Pol. J. Environ. Stud. Vol. 31, No. 5 (2022), 4167-4175
DOI: *10.15244/pjoes/147650* ONLINE PUBLICATION DATE: 2022-05-20

Original Research

Optimizing the Metabolic Performance of Mixed Bacterial Culture Towards Dibenzothiophene Desulfurization under the Effect of Varying Nutrient and Environmental Factors

Javed Khan[1], Muhammad Ishtiaq Ali[1*], Asif Jamal[1], Zaixing Huang[2], Jahangir Khan Achakzai[3], Nazim Nasir[4], Khursheed Muzammil[5], Muhammad Zahid Qureshi[6]

[1]Department of Microbiology, Quaid-I-Azam University, Islamabad 45320, Pakistan
[2]School of Chemical Engineering and Technology, University of Mining and Technology, Xuzhou 221116, China
[3]Institute of Biochemistry, University of Baluchistan, Quetta 87300, Pakistan
[4]Department of Basic Medical Sciences, CAMS, Khamis Mushait Campus, King Khalid University, Abha, Saudi Arabia
[5]Department of Public Health, CAMS, Khamis Mushait Campus, King Khalid University, Abha, Saudi Arabia
[6]Deanship of Educational Services, Department of Biochemistry, Qassim University, Buraydah, Al Qassim, Saudi Arabia

Received: 19 January 2022
Accepted: 23 March 2022

Abstract

Biodesulfurization is a promising approach, capable of reducing the sulfur content of recalcitrant sulfur-containing heterocyclic compounds such as dibenzothiophene and their alkylated derivatives. The performance of bio-desulfurization is undoubtfully dependent on different operating parameters. The effect of different process parameters on the growth rate and desulfurization capability of the bacterial consortium IQMJ-5 have been examined. The parameters that were optimized include the temperature of incubation, initial pH of the medium, and DBT concentration. In addition, the effect of several carbon and sulfur compounds on the growth of bacterial consortia IQMJ-5 was also analyzed. Moreover, the concentration of the most effective carbon compound was also examined in shake flask fermentation. The results showed that 25 °C temperature, 7.6 pH, and 0.3 mM DBT were the optimum conditions for the highest growth and desulfurization of the DBT. In addition, glycerol and Na_2SO_4 were the bioavailable carbon and sulfur sources respectively, at which the consortium IQMJ-5 showed maximum growth. Moreover, $2gL^{-1}$ glycerol appeared as the carbon concentration at which the consortium IQMJ-5 showed the highest activity. An enhanced rate of desulfurization was encountered when a medium with optimized conditions was employed, compared to non-optimized conditions.

*e-mail: Ishi_ali@hotmail.com

The current research study uncovered the potential of the bacterial consortium IQMJ-5 to desulfurize sulfur-containing organic compounds at the optimized conditions of different process parameters.

Keywords: biodesulfurization, dibenzothiophene, Gibb's assay, parameter optimization, consortia

Introduction

Energy plays an important role in the economic and social development of a country. The industrial, economic, and agricultural development of Pakistan is critically hindered by the worst ever catastrophe of energy [1]. After the discovery of huge coal reserves in the Tharparker district of Sindh, globally Pakistan is now ranked as the eleventh richest coal-containing country with 185 billion tons reserves [2]. A large increase in energy requirement has triggered a greater utilization of low rank and brown coal mainly for electricity generation in coal power plants, industry, and domestic heating purposes [3]. Large-scale combustion of sulfur-containing coal for industrial, power generation, and domestic purposes resulted in the release of various oxides of sulfur into the atmosphere [4]. The combination of such sulfur oxides (especially sulfur dioxide) with water vapor in the atmosphere generates sulfuric acid, resulting in acid rain [5]. Acid rain can have serious effects such as endangering aquatic life by causing water pollution, damaging forest life, disruption of chemical balance in the ecosystem, and corrosion of historical buildings. Sulfur oxides emission can also have an adverse effect on health like its inhalation can result in lung cancer and other cardiac-associated illnesses such as asthma and bronchitis (6). About 25% of overall fatalities in African and Asian countries have been estimated to be caused by exposure to water and air pollution [7].

At present, several physical, chemical, and biological techniques are being used to transform or eliminate impurities and noxious substances from fossil fuels [8]. Among these, hydrodesulfurization (HDS) is the most used physicochemical technique for eliminating sulfur-containing compounds from fossil fuels. This technique transforms sulfur-containing organic compounds to hydrogen sulfide (H_2S) in the presence of hydrogen and a metal catalyst under high temperature and pressure [9]. Hydrodesulfurization reduces sulfur-containing aliphatic compounds from fossil fuels up to significant levels with limited reduction of the heterocyclic sulfur-containing organic compounds like dibenzothiophene (DBT) and its alkylated derivatives, that account for up to 70% of the total organosulfur compounds in fossil fuel [10].

Biodesulfurization (BDS) has emerged as one of the most potent alternatives of the physiochemical techniques in which microorganisms or their enzymes serve as natural catalysts for reducing sulfur from heterocyclic sulfur-containing compounds in fossil fuels [11]. This technique has gained wide acceptance in scientific communities owing to its higher selectivity, less energy and capital cost requirements, zero emissions, and waste generation. Moreover, in BDS the bond between carbon and sulfur is cleaved by a sulfur specific 4S pathway, eliminating the sulfur atom in the form of sulfate while the carbon skeleton of the parent compound is leftover unharmed in the form of phenolic end products thereby preserving the combustion value of the fossil fuels [12].

The activity and performance of the isolated microorganism employing the 4S pathway are undoubtfully dependent on the operating parameters and while providing the optimum level of reaction parameters, maximum BDS efficiency could be achieved [13]. Up till now, a limited amount of literature is available in which the operational conditions are considered. Most of the microorganisms that have been isolated so far, carry out desulfurization of DBT at mesophilic conditions, typically at about 30ºC, and only limited literature where desulfurization takes place at thermophilic conditions. Whereas, in the case of pH, the optimum pH reported so far in the literature, ranges from 6.5 to 7.5 [14]. Response surface methodology (RSM), a way to find out the conditions needed for the highest desulfurization capacity of microorganisms, was suggested by [15], through which the optimum operational conditions could be achieved. However, the traditional one factor at a time (OFAT) was used in most of these cases with less information on the combined interaction of the parameters [16].

