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Biodegradation of the aromatic fraction from petroleum diesel fuel by *Oerskovia* sp. followed by comprehensive GC×GC-TOF MS

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Highlights

- PAHs degradation from diesel fuel aromatic fraction was investigated
- Novel method was applied for the visualization degraded compounds in the GC×GC-TOF MS chromatograms
- *Oerskovia sp*. CHP-ZH25 has successfully degraded different PAHs from diesel fuel aromatic fraction

Abstract

Polycyclic aromatic hydrocarbons (PAHs) from petroleum and fossil fuels are one of the most dominant pollutants in the environment. Since aromatic fraction from petroleum diesel fuel is mainly composed of PAHs, it is important to discover new microorganisms that can biodegrade these compounds. This article describes the biodegradation of the aromatic fraction separated from petroleum diesel fuel using the strain *Oerskovia* sp. CHP-ZH25 isolated from petroleum oil-contaminated soil. The biodegradation was monitored by gravimetry and GC×GC-TOF MS. An innovative method was applied to visualize degraded compounds in the data provided by a GC×GC-TOF MS. It was shown that *Oerskovia* sp. CHP-ZH25 degraded 77.4 % based on gravimetric analysis within 30 days. Average rate of degradation was 14.4 mg/L/day, 10.5 mg/L/day and 4.0 mg/L/day from 0-10 day, 10-20 and 20-30 day, respectively. The order of PAH degradation based on decrease in peak volume after 30 days of incubation was as follows: dibenzothiophene derivatives> benzo[b]thiophene derivatives > naphthalene derivatives > acenaphthene derivatives> acenaphthylene/biphenyl derivatives> fluorene derivatives > phenanthrene/anthracene derivatives. Here we demonstrated that *Oerskovia* sp. CHP-ZH25 could potentially be a suitable candidate for use in bioremediation of environments polluted with different PAHs.

Keywords: petroleum diesel fuel, PAHs, biodegradation, *Oerskovia* sp., GC×GC-TOF MS

1. Introduction

Petroleum diesel, a product of crude oil refining, is a complex mixture of paraffins (75%) and aromatic hydrocarbons (25%) [1]. When released into the environment, it is very toxic to plants, animals and humans [3]. Remediation of diesel-contaminated sites can be done using both physico-chemical and biological methods, but biological methods are more economical and efficient [4, 5]. Many authors have investigated the degradation of diesel fuel, but the greatest challenge is degradation of the aromatic fraction, which is mainly composed of polycyclic aromatic hydrocarbons (PAHs) [2, 6].

PAHs are a group of compounds with two or more fused aromatic rings [8, 9]. They are very hydrophobic, with low aqueous solubility and their bioavailability is limited by their high molecular weights and low water dissolution rates [10, 11]. Despite these facts, many microorganisms have been isolated primarily for their ability to transform and degrade PAHs to simple molecules [7, 12, 13]. Actinomycetes are a group of bacteria that are very often used in the bioremediation of various PAHs, due to their resistance to harsh conditions (drought, alkaline pH) and their production of extracellular enzymes and biosurfactants [12-14]. Strains that are used usually belong to the genera *Mycobacterium*, *Rhodococcus*, *Nocardia* and *Gordonia*, but it is assumed that a lot more of the actinobacteria members could degrade PAHs [6, 7, 14].

The aim of this study was to isolate new bacterial strains that have the potential to degrade PAHs. Six bacterial strains were isolated from a bacterial consortium that was collected from a petroleum oil-contaminated site and was previously used to biodegrade different complex hydrocarbons [15]. After evaluation of bacterial growth on various aromatic carbon sources it was concluded that strain CHP-ZH25 belonging to genus *Oerskovia* showed the highest potential for the biodegradation of PAHs. Bacteria of the genus *Oerskovia* belong to the phylum *Actinobacteria*, and they are capable of starch and cellulose degradation and sulfur removal from dibenzotiophene [16, 17]. Furthermore, it was shown that they can tolerate pure and mixtures of saturated, monoaromatic and polyaromatic hydrocarbons [18], and recently, Haleyur et al (2018) showed that they could degrade naphthalene, phenanthrene and pyren [19]. However, to our knowledge, the degradation of aromatic hydrocarbons from a complex mixture was never determined. The objective of this study was to assess the biodegradation of aromatic fraction from petroleum diesel fuel by *Oerskovia* sp. Since diesel fuel aromatic fraction is a mixture of various polycyclic hydrocarbons, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF MS) was used to evaluate and improve analysis of the PAHs removal.

