Ecotoxicology and Environmental Safety, Volume 198, July 2020, Article number 110645
DOI:10.1016/j.ecoenv.2020.110645

Bioremediation of petroleum hydrocarbons by vermicomposting process bioaugmentated with indigenous bacterial consortium isolated from petroleum oily sludge

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Abstract: Finding a sound ecological-based approach for the removal of petroleum hydrocarbons (PHCs) from petroleum oily sludge (POS) generated in oil refinery plants is still a challenge. This study investigated the removal of total petroleum hydrocarbons (TPHs) using bioaugmentated composting (BC) by hydrocarbon-degrading bacteria (HDB) and vermicomposting (VC) by *Eisenia fetida*, individually and in combination (BCVC). After isolating two native bacterial strains from POS prepared from an oil refinery plant in Iran, the degradation capability of their consortium was initially assessed in mineral Bushnell-Haas medium (MBHM). Then, the biodegradation rates of POS in the BC, VC, and BCVC treatments containing different concentrations of TPHs (5, 10, and 20 g/kg) were determined by measuring TPHs before and after the biodegradation. The results showed that the consortium degraded 20-62% of TPHs contents of Kerosene (1-5%) in the MBHM after 7 days. After 12 weeks, the TPHs removal percentages in the BC, VC, and BCVC treatments were respectively found to be 81-83, 31-49, and 85-91 indicating the synergistic effect of bacteria and worms in bioremediation of POS. The PHCs biodegradation in the BC, VC, and BCVC experiments was fitted to 1st order model kinetics. The results of toxicity tests indicated that the values of the no observed lethal concentration (NOLC) and median lethal concentration (LC_{50}) of TPHs were 2-5 and 14.64 g/kg, respectively after 28 days of earthworm exposure. Morphological impairments such as swelling, coiling, and curling were observed when TPHs concentration was even lower than NOLC. The study verified the effectiveness of vermicomposting bioaugmentated with the indigenous bacterial consortium for POS bioremediation.

Key words: Vermicomposting; Petroleum oily sludge; Bioaugmentated composting; Earthworms

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1. Introduction

Huge volumes of petroleum oily sludge (POS) are annually generated from the production and processing of crude oil. POS is a complex mixture of water, sediment, petroleum hydrocarbons (PHCs), and heavy metals. PHC_S are the most deleterious components in POS, due to their potential threats to human health and the environment. Hence, POS is classified as a priority pollutant and its release into the environment is strictly controlled. Over the years, a variety of treatment methods have been developed to remediate POS (Kuppusamy et al., 2017; Robichaud et al., 2019). Among them, bioremediation methods such as composting process have been receiving great attention due to their high efficiency and applicability, low costs, and also because they are environmentally friendly (Huang et al., 2019; Ren et al., 2018).

The application of vermiremediation and vermicomposting in which earthworms are used for contaminants removal has been frequently reported (Kuppusamy et al., 2017; Martinkosky et al., 2017; Rorat et al., 2017). The normal activities of earthworms can lead to aerate and mix the composting media and therefore make contaminants available for microbial community. As the composting materials pass through their gut, the secreted enzymes may increase the degradation rate of target pollutants. Earthworms also increase the biomass and enzymatic activities of microbial population resulting in increasing the PHCs degrading efficiency (Chachina et al., 2015; Martinkosky et al., 2017).

Despite the successful works recorded so far for POS bioremediation, attempt to elucidate more suitable and effective modifications has been continuing (Ekperusi and Aigbodion, 2015). Multiple reports have indicated that the combination of two or more bioremediation technologies can improve PHCs removal (Guarino et al., 2017; Rodriguez-Campos et al., 2019). In this regard, addition of bacterial strains (bioaugmentation) increases earthworms' survivability and

reproduction rates (Chachina et al., 2015). Accordingly, the combination of vermicomposting and bioaugmentation may offer a valuable improvement in the process effectiveness. Since allochthonous bacteria are not highly efficient in a new environment due to their low adaptability, addition of metabolically superior strains isolated from the same environment as bioaugmentation agents is preferred (Rabodonirina et al., 2019; Tao et al., 2017).