In previous research various conditions have been optimized for biological desulfurization, however, the synergistic effect of operational parameters received less attention. Therefore, in the present research work, bacterial consortia IQMJ-5 isolated from hydrocarbon-contaminated soil was employed to find out the optimized different operational parameters such as the temperature of incubation, initial pH of the medium, and DBT concentration of the growth and BDS activity. Likewise, the effect of different carbon and sulfur-containing compounds on the cell growth of bacterial consortia IQMJ-5 and the effect of carbon source concentration on the growth and desulfurization was also analyzed.

Material and Method

Microbes, Media and Growth Condition

The bacterial consortium IQMJ-5 used in the present research work was isolated from hydrocarbon-contaminated soil sample "Islamabad", Pakistan (NCBI Bio Project ID: PRJNA765671, data yet not

published). The consortium IQMJ-5 was maintained in 50% glycerol and preserved in -20ºC freezer in Environmental Microbiology Lab, Department of Microbiology, Faculty of Biological Sciences, Quaid I Azam University, Islamabad, Pakistan. The stock MSM media was prepared by dissolving the following in 1 L of distilled water: 1M phosphate buffer (KH_2PO_4: 53 g and Na_2HPO_4: 86.6 g), 1M $MgCl_2.6H_2O$, 1 M NH_4Cl, 0.3 M $CaCl_2.2H_2O$, 50 g/L yeast extract, and 1 M glucose. For making 1L working MSM from stock medium, the amount per liter of distilled water was Phosphate buffer: 50 mL, Glucose: 30 mL, NH_4Cl: 10 mL, $MgCl_2.6H_2O$: 1 mL, $CaCl_2.2H_2O$: 1 mL, and yeast extract: 1 mL. 0.3 mM final concentration of DBT as a prime sulfur source was added from a 100 mM stock (0.72 g dissolved in 40 mL ethanol without sterilization). The pH of the phosphate buffer was maintained at 7.2 with 1 M HCL/NaOH. The media was sterilized by autoclaving at 121ºC and 15 psi pressure for 15 min.

Effect of Different Process Parameters

For optimization of incubation temperature, the culture was inoculated into 250 ml flasks having 100 mL MSM and 0.3 mM of DBT (from stock 100 mM dissolved in ethanol) as sulfur and 5 g/L glucose as carbon source. The flasks were incubated at different temperature ranges such as 25ºC, 30ºC, 35ºC, and 40ºC with an initial pH of 7.2 of phosphate buffer and shaking rate of 100 rpm.

The growth and desulfurization activity of the isolated organisms seems to be dependent on the initial pH of the medium. To find out the optimum initial pH value for the growth and bio-desulfurization potential of the bacterial consortiumIQMJ-5, the bacteria were inoculated in 250 mL flasks containing 100 mL MSM. 0.3 mM DBT and 5 g/L glucose were added as the sole sulfur and carbon sources respectively, whereas the initial pH of different media was adjusted at the range of 6.6 to 8.0 with 1M NaOH/ HCL.

In order to find out the effect of the initial concentration of DBT, the reaction was performed in MSM medium supplemented with varying concentrations of DBT (ranging from 0.1 to 4.0 mM, dissolved in ethanol). The reaction was carried out at a constant temperature of 25ºC with a 100rpm shaking rate. The flasks were incubated on a rotary shaker with a 100rpm shaking rate for 8 days and measurements were made at regular time intervals (24 h) and growth and 2-HBP production were determined at OD600 and A610, respectively.

Effect of Carbon and Sulfur Substrate on the Growth of Bacterial Consortium IQMJ-5

Two sets of experiments were performed. In the first experiment, six types of carbon-containing compounds such as glucose, glycerol, ethanol, 2-HBP, DBT (dissolved in ethanol), and powdered DBT were added in MSM medium as the bioavailable source of carbon to find out their effect on the growing characteristic of the microbial consortium IQMJ-5. An initial concentration of about 2 g/L was maintained in all the experimental runs under the optimized condition of temperature, pH, and DBT concentration. As a sub-part of the first set of experiments, the most effective carbon compound was added in different concentrations (2, 5, 10, and 20 g/L) in order to find its optimum concentration. In the 2nd set of experiments, the effect of different types of sulfur compounds (both organic and inorganic) on the growth of bacterial consortiaIQMJ-5 was examined. The sulfur compounds were added to 250 mL flasks containing 100 mL MSM medium supplemented with DBT at a final concentration of 0.3 mM. In all the flasks, 2 g/L glycerol was added as a carbon source and 0.5 mL of microbial suspension with an initial biomass concentration of 0.06 g $DCWL^{-1}$.

Determination of Growth and BDS Activity

The growth of the bacterial consortium IQMJ-5 was determined by taking OD at 600 nm with Analytik Jena UV visible spectrophotometer (SPECORD 200 PLUS, Germany). The cell concentration was identified by comparing the obtained OD value with a linear relationship between OD and dry cell weight. One unit of OD corresponds to 0.164 g DCW L^{-1}.

The existence and amount of 2-hydroxybiphenyl (2-HBP) in the reaction mixture were confirmed by conducting Gibb's assay and HPLC analysis. This assay is based on the principle that, at alkaline pH, Gibb's reagent (2,6 - dichloroquinone-4-chloroimide) forms a blur color complex by reacting with the hydroxyl group of aromatic compounds [17]. Briefly, 1.5 mL of culture media in a 2 mL Eppendorf tube was centrifuged (HERMLE Z216 MK, Germany) at 10000 rpm for 10 min to remove bacterial isolates. 1 mL of the supernatant was transferred to another fresh Eppendorf tube, the pH of which was adjusted to 8 by the addition of 33 µL of 1M Na_2CO_3. The alkaline supernatant was incubated with 10 µL of Gibb's reagent (50 mg/L in absolute ethanol) for 30 min at room temperature. Blue coloration appeared in a positive reaction after incubation and was measured spectrophotometrically at 610 nm. The result obtained was compared with the standard curve made with authentic 2HBP. The standard curve was obtained by dissolving different concentrations of 2-HBP in a 1 mL medium and the rest of the assay was performed as above. A linear relationship took place between 0.05- and 0.4-mM concentrations of 2-HBP. The detection and quantification of substrate and product (both at optimized and non-optimized state) of the 4S pathway was conducted by High-Performance Liquid Chromatography analysis (Agilent, USA) equipped with a C18 column. The ethyl acetate extract of 50 µL

was injected in a 60:40 acetonitrile/water mobile phase and detected at 280 nm with a UV detector. Substrate and product Identification and quantification were accomplished by comparing the retention time and peak length with the verified reference standard one. Substrate and product retention time following HPLC analysis were found to be 5.481 and 10.505 (average of 10.358 and 10.653), respectively.