2. Experimental

2.1. Isolation, characterization and identification of bacteria

2.1.1. Isolation

Bacterial strains CHP-ZH25, CHP-NR31, CHP-315, CHP-A35, CHP-Y37 and CHP-YG38 were isolated from petroleum oil-contaminated soil sampled from Serbia [15], by repeated inoculation onto mineral medium with 2000 ppm of diesel as the only source of carbon and M3 medium [20,21].

2.1.2. Growth on different carbon sources

To evaluate bacterial growth on various carbon sources, each strain was inoculated on mineral medium agar [20] supplemented with different aromatic compounds: 3,4-dihydroxybenzoic acid (500 ppm), 4-hydroxybenzoic acid (1000 ppm), sodium-benzoate (500 ppm), phenol (200 ppm), phenanthrene (200 ppm), diesel fuel (2000 ppm) and motor oil (200 ppm). The bacterial strains were than incubated for 3 days on 28 °C.

2.1.3. Sensitivity to heavy metals

The sensitivity of the six bacterial strains to heavy metals was tested by the disk diffusion method in Mueller-Hinton agar [22]. Increasing concentrations of $Cd(CH_3COO)_2$, $NiCl_2$, $CuSO_4x5H_2O$, $Zn(CH_3COO)_2$, and $K_2Cr_2O_7$ were used.

2.1.4. Identification of bacterial strains

For preliminary identification of six bacterial strains, API tests (BioMerieux) were utilized. The API Coryne, 20E and 20NE were used according to manufacturer's instructions.

Fatty acid methyl esters of isolate CHP-ZH25 were determined based on the method previously described [23]. The samples were then analyzed by GC/MS. The gas chromatograph/mass spectrometer system used was Agilent 7890A–5975C inert XL EI/CI with HP-5 ms (30 m× 0.25 mm ×0.25 μm) column. Helium gas was used and the oven program was set to: 50 °C for 0 min, then 4.3–285 °C for 5 min. For FAME identification, standard bacterial acid methyl esters (BAME, Supelco) and the NIST5a.L database were used.

For analysis of 16S rRNA, genomic DNA was extracted with Dneasy Blood & Tissue kit (Qiagen). The 27F (AgAgTTTgA TCM Tgg CTC Ag) and 1492R (Cgg CTA CCT TgT TAC gAC TT) primers were used for the amplification of genomic DNA by PCR [22, 23]. The sequencing was performed by Macrogen Inc, Netherland. According to 16S rRNA gene sequence analysis, strains CHP-ZH25, CHP-NR31, CHP-315, CHP-A35, CHP-Y37 and CHP-YG38 were identified as *Oerskovia* sp. CHP-ZH25 (JX430000), *Rhodoccocus* sp. CHP-NR31 (JX965395), *Gordonia* sp. CHP-315 (JX429999), *Micrococcus* sp. CHP-A35 (JX965396), *Sphingobacterium* sp. CHP-Y37 (JX965397) and *Cupriavidus sp*. CHP-YG38 (JX965398), respectively. Obtained sequences were compared to NCBI GenBank database using the BLAST program. The software MEGA 7.0.21 was used to construct a phylogenetic tree. Strains were deposited at the Institute of Soil Science (Belgrade, Serbia) culture collection ISS WDCM375 with the following accession numbers: *Oerskovia* sp. HP-ZH25 - ISS 621; *Rhodococcus sp.* CHP-NR31 - ISS 622; *Gordonia* sp. CHP-315 - ISS 623; *Micrococcus* sp. CHP-A35 - ISS 620; *Sphingobacterium* sp. CHP-Y37 - ISS 624 and *Cupriavidus* sp. CHP-YG38 - ISS 625.

2.2. Fractionation of petroleum diesel fuel aromatic fraction

The aromatic fraction of diesel fuel, purchased at a local gas station in Serbia, was prepared using the procedure previously reported [26]. Briefly, diesel fuel (5 mL) was passed through a column (30 ×1.5 cm), with 10g of silica gel (70-230 mesh, Merck KGaA, Germany). The first two fractions (alkanes and branched/cyclic alkanes) were eluted with 30 mL and 10 mL of hexane, respectively. The third, aromatic fraction was eluted with a mixture of hexane:toluene (3:1, v/v). This procedure was repeated several times using fresh columns, to obtain enough aromatic fractions for the biodegradation experiment. The percentage of aromatic fraction in

diesel fuel was determined by gravimetry. The aromatic fraction and diesel fuel were analyzed by Vario EL III CHNS/O Elemental Analyzer (Hanau, Germany) to assess the content of carbon, nitrogen, oxygen and sulfur.

2.3. Biodegradation experiment

Bacterial strain CHP-ZH25 was inoculated onto solid mineral medium [20] with 200 ppm of petroleum diesel fuel aromatic fraction as the only source of carbon. It was incubated at 28 °C for 7 days. After three passes on this medium, the strain was then transferred from solid mineral medium with 200 ppm of aromatic fraction to an Erlenmeyer flask containing 100 ml of fresh liquid mineral media with 300 ppm of aromatic fraction and incubated at 28 °C for 7 days. The resultant culture was then centrifuged (4500 rpm) and re-suspended in sterile quarter strength Ringer's solution three times. Aliquots (1 mL) of the cell suspension were then inoculated into 100 mL of the liquid mineral medium [20] with 300 ppm of aromatic fraction. The inoculated medium was incubated at 28 °C on a rotary shaker at 150 rpm. An abiotic control was prepared, which contained everything except the bacterial culture and was processed the same way as the media with bacteria. Both bacterial culture and the abiotic control were prepared in triplicate. The biodegradation process was monitored every 10 days for 30 days. Bacterial numbers were estimated using the plate counting method on nutrient agar plates. Biodegradation was stopped after 30 days by adding 2 % $HgCl_2$, and the remaining aromatic fraction was extracted with three volumes of hexane. The amount of remaining aromatic fraction was determined by gravimetry. Extracts were dissolved in 8 mL of hexane and passed through a clean-up column (8 × 0.5 cm) filled with florisil (60-100 mesh ASTM, Merck KGaA, Germany) and anhydrous sodium sulphate (Merck KGaA, Germany). The resultant cleaned extracts were analyzed with GC×GC-TOF MS.

2.4. Comprehensive two dimensional gas chromatography/mass spectrometry

Extracts that passed through a clean-up column were evaporated to constant weight, dissolved in 1 mL of hexane and analyzed with GC×GC-TOF MS, system reported in our previous studies [27, 28]. The Agilent 7890A gas chromatograph (Agilent, Wilmington, DE, USA) was equipped with a GERSTELMPS2 autosampler. An InertCap 5MS/Sil (45m × 0.25 mmi.d., 0.10 µm) column was connected with a BPX50 (0.9 m×0.10 mmi.d., 0.10 µm) column with a loop jet modulator (Zoex KT2006, Zoex Corp.) and a high resolution (HR) TOFMS (JeolAccuTOFGCv 4G), which was tuned to enable measurement at high mass resolution, i.e. $m/\Delta m = 10{,}000$ (FWHM). The samples were injected at 280 °C splitless. Helium was used as carrier gas with a velocity of 1.8 mL/min. The oven was programmed from 50 °C (held for 2 min) to 300 °C at 3 °C/min. The modulation period was 4 s. The temperature of the transfer line was 300 °C, and the ion source temperature was 280 °C. The electron energy was 70 eV. The scan range was *m/z* 33-850. The GC×GC-TOF MS data were analyzed using GCImage R2.6 for HRMS (GCImage LLC).

3. Results and discussion

3.1. Identification and characterization of bacteria

Six bacterial strains were isolated from soil contaminated with petroleum oil. The ability of these strains to utilize various compounds as the only source of carbon and their sensitivity to heavy metals was evaluated. The results are shown in Table 1.

Among the examined strains, CHP-ZH25, CHP-Y37 and CHP-NR31 were capable of growth on different organic substrates, especially diesel fuel and motor oil, and showed relatively high resistance to heavy metals, especially nickel. Thus, these strains were further examined for their ability to grow on diesel fuel aromatic fraction. After three passes on solid mineral medium with aromatic fraction as the only source of carbon, strain CHP-ZH25 was estimated to grow the most efficiently compared to other isolated strains and was selected for further study.