Although the potential of vermiremediation for improving degradation of PHCs has been well demonstrated, several variables remain to be tested to develop it as a viable process (Kuppusamy et al., 2017; Martinkosky et al., 2017). It seems clear that earthworms increase degradation rates of low to medium levels of total petroleum hydrocarbons (TPHs); however, they have not been frequently tested for high concentrations. Only a few papers have reported a combination of vermicomposting and bioaugmentation with the indigenous bacterial strains isolated from POS. Taking the above mentioned into consideration, the aim of this study was to evaluate the removal of TPHs using these two technologies individually and in combinations. The toxicity of POS on earthworm (*Eisenia fetida*) survival and growth was also investigated. In the present work, we isolated two native bacterial strains from POS and used their mixture as a consortium. These two strains as hydrocarbon-degrading bacteria (HDB) exhibited high potential for PHCs degradation both in mineral Bushnell-Haas medium (MBHM) and in the vermicomposting process.

2. Materials and methods

2.1. Isolation and identification of the HDB

POS, obtained from an oil refinery plant (situated in Shazand, Iran), was used as a source of the HDB. Following transporting POS to the laboratory, the HDB were isolated by incubating a mixture of MBHM (100 ml) and POS (5 g) at 30 °C on an orbital shaker for 7 days. Then, 5 ml of

the incubated culture was inoculated to fresh MBHM. After repeating the abovementioned enrichment step, 100 µl of the medium was grown in the nutrient agar surface and the generated colonies were identified. Phenotypic characterization of the two isolates was carried out including gram staining, catalase, oxidase, urease, citrate, H_2S production, indole production, nitrate reduction, and triple sugar iron were performed. Molecular identification was done by the 16S rRNA gene amplification and sequencing based on the standard procedure described in the published papers (Farzadkia et al., 2019; Koolivand et al., 2019b; Koolivand et al., 2019c).

2.2. Measurement of bacterial growth and Kerosene removal in MBHM

The growth ability of the HDB in the presence of 1% crude oil was determined by measuring optical density (OD) at 600 nm in MBHM after 2, 4, 7, 10, and 12 days. In order to examine the effect of pH on bacterial growth and Kerosene removal, the HDB (0.5 McFarland) was inoculated to MBHM containing Kerosene (1%) at pH values of 4, 5, 6, 7, 8, and 9 and incubated for 7 days at 30 °C. The abilities of the HDB for degradation of multiple concentrations (1-5% v v^{-1}) of Kerosene were investigated in MBHM at neutral pH before it was inoculated into the vermicomposting reactors. In all the tests carried out in MBHM, the amount of Kerosene removal was calculated as the TPHs reduction against the control experiments with no bacterial inoculation. The removal rates of TPHs were calculated as follows:

$$\text{Removal rate of TPHs} = [(\text{initial TPHs} - \text{final TPHs})/\text{initial TPHs}] \times 100$$

where the initial TPHs and final TPHs are the levels of TPHs before and after treatment, respectively.

2.3. Earthworm preparation

A common composting earthworm, *Eisenia fetida*, was selected as this easily accessible species is tolerant of environmental conditions and reproduces quickly. This species is capable of moving into the composting materials and thus can be found at all depths of composting piles (Martinkosky et al., 2017). Earthworms were collected manually from a vermicomposting facility situated in Arak, Iran. The earthworms weighing 0.3-0.4 g were collected and used for biological reactors. All earthworms were held in the experiment for acclimatization purposes and were checked daily to ascertain their health condition.