Results and Discussion

Effect of Temperature

Temperature is considered one of the most influencing parameters for the growth and desulfurization capability of microorganisms. The results in Fig. 1a) revealed that after growing for eight days, the consortium IQMJ-5 showed the highest biomass concentration (1.02 g DCWL^{-1}) and maximum growth rate (0.0053g h^{-1}) when incubated at 25ºC. Other temperature treatments were not effective in terms of the growth and metabolic activities of the consortium. Desulfurization activity of the consortium IQMJ-5 was also maximum at 25ºC, producing 0.181 mM (highest 0.212 mM, after 6 days incubation) 2-HBP, followed by 0.14 mM 2-HBP at 30ºC (Fig. 2b). The consortium IQMJ-5 showed lower 2-HBP production at 35 and 40ºC (0.062 and 0.013 mM respectively). The growth and desulfurization activity were found to be the function of temperature where an increase in temperature caused a decreased growth and desulfurization activity. Possibly, the biocatalytic activity of microbial cells increased at a certain range of temperature (i.e., 25ºC). However, increased temperatures above this range have retarded or even diminished the biocatalytic activity. Some of the bacterial species such as *Gordonia alkanivorans* strain 1B [18], *Rhodococcus erythropolis* SHT87 [19], *Pseudomonas aeruginosa* S25 [20], and *Serratia marscens* S27 [21] isolated previously from different environmental sources, also performed desulfurization of dibenzothiophene at mesophilic temperature. However, none of the studies provided information regarding the effect of varying temperatures on bio-desulfurization activity. Derikvand et al, [22] have obtained about 55% desulfurization of 0.38 mM DBT in BSM medium at a mesophilic temperature of 27ºC with *Rhodococcus ertythropolis* PD1. In addition, Bhatia and Sharma [23] have applied thermophilic desulfurizing strain *Klebsiella* sp. 13T in the sulfur-free medium at 45ºC and gained about 53 % desulfurization activity. However, the desulfurization obtained in the present study with the bacterial consortium IQMJ-5 was about 70.6 %, which is higher than the above studies.

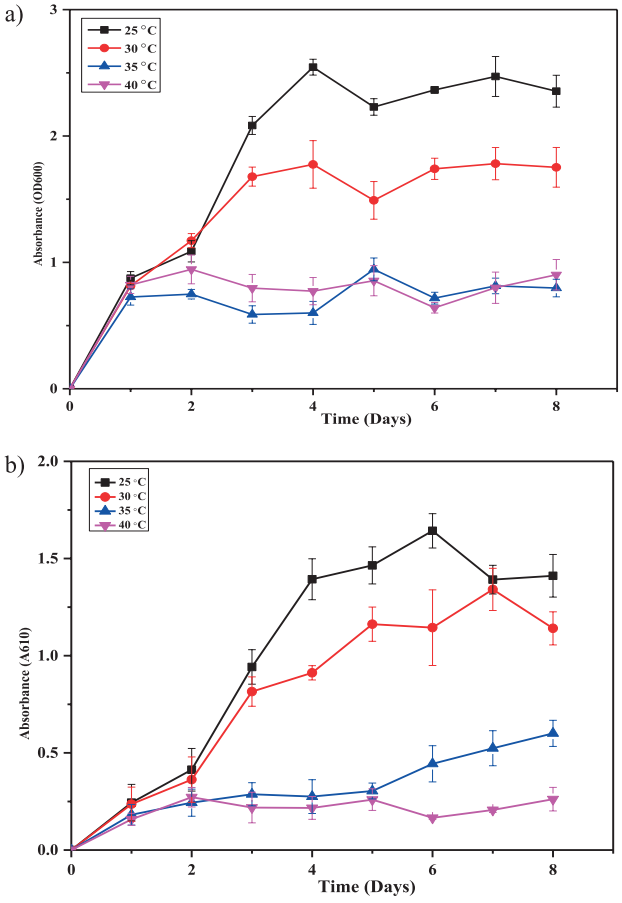

Fig. 1. Effect of temperature of incubation on Growth rate a) and 2-HBP production b) of consortium IQMJ-5.

Effect of pH

Like temperature, substrate concentration, and other important parameters of the culture medium, pH is also an active factor affecting the growth and desulfurizing activity of the microorganisms. The results in Fig. 2a) and 2b) showed the growth and biodesulfurization activity of the bacterial consortium IQMJ-5 at different pH of the medium. The result indicated that slightly alkaline pH conditions significantly affected the growth of the consortium IQMJ-5 with the highest biomass production (0.975 g DCWL^{-1}) and growth rate of 0.0051 g h^{-1}) at pH 7.6. The bacteria showed a slight increase in biomass concentration (0.37 gL^{-1} and 0.39 gL^{-1}) and growth rate (0.0019 gh^{-1} and 0.0020 g h^{-1}) when incubated in the medium having an initial pH of 6.4 and 6.8, respectively. Similarly, the production of 2-HBP was also highest at alkaline pH, exhibited a maximum 2-HBP production of 0.194 mM at 7.6 pH followed by pH 8.0 with a 0.187 mM 2-HBP concentration (Fig. 2b). However, lower production of 2-HBP was observed at a slightly acidic pH of 6.0 to 6.8. The initial hydrogen ion concentration of the culture medium has a regulatory effect on bacterial growth. Variation in hydrogen ion concentration has a striking influence on the structure and function of every enzyme. Moreover, alteration in the initial pH from optimum value affects the binding characteristics of substrate to the active site of the enzyme by altering substrate's structural and electrical properties [24].

