Degradation of Polycyclic Aromatic Hydrocarbons by Consortium 5H Under Hypersaline Conditions

Jiaqi Jin
Henan University
Zuotao Zhang
Tsinghua University
Lu Han
Henan University
Chicheng Yan
Henan University
Xinxing Ding
Henan University
Jiage Sun
Henan University
Jingyu Cui
Henan University
Chongyang Wang (wangchongyang2012@163.com)
Henan University

Research Article

Keywords: PAHs, halotolerant, Methylophaga, consortium, degradation pathway

Posted Date: June 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-524058/v1

Abstract

PAHs were widely detected accumulated in saline and hypersaline environments. The halotolerant and halophilic microbes were considered as the most suitable player for the elimination of PAHs in such environments. In this study, consortium 5H was enriched under 5% salinity that was able to completely degrade phenanthrene in 5 days. By high-throughput sequencing, consortium 5H was identified mainly composed of *Methylophaga*, *Marinobacter* and *Thalassospira*. Combined with the investigation of intermediates and enzymatic activities, the degradation pathway of consortium 5H on phenanthrene was proposed. Meanwhile, consortium 5H was identified with ability to tolerate a wide range of salinity (1% to 10%) and initial PAHs concentration (50 mg/L to 400 mg/L). It was also able to work under neutral to week alkaline conditions (pH from 6 to 9) and the phytotoxicity of the produced intermediates showed no significant difference with distilled water. This study expanded the knowledge of PAH-degradation under hypersaline environments and consortium 5H was proposed with a good potential for the elimination of PAHs pollution under saline/hypersaline environments.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a form of essential petroleum pollutant made up of two or more benzene rings (Haritash and Kaushik, 2009). PAHs are significant environmental persistent contaminants that are commonly found in various environments with high concentrations due to their structural complexity (Debajyoti et al., 2016; Patel et al., 2020). In recent years, PAHs are largely detected in saline environments, including saline soil, salt marshes, ocean sediments and mangrove forest, which will cause a serious threat to the local ecosystem as their potential bio-toxicity. As a result, the deterioration of PAHs in a saline environment has received a lot of attention (Crampon et al., 2018; Xu et al., 2021).

The elimination of PAHs by microbes are identified as the most cost-effective method for removing PAHs from the ecosystem. However, as the limitation of saline environments, including (1) decreased dissolved oxygen level with the increased salinity, (2) the increased osmotic pressure; (3) the decreased solubility of PAHs due the salting our effectiveness, PAHs degradation in saline environment is quiet hard (Yu et al., 2005; Wang et al., 2017). Moreover, the wide range of salinity variation in some saline environments can make it difficult for strains that can degrade PAHs to survive. Moderately halophiles with an optimal growth salinity ranging from 5–10% are found to be an alternative bacteria resource that can effectively degrade PAHs effectively in saline environments (Arulazhagan and Vasudevan, 2009).

To data, several halophilic or halotolerant microbes have been isolated with the ability to degrade PAHs. For example, *Matelella* sp. AD-3 which was isolated from saline soil was identified able to degrade PAHs under high salinity (1–15%) (Feng et al., 2012). *Marinobacter* sp. NCE312 isolated from marine sediment was able to degrade phenanthrene under 10% salinity (Hedlund et al.). Meanwhile, a number of halotolerant consortiums capable of degrading PAHs have been enriched solely on PAHs. Consortium CY-1 isolated by Wang et al. has been identified to tolerate a wide range of salinity (from 1–20%) (Wang et

al., 2018). Halophilic consortium Qphe enriched by Dastgheib et al. was able to degrade 100mg/L phenanthrene under 15% salinity (Dastgheib et al., 2012). Several related studies have identified that consortium can adapt a wider environmental range than pure cultures (Qasemian et al., 2012; Wang et al., 2015; Hentati et al., 2016). Moreover, the mechanism degradation of PAHs by mixed bacteria was more consisit with PAHs elimination in environments, especially in saline environments (Debajyoti et al., 2016; Xu et al., 2021). Therefore, further research into the PAH-degrading halophilic consortium is required, which can provide a more detailed version of PAH-degradation in saline/hypersaline environments.

Sea salt-defined media (SSDM) with a salinity of 5% was used to enrich consortium 5H in this study. Consortium 5H was discovered to be capable of degrading 100 mg/L phenanthrene in 5 days. The metabolic pathway of consortium 5H was investigated using a combination of GC-MS intermediates and enzymatic activities. Further study was processed on the influence of salinity, pH and other environmental factors on the degrading process and the phytotoxicity assay on the produced intermediates. Consortium 5H was described as having a high capacity for degrading PAHs and a broad range of environmental tolerance, indicating that it has a good potential of eliminating PAHs in saline/hypersaline settings.

2. Materials And Methods

2.1 Regents

The standards chemical, namely phenanthrene (98%), catechol (98%), gentisic acid (98%), 1,2-dihydroxynaphthalene (98%) and 1-hydroxy-2-naphthoic acid (97%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). MP Biomedicals Co., Ltd (American) provided the Fast DNA Spin Kit for Soil that was used to extract genome DNA.

2.2 The enrichment process and community structure detection

Mixed saline soil samples were obtained from a perennially PAH-contaminated site in Shanxi Province, China, for the enrichment of consortium 5H. 5g of the sifted soil sample was added into 200 mL clean SSDM with 100mg/L phenanthrene as sole carbon source. The 5% SSDM was prepared as previously described (Wang et al., 2017). The culture medium was placed in a shaker in darkness with 120 rpm, 30℃. 7 days after incubation, 20mL cell suspension was transferred to 180 mL fresh culture medium. The whole enrichment lasted for 3 months for obtaining a stable consortium.

The genomic DNA of consortium 5H was extracted from the consortium cell suspension 4 days after incubation. 50 mL cell suspension was collected and centrifuged at 12000 rpm for 5 min. The genomic DNA of consortium 5H was further extracted using Fast DNA Spin Kit for Soil following the manufacturer's procedure. V3 - V4 regions of 16S rRNA in the genomic DNA of consortium 5H was further investigated by Illumina MiSeq PE250 high-throughput sequencer (Genewiz Co. Ltd, Suzhou, China). The sequence of OTUs were further submitted to NCBI database (accession number: MZ099737 to MZ099769).

2.3 The detection of residual phenanthrene concentration and metabolic intermediates

Consortium 5H was cultured in 200mL fresh SSDM with 5% salinity using 100 mg/L phenanthrene as sole carbon source. The culture systems were further placed in a shaker under 120rpm, 30℃. Samples of 3 mL cell suspension were collected once or twice per day after incubation and extracted using 5 mL dichloromethane for 30 minutes. After filtration, the residual phenanthrene was further measured by HPLC (Shimudzu, Japan) as previously described (Wang et al., 2019).

After incubation for 4 days, 100 mL cell suspension was collected and centrifuged at 12000 rpm for 5 minutes under 4℃. Then the collected cell pellet was disrupted using high pressure homogenizer (JN-Mini, JNBIO Co., Ltd, