rate, removing 60 mg/kg (50%) during 16 hours. It demonstrated that the efficacy of sulfur removal differs between microbial strains, with *Rhodococcus* exhibiting the most promising findings for DBT degradation.

Table 1. Comparison of BDS capability with various microbes

Microorganisms	BDS results (Sulfur removed)	Sulfur source	Process time (h)
Thiobacillus	75 mg/kg 60%	DBT	24
Bacillus	85 mg/kg 70%	DBT	18
Sulfobacillus	90 mg/kg 75%	DBT	24
Rhodococcus	110 mg/kg 85%	DBT	24
Sphingomonas	100 mg/kg 80%	DBT	20
klebsiella	60 mg/kg 50%	DBT	16
Geobacillus	95 mg/kg 78%	DBT	20

Analysis of Variance (ANOVA) for Petroleum and Fuel Desulfurization

Table 2 presents analysis results for different sources of variation affecting BDS and the degradation of DBT ($p<0.05$).

Table 2. ANOVA for petroleum and fuel desulfurization

Source of variation	Effects	Standard error	Student's t-test	P-value
(1) BDS	4689.49	79.72	58.8245	0.0000
(2) DBT	-949.69	210.92	-4.5026	0.0205
(3) Process time	258.95	210.92	1.2277	0.3071
(1) and (2) interaction	381.78	210.92	1.8100	0.1680

Discussion

The BDS experiments show that reaction time, biocatalyst concentration, and temperature significantly influence the sulfur removal efficiency. The sulfur removal increased with longer reaction times, as determined by the enhancement from 85 mg/kg at 18 hours to 75mg/kg at 24 hours using a 2.5% bacterial consortium (BC) at 30°C. Increasing BC concentration improved efficiency, with 5% BC removing 30% more sulfur than 2.5% BC after 6 hours. The desulfurization rate decreased over time, indicating the decline in efficiency at later stages. An increase in temperature to 35°C further improved the sulfur removal, with 5% BC removing 400 mg/kg of sulfur at 35°C, showing 40% sulfur removal efficiency and the rise in the BDS rate from 50 to 66.7 mg sulfur/kg.h. The comparison of microbial strain reveals the difference in bio-desulfurization capacities, with Rhodococcus achieving the highest sulfur removal (110mg/kg, 85% after 24 hours), followed by Sphingomonas (100 mg/kg, 80%) and Geobacillus (95 mg/kg, 78%). The ANOVA results validate that BDS has a highly significant influence on sulfur removal (p-value = 0.0000), while DBT negatively affects sulfur removal (p-value = 0.0205). Process time and the interaction between BDS and DBT have less impact on the bio-desulfurization process.

Conclusion

The major goal of the research was to investigate the potential of microbial consortia for the BDS of DBT in petroleum and fuel applications, with a focus on improving sulfur removal efficiency using a diverse set of microorganisms isolated from oil-contaminated soil samples. The objective was to isolate and characterize microbial consortia capable of degrading DBT, assess their sulfur removal efficiency, and compare the performance of microbial consortia with individual microbial strains. Additionally, it aimed to identify the microbial genera responsible for DBT degradation and evaluate the growth dynamics of the consortia through OD measurements. Soil samples were collected from multiple petroleum extraction sites, and microbial were isolated using serial dilution techniques on nutrient agar and BSM augmented with DBT as the only sulfur and carbon source. Growth monitoring was performed using OD, and sulfur removal efficiency was assessed via GC and AAS. The MPN method was employed to estimate viable microorganism concentrations. The microbial consortia were compared to individual strains for DBT degradation and sulfur removal efficiency. The microbial consortia demonstrated significantly improved sulfur removal compared to individual isolates, with a notable decrease in DBT concentration over time. Growth monitoring revealed that the microbial consortia reached an OD of 1.2 after 48 hours, while individual isolates averaged an OD of 0.71, indicating higher microbial activity in the consortium. Various bacterial genera were identified, including Thiobacillus, Bacillus, Sulfobacillus, Rhodococcus, Sphingomonas, Klebsiella, and Geobacillus contributes to the successful degradation of DBT.

Limitations and Future Scope

DBT in petroleum refiners and fuel production has some limitations, including focusing on a limited number of microbial genera and not extensively varying environmental factors like temperature, PH, and nutrient availability. The scalability of the BDS process also needs further investigation to assess its applicability in industrial settings. Future research should optimize conditions for microbial consortia growth, and advanced molecular approaches, such as metagenomics and transcriptomics, should be investigated to give greater insights into microbial interactions and fuel products.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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Appendix

BDS	Biodesulfurization
DBT	Dibenzothiophene
BSM	Basal Salt Media
GC	Gas Chromatography
AAS	Atomic Absorption Spectroscopy
MPN	Most Probable Number
TH	thiophene
BT	benzothiophene
BTF	Biotrickling filters
SOB	Sulfur-oxidizing bacteria
H_2S	Hydrogen sulphide
NO_3	Nitrate
O_2	oxygen
C_3N_3	Carbon Nitrate
Na_2SO_4	sodium sulfate
otrA	trehalose-6-phosphate synthase
otrB	trehalose-6-phosphate phosphates
otrZ	trehalose-6-phosphate hydrolase
$MgCl_2{\cdot}6H_2O$	Magnesium chloride hexahydrate
$K_2HPO_4{\cdot}3H_2O$	Dipotassium Phosphate
$CaCl{\cdot}2H_2O$	Calcium chloride
$NaH_2PO_4{\cdot}H_2O$	Sodium dihydrogen phosphate
$FeCl_3{\cdot}6H_2O$	Iron (III) chloride hexahydrate
SFM	sulfur-free medium
Na_2S_204	Sodium dithionite
g-C_3N_4	Graphitic carbon nitride
LKB	Luminometer (LKB Wallac)
FID	Flame lionization detector
CFU	Colony Forming unit