2.4. Experimental set up and maintenance of vermicomposting experiments

The translucent chemical resistant polypropylene containers equipped with vented lid with clips on two sides of the edges were purchased from the market. The containers were filled with 3 kg of immature compost (IC) with the organic carbon (C_{org}) level of 290 g/kg (Koolivand et al., 2019a). The IC obtained from a composting facility was passed through a 1 cm pore size sieve to remove impurities like plastic and glass and enhance homogeneity. Based on the results of the MBHM experiments as well as toxicity tests, various amounts of the POS with the initial TPHs of 255 g/kg (Parhamfar et al., 2019) were mixed with IC to reach the initial TPHs levels of 5, 10, and 20 g/kg and added to the treatments. Ten treatments were performed according to Table 1. The IC and POS were sterilized with three autoclave cycles (121 °C for 30 min) with 24-h intervals and then kept at 4 °C for less than 48 h before starting the experiments.

Ten earthworms were separated, washed with clean water, cleaned by keeping on wet filter paper, and their superficial layer was gently wiped. Then, they were weighed and added to each treatment. Prior to degradation experiments, earthworms were acclimated to the IC and laboratory conditions

for at least one week to diminish mortality caused by sudden environmental changes. The earthworms were nourished just at the start of the process since the addition of the feed during the process may dilute TPHs levels. The mixture of carrot and potato pulp (1:1 w/w) was chosen as the food supply for the worms since it was inexpensive and accessible and worms can survive well in it. A netting material and the cover lid were placed on top of each container to avoid escape of the earthworms and to allow free flow of oxygen into the treatments. Regarding the bacterial consortium utilization, equal mix of the two isolates were mixed and cultured until logarithmic growth phase. Then it was inoculated into the reactors at the concentration of 1.5×10^8 CFU/mL at 5% v/v of work volume. The control treatment was prepared without worms and bacterial strains additions in order to analyze TPHs change due to abiotic processes such as natural evaporation of light hydrocarbons.

The experimental setup was placed inside the laboratory, shielded from light, and checked on a daily basis for 12 weeks. The treatments were moistured by adding distilled water twice a week. The C/N/P ratio was adjusted at 100/5/1 by adding NH_4Cl and KH_2PO_4 (Koolivand et al., 2014; Koolivand et al., 2018).

2.5. Sampling and quantification methods

The samples for TPHs and C_{org} analyses in the composting materials were collected seven times on days 0, 14, 28, 42, 56, 70, and 84. At the termination of the experiment, TPHs contents of earthworms were also measured to estimate the amounts of TPHs bioaccumulation in the earthworms' bodies. After blending the contents of each treatment, three subsamples were taken from various depths, mixed, and homogenized into one composite sample. The homogenized samples were transferred into a glass jar and immediately analyzed. Moisture readings were

conducted gravimetrically by drying at 105 °C for 24 h. The C_{org} was determined by loss-on-ignition at 600 °C (TMECC, 2002). TPHs were extracted with n-pentane and then quantified by means of a gas chromatograph-flame ionization detector (GC-FID) (Shimadzu, Japan) based on TNRCC (2001). The operating conditions of the GC are accessible in the previous papers by Koolivand et al. (2013a; 2013b).

2.6. Toxicity tests

Eisenia fetida has been extensively used as one of the recommended model species for evaluating the toxicity of chemicals (OECD, 1984). The toxicity was monitored by the following tests: (i) the survival and weight loss of mature adult earthworms; and (ii) the numbers and hatchability of cocoons and the survival of juveniles. The toxicity treatments were performed in duplicate.

2.6.1. The survival and weight loss of adult earthworms

The earthworms were removed from the treatments by hand, rinsed to remove particles, patted dry, and then mortality was determined after 28 days, based on the previously established protocols (OECD, 1984). The TPHs concentrations of 0 (as control), 2, 5, 10, 20, 30, 40, and 50 g/kg were used to find the concentration that causes mortality in worms. The median lethal concentration (LC_{50}) was expressed in relation to the initial concentrations of TPHs after exposing worms for 28 days (OECD, 1984; Ramadass et al., 2017). In order to determine weight change, the worms were depurated and their weight was recorded. The effects of contamination on morphology were also examined visually. Toxicity was determined as percent survival and weight loss when compared to the controls.