For the assessment of biochemical properties of strain CHP-ZH25, the Api Coryne test was used. Strain CHP-ZH25 was positive for the following reactions: oxidase, catalase, nitrate reduction to nitrite, pyrazimidase, α-glucosidase, β-galactosidase, urease, β-glucosidase, gelatin hydrolysis, and fermentation of glucose, ribose, xylose, maltose, lactose, sucrose and glycogen. The negative reactions were pyrrolidonylarylamidase, N-acetyl-β-glucosidase, alkaline phosphatase, β-glucuronidase, pyrazinamidase and mannitol fermentation.

FAME analysis showed that the predominant fatty acids produced by CHP-ZH25 were *anteiso*-pentadecanoic, *iso*-pentadecanoic and hexadecanoic acid (Table 1). These results are in accordance with previously published data for genus *Oerskovia* [29].

Table 1. Growth on different carbon sources and sensitivity to heavy metals.

	Oerskovia sp. CHP-ZH25	*Rhodoccocus* sp. CHP-NR31	*Gordonia* sp. CHP-315	*Micrococcus* sp. CHP-A35	*Sphingobacterium* sp. CHP-Y37	*Cupriavidus* sp. CHP-YG38
Growth on different carbon sources[1]						
Phenol	+	+	+	-	+	-
Phenanthrene	+	+	-	-	+	-
3,4-dihydroxybenzoic acid	-	-	-	-	-	-
4-hydroxybenzoic acid	+	+	+	+	+	+
Sodium benzoate	-	+	-	-	+	-
Motor oil	+++	+++	+++	+	+	+
Petroleum diesel fuel	+++	+++	++	+	+++	+
Sensitivity to heavy metals[2]						
Cd	2.5	<1	10	2.5	10	10
Ni	50	>50	10	50	10	10
Cu	10	5	25	10	10	10
Zn	10	2.5	10	10	10	2.5
Cr	10	2.5	2.5	10	>10	5

Cellular fatty acid composition of the strain CHP-ZH25, % of total detected[3]										
Fatty acid	*i*14:0	*n*14:0	*i* 15:0	*ai* 15:0	*n*15:0	*i* 16:0	*n*16:0	*i* 17:0	*ai17:0*	*n*17:0
%	0.62	4.49	10.24	62.32	1.65	3.27	10.6	5.48	0.21	1.13

[1]The absence of growth or growth are marked as – or +, respectively. For petroleum diesel fuel and motor oil, the growth intensity is marked with + (minimum growth), ++ (medium growth) or +++ (best growth) [2]The resistance is expressed as minimal inhibitory concentration (mmol/L).[3]Fatty acids are designated in terms of the total number of carbon atoms: number of double bonds. The prefixes *n*, *i* and *ai* indicate normal (unbranched) chain, *iso* and *anteiso* branching, respectively. Values <0.20 % are omitted.

The identification of the six bacterial species according to BLAST analysis is given in the Supplementary material (Table S1). According to 16S rRNA gene sequence analysis, strain CHP-ZH25 was identified as *Oerskovia* sp. CHP-ZH25 (JX430000). This sequence was compared to the NCBI GenBank database using the BLAST program, and the most similar type strain was *Oerskovia enterophila* DSM strain 43852 (NR_026239). The relationship between *Oerskovia* sp. CHP-ZH25 and five other bacterial strains that we considered is shown in Figure S1 (Supplementary material). *Oerskovia* sp. CHP-ZH25 differs biochemically from the type strain only in two reactions, pyrazinamidase and gelatin hydrolysis. Also, compared to the type strain *Oerskovia enterophila* DSM 43852, CHP-ZH25 produces a higher percentage of *i*-$C_{15:0}$ and a lower percentage of *ai*-$C_{17:0}$ [30]. The bioremediation potential of *Oerskovia* sp. CHP-ZH25 for degradation of aromatic compounds was further evaluated in biodegradation experiment.

3.2. Biodegradation experiment

The aromatic fractions separated from ten volumes of 5 mL of petroleum diesel fuel were evaporated to constant weight. The obtained weights ranged between 88 mg and 116.7 mg, which accounted for 1.76-2.3% of total diesel fuel mass. Elemental analysis showed that diesel fuel contained 88.8% carbon, 15.0% hydrogen, 0.5% nitrogen and 0.5% sulfur. The aromatic fraction separated from diesel fuel contained 86.9% C, 11.0% H, 0.4% N and 1.7% S. All aromatic fractions were combined in composite sample to be used in further study.
The aromatic fraction degradation potential of *Oerskovia* sp. CHP-ZH25 was assessed gravimetrically after 10, 20 and 30 days in triplicate experiment. Percentage of aromatic fraction degradation with bacteria and in abiotic control together with the bacterial counts at 10, 20 and 30 days are shown in Figure 1. Average rate of degradation, calculated from the gravimetric results, was 14.4 mg/L/day, 10.5 mg/L/day and 4.0 mg/L/day from 0-10 day, from 10-20 and from 20-30 day, respectively. The gravimetric loss in the abiotic control after 30 days incubation was similar to that previously reported [31, 32]. The number of bacteria had increased steadily until the 20th day, when the reduction in number was detected, as a result of accumulation of products of microbial metabolisms and substrate depletion. For the analysis of biodegradation of naphthalenes, acenaphthylene/biphenyls, acenaphthenes, fluorenes, phenanthrenes/anthracenes, benzo[b]thiophene and dibenzothiophenes using *Oerskovia* sp. CHP-ZH25, GC×GC-TOF MS analysis was applied.

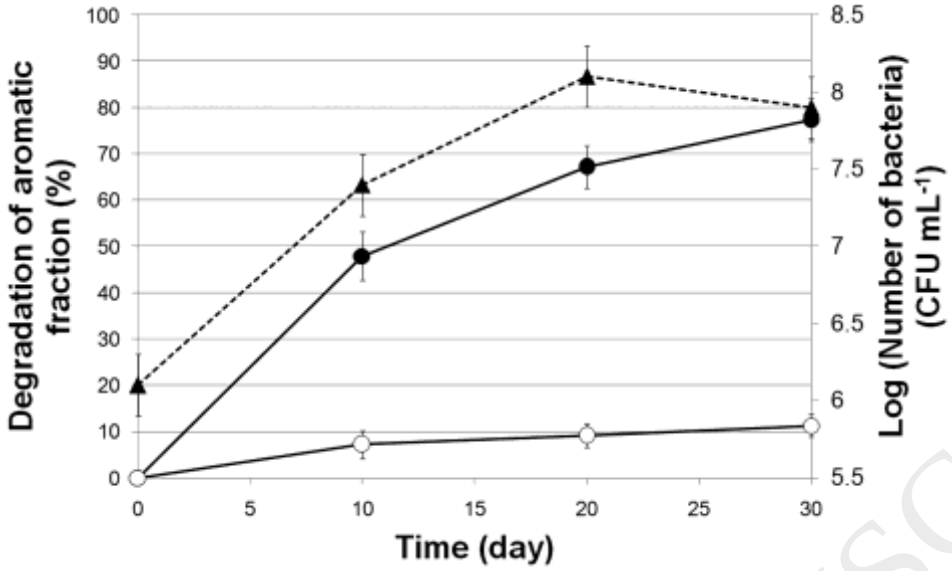

Figure 1. Percentage of aromatic fraction degradation and the number of *Oerskovia* sp. CHP-ZH25. Closed and open circle symbols on solid lines denote the percentage of aromatic fraction degradation with bacteria and in abiotic control, respectively, and closed triangles on dash line number of bacteria.

3.3. GC×GC-TOF MS analysis

Figures 2A and 2B show the two-dimensional total ion chromatograms (2D TICs) of cleaned extracts from the abiotic control and *Oerskovia* sp. CHP-ZH25 cultured on diesel fuel aromatic fraction after 30 days of incubation. Changes after 30 days of incubation for various aromatic compounds can be seen. Also, since the fractionation of diesel fuel isn't complete and some aliphatics remain in the separated aromatic fraction, decrease in aliphatic alkenes can be observed. The most noticeable decrease is found in the area up to a 40 min retention time, where the lower molecular weight compounds are found. This was expected, since these compounds are more volatile and more susceptible to microbial degradation [33, 34].

Many studies have shown that microbial consortia are more efficient in biodegradation than single cultures, especially in the case of complex pollutant mixtures [10, 35, 36]. However, testing of individual strains of microorganisms with respect to degradation ability and the ability to survive is necessary for the selection of strains for use in bioaugmentation. Most of the studies on PAH degradation were carried out with different Actinobacteria, mainly *Rhodoccocus, Micrococcus* and *Mycobacteria* [6, 7, 10, 14]. The *Oerskovia sp*. strain was only recently confirmed to be able to utilize PAHs as a sole source of carbon [19], however no data is available on the degradation of PAH mixtures.

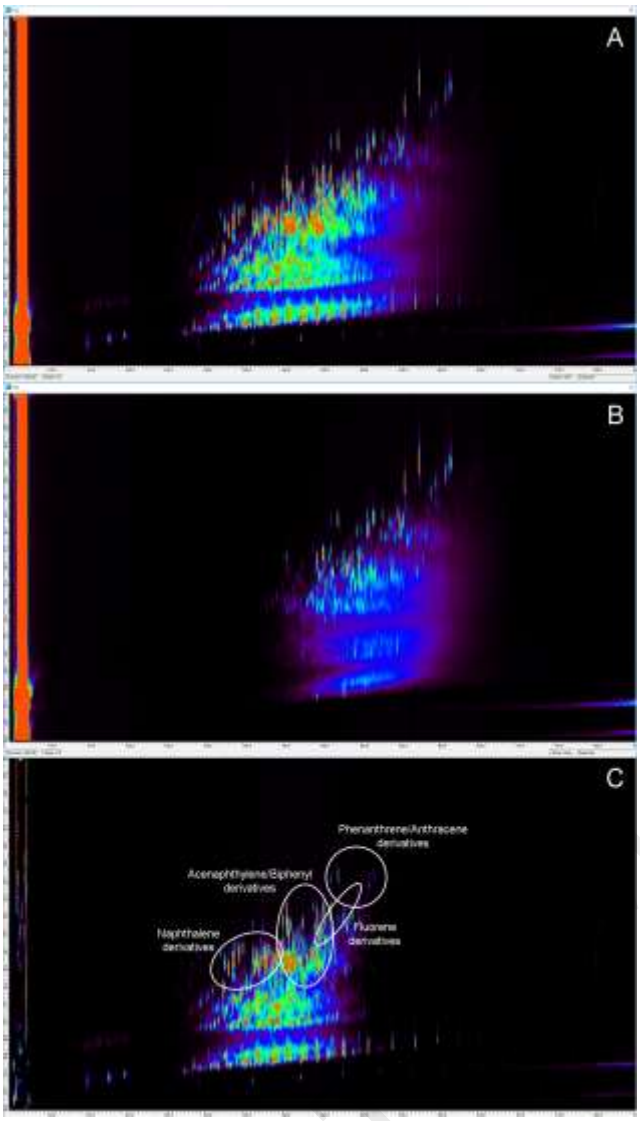

Figure 2. GC × GC-TOFMS chromatograms of: (A) Petroleum diesel fuel aromatic fraction in abiotic control after 30 days; (B) Petroleum diesel fuel oil aromatic fraction in test with *Oerskovia* sp. after 30 days; (C) Compounds from petroleum diesel fuel aromatic fraction degraded by *Oerskovia* sp. CHP-ZH25 (chromatogram obtained using equation 1)

To analyze in more detail the compounds metabolized by *Oerskovia* sp. CHP-ZH25, change of mass spectra during the degradation was estimated. Firstly, a factor, *F(i)*, representing the change of each compound, *i.e.* each data point, *(i)*was calculated by the following equation:

$$F(i) = (T_{AC}(i) - T_{ZH}(i))/T_{AC}(i) \quad (1)$$

Where $T_{AC}(i)$ and $T_{ZH}(i)$ is the total intensity of each compound (data point) *(i)* of abiotic control and *Oerskovia* sp. CHP-ZH25 after 30 days of degradation, respectively. Each changed mass spectrum was estimated by multiplying the factor *F(i)* by each compound (data point) of abiotic

control. Total intensity values were used to provide the factor since mere subtraction of each mass spectrum would likely create artificial mass spectra which do not exist in reality, because intensity and precise mass of spectra fluctuated on each measurement due to instrumental characteristic. Therefore exact mass spectra could not be compared by simple method such as subtraction. This method allowed the visualization of degraded compounds instead of the compounds remaining after the degradation. The results of this novel method for depicting changes in the mass spectra are shown in Figure 2C, depicting 2D-TIC of compounds from the diesel fuel aromatic fraction degraded by *Oerskovia* sp. CHP-ZH25 (difference between Fig 2A and Fig 2B).