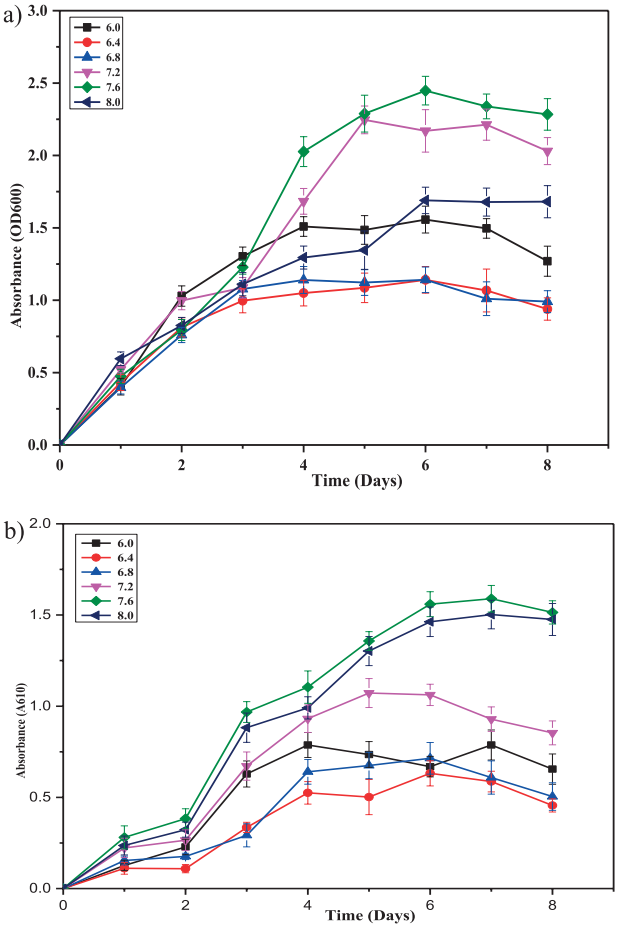

Fig. 2. Effect of initial pH of the medium on Growth rate a) and 2-HBP production b).

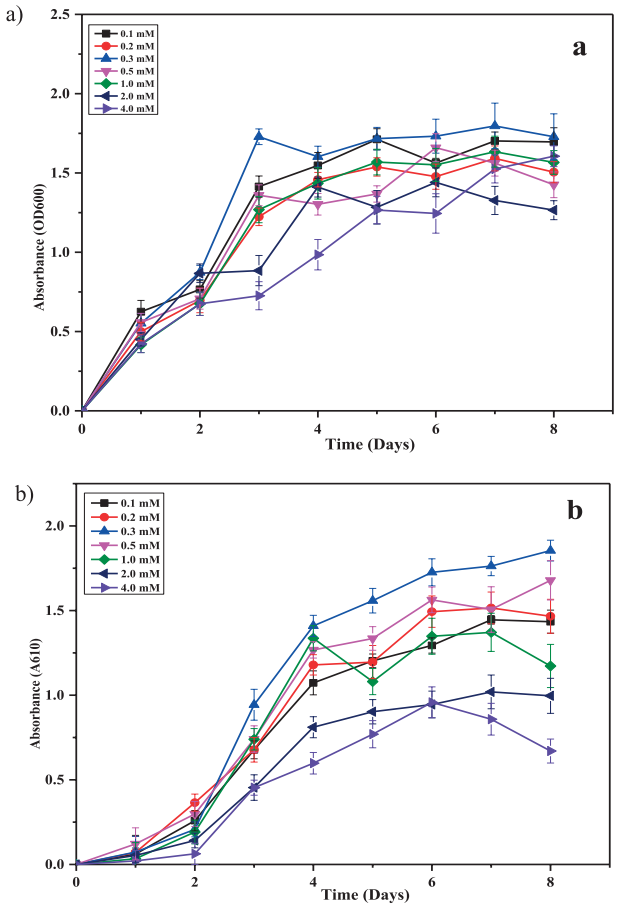

Fig. 3. Effect of initial concentration of DBT on Growth rate a) and 2-HBP production b) of consortium IQMJ-5.

Previous literature regarding biodesulfurization of DBT exhibited that the highest rate of desulfurization was achieved at a pH range close to neutral [25]. While Dejaloud et al., [26] have found maximum biomass of 0.02 g/L at an initial pH of 8 by growing *Ralstonia eutropha* PTCC 1615 in the media with different pH ranged from 6 to 9. Jia Xu et al, [27] performed desulfurization of coal with *Pseudomonas putida*, E. *coli*, and T. *ferrooxidans* in the medium at different initial pH ranges. Maximum desulfurization of 58.12% was obtained with P. *putida* at a pH range of 6. In another study conducted by Tong Liu et al., [28] have obtained about 44 % desulfurization with S. *flava* XL4 at an optimum pH of 4. However, about 66 % desulfurization was obtained in the present study with bacterial consortium IQMJ-5 at pH close to neutral.

Effect of DBT Concentration

The microbial consortia IQMJ-5 was inoculated in a medium containing different concentrations of DBT. The result in Fig. 3a) revealed that the consortium IQMJ-5 showed the highest biomass production (0.73 g $DCWL^{-1}$) and growth rate (0.0038 gh^{-1}) in the medium supplemented with 0.3 mM DBT. The growth slowly decreased with the increase in the concentration of DBT, although growth was not found to be completely retarded even at about 4 mM concentration. In addition, the consortium IQMJ-5 produced the highest concentration of 0.24 mM (Fig. 3b) of 2-HBP when the medium was supplemented with 0.3 mM of initial concentration of DBT. This was followed by 0.215 and 0.187 mM 2HBP production at 0.5 and 0.2 mM DBT, respectively. The production of 2HBP gradually decreases with the increasing concentration of DBT. Naturally, DBT is a xenobiotic compound and at the elevated level, it had a negative effect on cell growth and desulfurization performance of microorganisms. Such kind of effect was also shown by Thayse et al. [29] by providing DBT as the bioavailable source of sulfur to *Pseudomonas fluorescens* UCP 1514. The bacterium exhibited a maximum of 73% desulfurization when supplied 2 mM of DBT, however, the desulfurization achieved with the consortium IQMJ-5 (current paper) is 80%. The bacterium *Pseudomonas putida* CECT 5279 also showed a decreased rate of desulfurization when the concentration of DBT was increased or decreased than the optimal level. Patricia et al. [30] have provided 0.5, 1.0, and 2.0 mM initial concentrations of DBT to the fungus *Cunninghamella elegans* UCP 0596. Although an effective degradation of DBT (about 100 % with

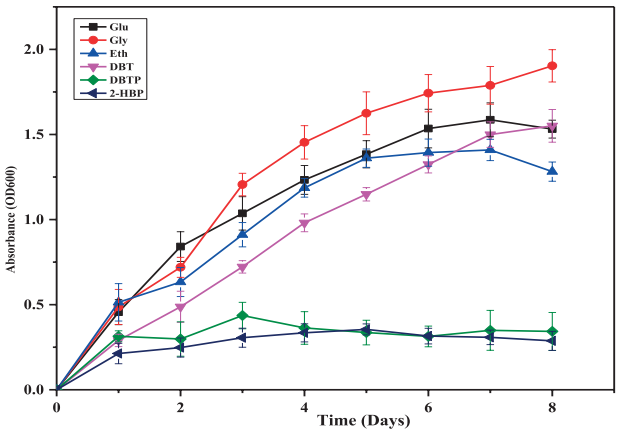

Fig. 4. Growth rate of consortia IQMJ-5 on different carbon compounds.

2 mM) was obtained, the fungus remained unsuccessful in desulfurizing the DBT.

Effect of Carbon Compounds on the Growth of Consortium IQMJ-5

The effect of carbon substrates on the performance of bacterial consortium IQMJ-5 was also examined. The results in Fig. 4 showed that glycerol appeared as a suitable source of carbon with maximum biomass production (0.795 g DCW/L) and the highest growth rate (0.0041 gh^{-1}). The results also showed that other carbon sources like glucose, ethanol, and DBT dissolved in ethanol also supported bacterial growth and metabolism however, comparatively lower than the glycerol. The consortium IQMJ-5 exhibited poor biomass production and growth rate in the medium containing powder DBT (0.07 g DCW/L and 0.0004 gh^{-1}, respectively) and 2-HBP (0.04 g DCW/L and 0.00021 gh^{-1}, respectively). The inability of the consortium to use DBT or 2-HBP as a carbon source or poor growth on these carbon compounds excludes the existence of the ring destructive or Kodama pathway [31], which is an advantage of retaining the calorific value of fossil fuels. Although the growth on DBT (dissolved in ethanol) was also high, this can be attributed to the presence of ethanol in the medium. This was confirmed by Aggarwal et al., [32] by growing different bacteria while supplying ethanol as a carbon source. Previously, Papizadeh et al., [33] have supplied different carbon compounds (such as glucose, glycerol, and benzoate) in MSM medium along with Enterobacter sp. Strain NISOC-03. The maximum desulfurization activity of 64 % was obtained when benzoate was added to the medium.When the consortia were supplemented with different glycerol concentrations (Table. 1), IQMJ-5 exhibited the highest biomass concentration and growth rate when the medium was supplemented with 2 g/L glycerol as a carbon source. Likewise, the production of 2-HBP was also maximum at the same concentration of glycerol. However, the production of 2-HBP gradually decreased with an increasing concentration of glycerol. This indicated that 2 g/L was the optimum concentration at which maximum growth and 2-HBP production were achieved. Previously, a similar kind of research was conducted, in which sulfur-free mineral culture medium (SFM) was supplemented with different carbon compounds and DBT as a sulfur source. These studies were conducted with the pure bacterial culture of *Gordonia alkanivorans* strain 1B [34], *Sulfolobus solfataricus* P2 [35], and *Rhodococcus sp.* Strain SA11, *Stenotrophomonas sp.* strain SA21, and *Rhodococcus sp.* strainSA31 [36]. In the first two studies, glucose serves as the bioavailable source of carbon that is more costly than glycerol. Another advantage of using glycerol as the bioavailable source of carbon is, glycerol also is a byproduct of fossil fuel catabolism. Though Magdy has also used glycerol as a carbon source, the concentration (10 g/L) was more than that of the present research work. Therefore, the present research work is considered more promising and cost-effective in terms of raw materials for the biodesulfurization process.

Effect of Sulfur on the Growth of Bacteria

Fossil fuels have a very complex composition with respect to sulfur compounds and it is advantageous to have bacteria, capable of desulfurizing most of these sulfur compounds. Fig. 5 revealed the effect of different organic and inorganic sulfur compounds on the growth of bacterial consortium IQMJ-5. The results showed that all the sulfur-containing compounds supported the growth of the bacterial consortium IQMJ-5, indicating the ability to desulfurize a broad range of sulfur compounds. However, maximum biomass production (0.82 g $DCWL^{-1}$) and growth rate (0.0043 gh^{-1}) were obtained when Na_2SO_4 was added to the medium as a sole source of sulfur. The consortium IQMJ-5 also

Table 1. Effect of carbon concentration on the growth rate and 2-HBP production.

Carbon Concentration (gl^{-1})	Cell density ($gDCWL^{-1}$)	Growth rate ($g\ h^{-1}$)	2- HBP Production (mM)
2 g	1.0267±0.0874	0.00533±0.00047	0.195±0.015
5 g	0.8367±0.1007	0.00433±0.00050	0.162±0.025
10 g	0.5767±0.1387	0.00300±0.00072	0.135±0.014
20 g	0.6033±0.0737	0.00313±0.00038	0.052±0.017

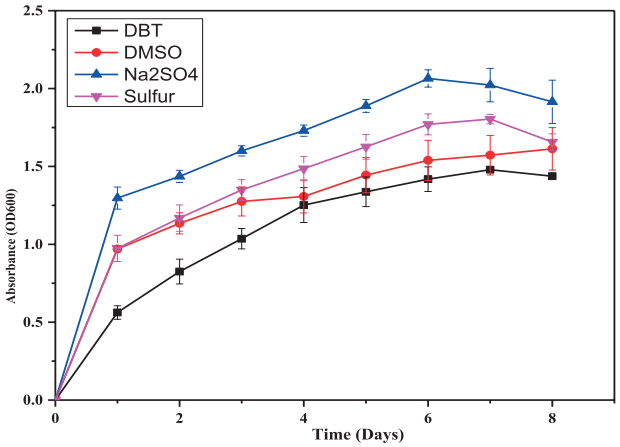

Fig. 5. Growth rate of consortium IQMJ-5 on different organic and inorganic sulfur compounds.

exhibited good biomass concentration and growth rate on elemental sulfur (0.69 g DCWL^{-1} and 0.0036 gh^{-1}, respectively) and DMSO (0.67 g DCWL^{-1} and 0.0035 gh^{-1}, respectively) but was less than that observed with Na_2SO_4. The biomass and growth rate of the bacteria in the medium containing DBT (0.59 g DCWL^{-1} and 0.0031 gh^{-1}, respectively) as the sulfur source was lower than other sulfur sources. This can be attributed mainly due to the lower solubility of DBT in water and the toxic intermediates of the DBT desulfurization pathway like 2-HBP that can inhibit the additional desulfurization process of DBT [22]. Wael Ismael [37] has grown AK6 synthetic bacterial consortium in a sulfur-free chemically defined medium containing various kinds of sulfur-containing organic compounds.

Although, the consortium showed good growth on all the sulfur compounds but showed maximum growth in the medium containing DBT as a sulfur source. In another study conducted by Hussain et al. [38] using *Brevibacillus invocatus* C19 containing different organic sulfurous compounds showed that maximum growth was obtained when DMSO was added in the medium as a sulfur source. Considering the broad organic and inorganic sulfur compounds specificity revealed by the consortium IQMJ-5, it can be applied for the biodesulfurization of different ranks of coal.

Effect of Optimized and Non-optimized Conditions as Revealed by HPLC Analysis

Fig. 6 showed HPLC chromatogram of the effect of both optimized and non-optimized environmental conditions of the medium on the desulfurization activity of consortium IQMJ-5. The results in Fig. 6a) showed that when grown in a non-optimized medium, a lower

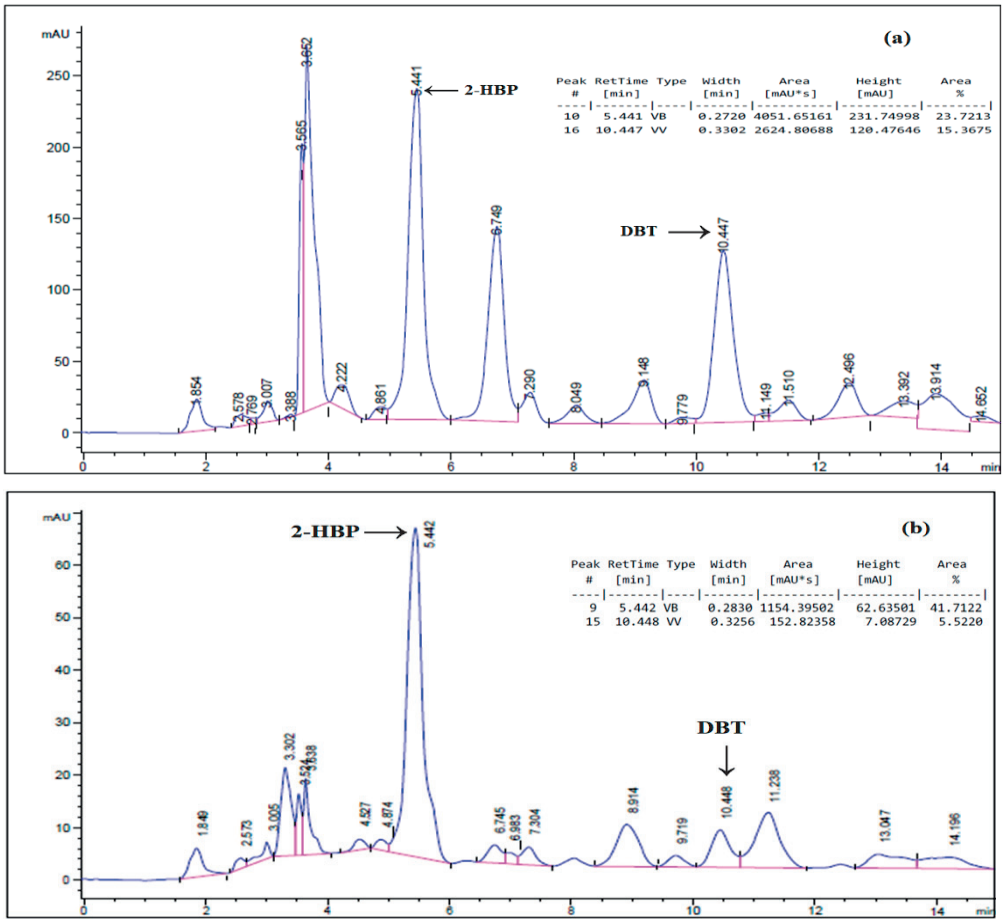

Fig. 6. HPLC analysis showing effect of non-optimized a) and optimized medium condition b).

amount of 2-HBP (above 23%) was produced, although the degradation of DBT was about 85%. On the other hand, at optimized growing conditions of the medium, about 95% of DBT in the medium was degraded while the amount of 2-HBP produced was above 41% (Fig. 6b). The study revealed that, although the generation of 2-HBP was only about 41%, the amount of substrate consumed was about 95% as indicated in the past by Caro et al. [39], which indicated the presence of intermediates along with the product in the reaction mixture. Previous literature using *Stenotrophomonas* sp. NISOC-04 has shown that the concentration of DBT was reduced to about 82% after the completion of desulfurization experimentation [40]. Darikvand et al. applied Box-Behnken response surface methodology for optimization of different parameters at a time and desulfurized about 84% DBT with *Paenibacillus validus* Strain PD2 [13]. Pure cultures were used in both above-mentioned studies, while in the present study a microbial consortium was used that exhibited better desulfurization activity than using pure cultures.

Conclusion

The present research was conducted for optimizing novel bacterial consortium IQMJ-5 for improving the growth and desulfurization of the coal. The performance of the consortium IQMJ-5 was improved significantly by using 2 g/L glycerol as optimum carbon source and 0.3 mM DBT as a sulfur source at 27ºC and slightly alkaline pH, as revealed by Gibb's assay. Moreover, HPLC analysis exhibited that the consortium carried out about 92% desulfurization activity when incubated in the medium with optimized operating parameters. Thus, under such operating conditions, the consortium IQMJ-5 can be used for future in-depth desulfurization of fossil fuels.

Conflict of Interests

The authors declare no conflicts of interest.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through a research group program under grant number RGP.1/345/42. The authors are grateful to the Higher Education Commission of Pakistan for awarding a Ph.D. fellowship Under the Project # MS Leading to Ph.D. under Aghaz e Haqooq e Balochistan Package and NRPU research grant 4568.

References

1. MALIK A.Y., ALI M., JAMAL A., ALI M.I. Isolation and Characterization of Coal Solubilizing Aerobic Microorganisms from Salt Range Coal Mines, Pakistan. Geomicrobiol. J. **34** (2), 109, **2016**.
2. MALIK A.Y., ALI M.I., JAMAL A., FAROOQ U., KHATOON N., OREM W.H., BARNHART E.P., SANFILIPPO J.R., HE H., HUANG Z. Coal biomethanation potential of various ranks from Pakistan: A possible alternative energy source. J. Cleaner. Prod. **255**, 1, **2020**.
3. SABAR M.A., ALI M.I., FATIMA N., MALIK A.Y., JAMAL A., FARMAN M., HUANG Z., URYNOWICZ M. Degradation of low-rank coal by Rhizopus oryzae isolated from a Pakistani coal mine and its enhanced releases of organic substances. Fuel. **253**, 257, **2019**.
4. MACIT M.B., GUMRUKCUOGLU M. Determination of Industrial Sulfur Dioxide Emission and Mapping by Geographic Information System. Pol. J. Environ. Stud. **21** (3), 549, **2012**.
5. KHOSRAVINIA S., MAHDAVI M.A., GHESHLAGHI A.M., DEHGHANI H., RASEKH B. Construction and Characterization of a New Recombinant Vector to Remove Sulfate Repression of dsz Promoter Transcription in Biodesulfurization of Dibenzothiophene. Front. Microbiol. **9** (1578), 1, **2018.**
6. BJELIC L.S., MARKIC D.N., ILIC P., FAROOQI Z.R. polycyclic Aromatic Hydrocarbons in Soils in Industrial Areas: Concentration and Risks to Humans Health. Pol. J. Environ. Stud. **31** (1), 1, **2022.**
7. LATEEF S.A., AJUMOBI O.O., ONAIZI S.A. Enzymatic Desulfurization of Crude Oil and Its Fractions: A Mini-Review on the Recent Progress and Challenges. Arab. J. Sci. Eng. **44**, 518, **2019**
8. GONZÁLEZ N., SIMARRO R., MOLINA M.C., BAUTISTA L.F., DELGADO L., VILLA J.A. Effect of surfactants on PAH biodegradation by a bacterial consortium and on the dynamics of the bacterial community during the process. Bioresour. technol. **102**, 9438, **2011.**
9. BONIEK D., FIGUEIREDO D., PYLRO V.S., DUARTE G.F.D. Characterization of bacterial strains capable of desulphurization in soil and sediment samples from Antarctica. Extremophiles. **14**, 475, **2010**.
10. ALEJANDRO M.D., ROJAS A., BAEZA P., ESPINOZA G., IBACACHE C.Q., OJEDA J. Optimizing the bio-desulfurization of gas oil by adding surfactants to immobilized cell systems. Fuel. **116**, 237, **2014.**
11. MARTINEZ I., MOHAMAD M.E., SANTOS V.E., GARCIA J.L., GARCÍAA-OCHOA F., DÍAZA F. Metabolic and process engineering for biodesulfurization in Gram-negative bacteria. J. Biotechnol. **262**, 47, **2017**.
12. CHEN S., SUN S., ZHAO C., LIU Q., ZANG M. Bio-desulfurization of model oil using growing cells of *Gordonia* sp. SC-10. Pet. Sci. Technol. **37** (8), 907, **2019**.
13. DERIKVAND P., ETEMADIFAR Z., SABER H. Sulfur Removal from Dibenzothiophene by Newly Isolated *Paenibacillus validus* Strain PD2 and Process Optimization in Aqueous and Biphasic (Model-Oil) Systems. Pol. J. Microbiol. **64** (1), 47, **2015**.
14. MARTIN A.B., ALCON A., SANTOS V.E., GARCÍAA-OCHOA F. Production of a Biocatalyst of *Pseudomonas*