2.6.2. The numbers and hatchability of cocoons and the survival of juveniles

Cocoon and juvenile numbers, as a measure of the reproductive output, were measured through wet sieving over the test duration. Briefly, a 1-mm sieve was placed on top of a 0.15-mm sieve and the composting material was washed with tap water. The cocoons and juvenile trapped on the upper and lower sieve, respectively. After picking up cocoons and juvenile, they were transferred to a beaker containing a little water. The juvenile worms were picked out of the water using a needle and counted. By counting the number of juveniles hatched from the collected cocoons, the hatchability percentage was measured (Ramadass et al., 2016).

2.7. Modeling of TPHs degradation

TPHs degradation was modeled assuming first-order kinetics (Nwankwegu et al., 2020) such that:

$$dC/dt = kC$$

$$t_{1/2} = \ln 2/k = 0.693/k$$

where dC/dt is the rate of degradation (g/kg.d), k is the degradation rate constant (d^{-1}), C is the TPHs concentration (g/kg), and $t_{1/2}$ is the time (d) required for decomposing half of the initial TPHs.

2.8. Statistical analysis

To get reliable data, all the tests were performed in triplicate. The obtained results were indicated as means ± standard deviations. Statistical significance of the data ($p \leq 0.05$) was evaluated using one-way analysis of variance (ANOVA) of SPSS package (version 11.0). Linear regression analysis of Microsoft Excel (2013) was applied to show the relation between the variables. LC_{50} values were calculated from the percentage of mortality relative to those of controls by Probit

analysis. The sequences were analyzed with Chromas and aligned with the CLUSTAL. BLAST was applied to perform similarity searches of the sequences compared to NCBI database.

3. Results and discussion

3.1. Characteristics of the HDB

The NCBI Genbank database similarity search based on 16S rRNA gene sequence analysis indicated that the two isolated strains were *Acinetobacter radioresistens* strain KA2 and *Enterobacter hormaechei* strain KA3. The nucleotide sequences were deposited in NCBI GenBank under the accession numbers of MK127544 and MK127545, respectively for the strains KA2 and KA3. Table 2 provides the biochemical characteristics of the bacterial consortium. The values of OD_{600}, as an indication of biomass production by the consortium, in MBHM containing Kerosene (1% concentration) were found to be 0.33, 0.93, 1.55, 1.60, and 1.31, respectively after 2, 4, 7, 10, and 12 days. These values indicated that the consortium can grow well in the presence of Kerosene as a sole source of carbon. It was also observed a lag period in growth over the first 2 days and then, rapid generation of biomass during the day 7-10. Thus, the incubation time for all degradation tests performed in MBHM was 7 days when the consortium reached to the logarithmic growth phase.

Table 2

3.2. Kerosene biodegradation in MBHM

The effect of pH as an important factor influencing the activities of bacterial consortium and the solubility of PHCs was tested to determine the best pH values for composting treatments. As provided in Table 3, the higher biodegradation efficiency (51-62%) and OD at the pH values 6-8,

verified the consortium preference to neutral conditions. The growth and TPHs reduction remarkably decreased at the pHs of 5 and 9. These findings are in line with other studies reporting that HDB prefer neutral pH for their growth and degradation of TPHs (Muangchinda et al., 2018; Wang et al., 2016).

Table 3 also showed the mineralization of multiple concentrations (1-5%) of Kerosene by the consortium. It was indicated that the HDB efficiently (60-66%) degraded Kerosene of 1-3% concentrations and no effective degradation was found for the levels of 4-5%. In kinetic terms, bacteria have different reactions to the pollutants concentrations present in the contaminated environments. A very low concentration of PHCs would limit degradation rate since there is a threshold level below which the PHCs are not effectively detected by HDB. On the other hand, high concentration of PHCs can block the growth and activities of the consortium (Ekperusi and Aigbodion, 2015; Zhang et al., 2017).