To assess the degradation of different classes of aromatic compounds, selective ion chromatograms were created using ±0.1 of the theoretical mass. The following classes of compounds were chosen to analyze: naphthalenes, acenaphthylenes/biphenyls, acenaphthene, fluorenes, phenanthrenes/anthracenes, benzo[b]thiophene and dibenzothiophenes. To analyze the relative change of each compound during the degradation, the above formula was applied. Selected Ion Chromatograms (SICs) or Recomposed Ion Chromatograms (RICs) for PAHs were drawn from the data extracted and summed the intensities of mass spectra in the mass range as Table S2.The two dimensional single ion chromatograms (2D-SICs) representing the degradation of various compounds are depicted in Figures S2-15. Based on obtained chromatograms, it is clear that the lower molecular weight compounds (PAHs derivatives) were largely degraded by *Oerskovia* sp. CHP-ZH25. Relative percentage of degradation of selected PAHs derivatives based on decrease of total peak volume value during incubation is given in Table S3. The order of degradation was as follows: dibenzothiophene derivatives > benzo[b]thiophene derivatives > naphthalene derivatives > acenaphthene derivatives > acenaphthylene/biphenyl derivatives > alkyl naphthalenes > fluorene derivatives > alkyl acenaphthenes > alkyl benzo[b]thiophenes > alkyl dibenzothiophenes > phenanthrene/anthracene derivatives > alkyl acenaphthylenes > alkyl fluorenes > alkyl phenanthrenes/anthracenes. According to this, it was shown that the most susceptible to biodegradation by *Oerskovia* sp. CHP-ZH25 were dibenzothiophene derivatives (*m/z* 184.0347) and benzo[b]thiophene derivatives (*m/z* 134.0190) which were almost completely degraded (97.9 and 72.7%, respectively), followed by naphthalene derivatives (68.8%, *m/z* 128.0626). The acenaphthene derivatives (*m/z* 154.0782), acenaphthylene/biphenyl derivatives (*m/z* 154.0783) and alkyl naphthalenes, were degraded to more than 50%. Fluorene derivatives (*m/z* 166.0783) and phenanthrene/anthracene derivatives (*m/z* 178.0783) were degraded up to 46.6% and 22.7%.

The degradation of alkyl PAHs (*m/z* values used are shown in Table S2, Figure S2-15) was dependent on the degree of alkylation, as the highly alkylated compounds were not efficiently degraded [34]. The order of degradation of alkyl PAHs was as follows: alkyl naphthalenes (55.2%), alkyl acenaphthenes (35.0%), alkyl benzo[b]thiophenes (29.6%), alkyl dibenzothiophenes (23.3%), alkyl acenaphthylenes (19.0%), alkyl fluorenes (5.4%) and alkyl phenanthrenes/anthracenes (0.5%).

It is widely acknowledged that lower molecular weight PAHs are a more suitable carbon sources for microbes [34, 37, 38]. The higher molecular weight PAHs can be degraded after the lower weight ones, which are the preferred substrates, are depleted or they can be co-metabolized with other substrates [39, 40]. However, the presence of phenanthrene could inhibit pyrene degradation, or fluoranthene could inhibit anthracene and pyrene degradation [9, 41].

The most interesting is the fact that dibenzothiophene and benzo[b]thiophene derivatives were degraded to a higher extent compared to other studied PAH derivatives. It should be emphasized that, as previously stated, bacteria of the genus *Oerskovia* belong to the phylum *Actinobacteria*, which are capable of sulfur removal from dibenzotiophene [17] This suggest that *Oerskovia* sp. CHP-ZH25 is not only capable to grow on PAH molecules and to use them as a only source of carbon, but also to use heteroatom containing PAHs, such as dibenzothiophene and benzo[b]thiophene.

Conclusion

Based on the available literature, the current study is the first report of *Oerskovia* sp. using the PAH mixtures as the only source of carbon and the first report on evaluation of biodegradation of the aromatic fraction from petroleum diesel fuel using GC×GC-TOF MS. This work has demonstrated that bacterial strain *Oerskovia* sp. CHP-ZH25 (JX430000) successfully degraded derivatives of dibenzothiophene, benzo[b]thiophene, naphthalene, acenaphthene, acenaphthylene/biphenyl, alkyl naphthalenes, fluorene, alkyl acenaphthenes, alkyl benzo[b]thiophenes, alkyl dibenzothiophenes, phenanthrene/anthracene, alkyl acenaphthylenes, and to some extent alkyl fluorenes and alkyl phenanthrenes/anthracenes. Hence, *Oerskovia* sp. CHP-ZH25 could potentially be a suitable candidate for use in bioremediation of environments polluted with different PAHs.

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