putida CECT5279 for DBT Bio-desulfurization: Influence of the Operational Conditions. Energy Fuel. **19**, 775, **2005**.
15. HOKMABADI M., KHOSRAVINIA S., MAHDAVI M.A., GHESHLAGH R. Enhancing the biodesulphurization capacity of *Rhodococcus* sp. FUM94 in a biphasic system through optimization of operational factors. J Appl Microbiol. **00**, 1, **2022**.
16. NOR N.M., MOHAMED M.S., LOH T.C., FOO H.L., RAHIM R.A., TAN J.S., MOHAMAD R. Comparative analyses on medium optimization using one-factor-at-a-time, response surface methodology, and artificial neural network for lysine-methionine biosynthesis by *Pediococcus pentosaceus* RF-1. Biotechnol. Biotechnol. Equip. **31**, 935, **2017**.
17. KARIMI E., YAZDIAN F., RASEKH B., JEFFRYES C., RASHEDI H., SEPAHI A.A., SHAHMORADI S., OMIDI M., AZIZI M., BIDHENDI M.E., HATAMIAN A. DBT desulfurization by decorating bacteria using modified carbon nanotube. Fuel. **216,** 787, **2018**.
18. SILVA T.P., ALVES S., SUSANA M., PAIXAO S.M. Effect of dibenzothiophene and its alkylated derivatives on coupled desulfurization and carotenoid production by *Gordonia alkanivorans* strain 1B. J. Environ. Manage. **270**, 1, **2020**.
19. DAVOODI F.D., VOSOUGHI M., ZIAEE A. Biodesulfurization of dibenzothiophene by a newly isolated *Rhodococcus erythropolis* strain. Bioresour. Technol. **101**, 1102, **2010**.
20. AL-JAILAWI M.H., AL-FARAAS A.F., YAHIA A.I. Isolation and Identification of Dibenzothiophene Bio-desulfurizing Bacteria. Am. J. Biosci. Bioeng. **3** (5), 40, **2015**.
21. EL-BASSI L., OUERTANI R.N., SHINZATO N., GHRABI A. Biotransformation of Dibenzothiophene by Resting Cells of a Newly Isolated *Serratia marscens* Sp. Strain Originated from Industrial Wastewater. J. Bioerm. Biodegrad. **9** (3), **2018**.
22. DERIKVANDA P., ETEMADIFARA Z., BIRIA D. RSM Optimization of Dibenzothiophene Biodesulfurization by Newly Isolated Strain of Rhodococcus erythropolis PD1 in Aqueous and Biphasic Systems. Microbiol. **84** (1), 65, **2015**.
23. BHATIA S., SHARMA D.K. Thermophilic desulfurization of dibenzothiophene and different petroleum oils by Klebsiella sp. 13T. Environ Sci Pollut Res. **19**, 3491, **2012**.
24. GUNAM I.B., IQBAL M., ARNATA I.W., ANTARA N.S., ANGGRENI A.A., SETIYO Y., GUNADNYA I.B. P. Biodesulfurization of Dibenzothiophene by a Newly Isolated *Agrobacterium tumefaciens* LSU20. Appl. Mech. Mate. **855**, 143, **2016**.
25. HIRSCHLER A., CARAPITO C., MAURER L., ZUMSTEG J., VILLETTE C., HEINTZ D., DAHL C., AL-NAYAL A., SANGAL V., MAHMOUD H., DORSSELAER A.V., ISMAILE W. Biodesulfurization Induces Reprogramming of Sulfur Metabolism in Rhodococcus qingshengii IGTS8: Proteomics and Untargeted Metabolomics. Microbiol. Spectr. **9** (2), 1, **2021**.
26. DEJALOUD A., HABIBI A., VAHABZADEH F., AKBARI E. Bioenergetic Aspects of Dibenzothiophene Desulfurization by Growing Cells of Ralstonia eutropha. Pol. **5** (4), 709, **2019**.
27. XU J., LIU X., SONG C., DU Z., WANG F., LUO J., CHEN X., ZHOU A. Biodesulfurization of high sulfur coal from Shanxi: Optimization of the desulfurization parameters of three kinds of bacteria. Energy Sources A: Recovery Util. Environ. Eff. 1, **2019**.
28. LIU T., HOU J., PENG Y. Bacterial Removal of Sulfur from the China Lignite by a Newly Isolated Bacterium, Sinomonas flava XL4. Environ. Prog. Sustainable Energy. **35** (2), 374, **2015**.
29. SILVA T.A.L., SCHWARTZ M., SOUZA P.M., GARRARD I., CAMPOS-TAKAKI G.M., TAMBOURGI E.B. Desulfurization of Dibenzothiophene by Pseudomonas flourescens (UCP 1514) Leading to the Production of Biphenyl. Recent Insights in Petroleum Science and Engineering. ISSN: 953513809X, Pages: 293, **2018**.
30. DESOUZA P.M., SILVA T.A.L., LIMA M.A.B., FRANCO L.D., SCHWARTZ M., SILVA P.H., BARBOSA L.R., NASCIMENTO A.E., OKADA K., CAMPOS-TAKAKI G M. Reduction in the Sulfur Content of Fossil Fuels by *Cunninghamella elegans* (UCP 0596) to Dibenzothiophene Compound. Recent Insights in Petroleum Science and Engineering. ISSN: 953513809X, Pages: 309, **2018**.
31. WANG L., JI G., HUANG S. Contribution of the Kodama and 4S pathways to the dibenzothiophene biodegradation in different coastal wetlands under different C/N ratios. J. Environ. Sci. **76**, 217, **2019**
32. AGGARWAL S., KARIMI I.A., IVAN G.R. In silico modeling and evaluation of *Gordonia alkanivorans* for biodesulfurization. Mol. Biosyst. **9**, 2530, **2013**.
33. PAPIZADEH M., ROAYAEI ARDAKANI M.R., MOTAMEDI H. Growth-phase dependent biodesulfurization of Dibenzothiophene by Enterobacter sp. strain NISOC-03. Pol. **3** (1), 101, **2017**.
34. ALVES L., SALGUEIRO R., RODRIGUES C., MESQUITA E., MATOS J., GIRIO F. M. Desulfurization of dibenzothiophene, benzothiophene and other thiophene analogs by a newly isolated bacterium, *Gordonia alkanivorans* strain 1B. Appl. Biochem. Biotechnol. **120**, 199, **2005**.
35. GUN G., YURUM Y., DOGANAY G.D. Revisiting the biodesulfurization capability of the hyperthermophilic archaeon *Sulfolobus solfataricus* P2 revealed DBT consumption by the organism in an oil/water two-phase liquid system at high temperatures. Turk. J. Chem. **39**, 255, **2015**.
36. MAGDY E.S.M., ZAKARIYA H.A.Y., JOHN V.V. Biocatalytic desulfurization of thiophenic compounds and crude oil by newly isolated bacteria. Front. Microbiol. **6** (112), 1, **2015**.
37. ISMAIL W., EL-SAYED W.S., ABDUL RAHEEM A. S., MOHAMED M.E., EL NAYAL A.M. Biocatalytic Desulfurization Capabilities of a Mixed Culture during Non-Destructive Utilization of Recalcitrant Organosulfur Compounds. Front. Microbiol. **7**, 1, **2016**.
38. NASSAR H.N., EL-GENDY N.S., ABO-STATE M.A., MOUSTAFA Y.M., MEHDY H.M., EL TEMTAMY S.A. Desulfurization of Dibenzothiophene by a Novel Strain *Brevibacillus Invocatus* C19 Isolated from Egyptian Coke. Biosci. Biotechnol. Res. Asi. **10** (1), 29, **2013**.
39. CARO A., LETON P., GARCIA-CALVO E., SETTI L. Enhancement of dibenzothiophene biodesulfurization using β-cyclodextrins in oil-to-water media. Fuel. **86,** 2632, **2007**.
40. PAPIZADEH M., ARDAKANI M.R., MOTAMEDI H., RASOULI I., ZAREI M. C–S Targeted Biodegradation of Dibenzothiophene by *Stenotrophomonas* sp. NISOC-04. Appl. Biochem. Biotechnol. **165**, 938, **2011**.