Table 3

3.3. POS removal in the BC, VC, and BCVC treatments

In the present study, the HDB capabilities for Kerosene degradation (Table 3) as well as the results of the toxicity tests were the basis for selecting the mixing ratios of POS to IC in the composting reactors. Fig. 1 showed that the TPHs removal rates were 83.00, 85.90, 81.15, 49.20, 42.10, 30.80, 91.20, 90.90, 85.35, and 6.60%, respectively for the reactors BC_1, BC_2, BC_3, VC_1, VC_2, VC_3, $BCVC_1$, $BCVC_2$, $BCVC_3$, and control. The high amounts of TPHs degradation in the experiments BC_1, BC_2, and BC_3 demonstrated the effectiveness of the isolated consortium for POS bioremediation.

Fig. 1

The results of the VC treatments revealed that the earthworms were able to survive and adapt the TPHs concentration of 5-20 g/kg. In the experiments containing the TPHs concentrations of 5 and 10 g/kg (BC_1, BC_2, VC_1, VC_2, $BCVC_1$, and $BCVC_2$), the biodegradation proceeded more actively. Various studies reported that earthworms have the capability for adaptation in the presence of certain amounts of TPHs. In a study conducted by Chachina et al. (2015), an amount of 97-99% removal of TPHs (20 g/kg concentration) was achieved in soil samples with 10 worms after 4 months. Martinkosky et al. (2017) reported a decrease of 80% of TPHs (C_{16}-C_{35}) with *Eisenia fetida* in soil contaminated with 30 g/kg crude oil. The lower reduction rate of TPHs in the VC treatments compared to those in the BC indicated that the bacterial consortium was more potent than the earthworms for TPHs degradation. Also, the earthworms were more sensitive than the HDB to high concentrations of TPHs since the removal rate in the treatment VC_3 were significantly lower than that of BC_3.

The inoculation of the bacterial consortium into the treatments with earthworms showed the better performances of BCVC treatments. This indicates that the simultaneous application the isolated HDB and earthworms exhibits a synergistic effect for TPHs removal. The results of regression analysis (Table 4) verified that the rate of TPHs loss in the experiments can be explained by 1st order degradation models. Table 4 also provides the calculated values of biodegradation rate constants for the BC, VC, and BCVC treatments. The higher values of the biodegradation constants in the BCVC experiments demonstrate the synergistic interaction of the *E. fetida* and the isolated bacterial consortium. Rodriguez-Campos et al. (2019) found that that the interaction of bacteria and earthworms resulted in the highest TPHs removal (77.2%) for the initial level of 309 mg/kg after 112 days. It was also reported that in petroleum-contaminated soil with 20-60 g/kg of petroleum, the amount of hydrocarbons decreased by 99% after 22 weeks in the case of

simultaneous application of bioaugmentation and worms (Chachina et al., 2016). On the other hand, Chachina et al. (2015) reported that the introduction of bacterial strains in samples with 20 g/kg concentration of oil had no effect on soil bioremediation process by of *E. fetida.* Hence, more studies should be done to determine the mechanisms responsible for the synergistic or antagonistic effects of earthworm and bacteria for PHCs remediation.

Table 4

As can be seen, in all the treatments, a similar downward trend was observed for TPHs degradation. The degradation was started rapidly and then the rate of removal declined. This can be due to the fact that in bioremediation processes, easily-biodegradable PHCs are decomposed first and the remaining PHCs or intermediate metabolites, which are persistent to biodegradation, are slowly degraded (Abtahi et al., 2020; Poorsoleiman et al., 2020). Rodriguez-Campos et al. (2019) also found that most of the PHCs removed within 28 days, while another study (Ekperusi and Aigbodion, 2015) reported that the bioremediation was more effective after 60-90 days of the study indicating that the worms needed sufficient time to adapt to the contaminated environment. The reason for these different results should be investigated by performing further studies.

3.4. Adult worms' survival

Acute toxicity test to quantify the impact of TPHs on the earthworms' survival is suitable for evaluating the ecotoxicological aspect of POS. *E. fetida* has been suggested by the OECD as the ideal species for assessing the effect of POS contamination (Ramadass et al., 2017). The survival of *E. fetida* after a 28-day exposure to POS has been provided in Table 5. Data from the present

study showed that POS is toxic to earthworms at some TPHs concentrations. Earthworm survival was naturally 100% in control sample containing no POS. Also, 100 and 97% survival of worms was observed, respectively for the TPHs levels of 2 and 5 g/kg during 28 days. Thus, the no observed lethal concentration (NOLC) for TPHs was 2-5 g/kg. Concentrations of 10, 20, and 30 g/kg resulted in 57, 30, and 3% survival, respectively. All the worms died at 40 and 50 g/kg after a period of 28 days. The variation in observed mortalities is due to the different concentrations of TPHs applied in the research. In the present study, the 90% survival of *E. fetida* computed by probit analysis was 6.24 g TPHs/kg after 28 days, while Aslund et al. (2013) observed it at a petroleum concentration of 20 g/kg. The LC_{50} obtained in the present study (14.64 g TPHs/kg) was also comparatively different from the value reported by Ramadass et al. (2017) (1.43 mg TPHs /kg soil) since it varies greatly depending upon multiple factors like the type and characteristics of the worms and petroleum compounds used.

Table 5

3.5. Adult worms' weight loss

The body weights of adult worms measured before and after exposure to varying concentrations of POS, indicated weight loss in all worms (Table 5). Loss in biomass in control treatments (with no POS) did not exceed the reference value of 20% weight loss (Iso, 1993). However, the worms exposed to POS exhibited significant weight loss ($P < 0.05$) compared to those of control. The worms lost 8, 14, 26, 32, and 30% of their body weight, respectively at the TPHs concentrations of 2, 5, 10, 20, and 30 g/kg. The observed weight loss in worm biomass may be due to the inability of worms to feed during their exposure to POS. The decrease in adult body weight with the increase

of pollutants concentrations was also reported by other researchers (Ramadass et al., 2017; Wang et al., 2010). The observed weight loss for the TPHs level of 2 g/kg which did not have worms' mortalities showed the sub-lethal effects of TPHs. Some behavioral and morphological changes were noticed in worms throughout the study period. The earthworms became restless immediately after exposure to POS even at TPHs concentrations of 2 and 5 g/kg. Other impairments like coiling and swelling were also observed during the process. Other studies also reported that the earthworms exhibit abnormalities when the contaminant concentrations exceed tolerable limits (Ramadass et al., 2017; Wang et al., 2010).

3.6. Production and hatchability of cocoons and survival of juveniles

Exposure of worms to POS resulted in the reduction of cocoon production, cocoon hatchability, and juvenile numbers (Table 5). The average (±SD) cocoon production in the control were 18 (±1.7). When the TPHs concentrations reached to 2, 5, 10, and 20 g/kg, the numbers of cocoons decreased to 17, 14, 5, and 2, respectively. No cocoon was produced at a TPHs level of 30 g/kg and EC_{50} for cocoon production was calculated to be 9.71 g/kg. Our observations indicated that egg capsules productions were started after 4-6 and 6-8 weeks in the control and contaminated experiments, respectively.

Hatching rates were nearly 83% in the control and in the treatment with 2 g TPHs/kg. The computed rates for the TPHs of 5, 10, and 20 g/kg were nearly 81, 60, and 47%, respectively. Juvenile production appeared sensitive to POS contamination since a total mortality of juveniles was observed at a concentration of 20 g/kg. The EC_{50} value for juvenile survival was 3.46 g/kg since this value reduced half of the juveniles as compared to those in the control treatment. Our

findings on the cocoon production and hatchability and juvenile numbers are in line with other similar studies (Ramadass et al., 2016; Wang et al., 2010).

3.7. TPH bioaccumulation in worms' tissues

In bioremediation studies, it is of great importance to notice the fate of TPHs so as to reach a clear understanding of the bioremediation pathway. Although 31-49% of TPHs were removed in the VC treatments, about 120 (±6) µg/g was bioaccumulated inside the worms' bodies after 12 weeks. The amount of TPHs accumulations in the worms' tissues (*E. fetida*) reported by Rammadass et al. (2017) were 80-85 µg/g for the TPHs application of 0.25-2 g/kg in soil. Ekperusi and Aigbodion (2015) reported that out of 84.99% of TPHs removal, 57.35% was bioaccumulated in Earthworm (*E. eugeniae*) tissue after 12 weeks. There is the likelihood that some constituents of PHCs are recalcitrant to transformation by the worms and they need more time (beyond 12 weeks) to degrade the entire TPHs in their tissues. It is also probable that the pollutants are to some extent bioaccumulated and immobilized first in earthworm's tissues and then biodegradation is initiated when the amounts of the contaminants can threaten the earthworm survival (Ekperusi and Aigbodion, 2015). To what extent the worms can bioaccumulate TPHs and when the breakdown is started were not revealed from this research and will be a matter for further studies.

4. Conclusions

The removal of TPHs using composting bioaugmentated with the indigenous bacterial consortium and vermicomposting by *Eisenia fetida* individually and in combination was investigated in the present study. The results showed that the isolated bacterial consortium was effectively capable of degrading TPHs in MBHM and composting process. It was observed a synergistic effect of the

HDB and earthworms in TPHs removal from POS. TPHs biodegradation proceeded according to the first order model kinetics. The results of ecotoxicity tests indicated various toxic effects of POS on juvenile and adult earthworms depending on concentrations. The study demonstrated that the combination of the earthworm and the bacterial consortium exhibited higher removal efficiency for TPH removal.

Acknowledgements

Authors would like to thank Arak University of Medical Sciences for supporting and funding this research [Grant Number 3099].

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Table 1. The characteristics of bioremediation treatments used in the present study

Bioremediation treatments	TPHs concentration (g/kg)	Bacterial inoculation	Worm addition
BC_1	5	Yes	No
BC_2	10	Yes	No
BC_3	20	Yes	No
VC_1	5	No	Yes
VC_2	10	No	Yes
VC_3	20	No	Yes
$BCVC_1$	5	Yes	Yes
$BCVC_2$	10	Yes	Yes
$BCVC_3$	20	Yes	Yes
Control	10	No	No

Table 2. The biochemical identifications of the bacterial strains isolated from petroleum oily sludge

Tests	*Acinetobacter radioresistens* strain KA2	*Enterobacter hormaechei* strain KA3
Nitrate reduction	−	+
Oxidase	−	−
Catalase	+	+
Gram stain	Gram negative	Gram negative
Citrate	−	−
Urease	−	−
Triple sugar iron	Alkaline/Alkaline	Alkaline/Alkaline
H_2S production	−	−
Indole production	−	−

Table 3. Kerosene biodegradation by the isolated consortium in mineral Bushnell-Haas medium

Variables	Values	OD_{600}	Kerosene degradation (%)
pH	5	0.97	33.33
	6	1.34	51.40
	7	1.55	62.02
	8	1.26	52.18
	9	0.86	40.70
Kerosene concentrations (%)	1	1.55	62.02
	2	1.69	66.35
	3	1.51	60.08
	4	0.93	35.78
	5	0.50	20.21

Table 4. The values of the first-order kinetics in the bioremediation treatments

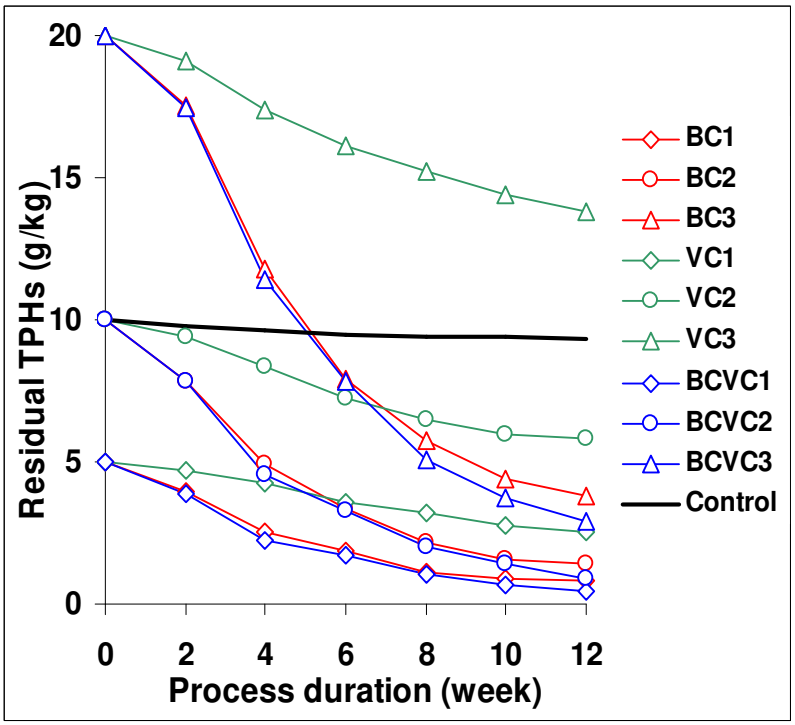
Bioremediation treatments	k (d^{-1})	$t_{1/2}$ (d)	R^2
BC_1	0.161	4.30	0.974
BC_2	0.176	3.94	0.985
BC_3	0.152	4.56	0.984
VC_1	0.061	11.36	0.989
VC_2	0.050	13.86	0.976
VC_3	0.032	21.66	0.987
$BCVC_1$	0.209	3.32	0.995
$BCVC_2$	0.203	3.41	0.996
$BCVC_3$	0.173	4.01	0.989

Table 5. Various toxicity tests performed for *Eisenia fetida* in the different concentrations of TPHs

TPHs concentrations	**Adult worms' survival (%)**	**Adult worms' weight loss (%)**	**No. of cocoons**	**Cocoons' hatchability (%)**	**Juveniles' survival (%)**
0	100 (0)	5.5 (4.2)	18 (1.7)	83.2 (1.7)	63.3 (3.3)
2	100 (0)	8.3 (3.2)	17 (1.7)	83.2 (1.7)	52.2 (5.1)
5	97 (5.8)	14.1 (7.1)	14 (1)	80.8 (6.9)	41.5 (2.1)
10	57 (5.8)	26.2 (4.3)	5 (0)	59.6 (2.2)	36 (6)
20	30 (10.0)	31.7 (5.9)	2 (1)	46.7 (11.6)	0
30	3 (5.8)	30.4 (7.0)	0 (0)	ND	ND
40	0 (0)	ND	ND	ND	ND
50	0 (0)	ND	ND	ND	ND

Data are mean of three replicates (± SD).
ND = not determined.

Fig. 1. The trend of TPHs changes in the bioremediation treatments over the process duration



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2020-04-25

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Koolivand A, Saeedi R, Coulon F, et al., (2020) Bioremediation of petroleum hydrocarbons by vermicomposting process bioaugmentated with indigenous bacterial consortium isolated from petroleum oily sludge. Ecotoxicology and Environmental Safety, Volume 198, July 2020, Article number 110645

https://doi.org/10.1016/j.ecoenv.2020.110645

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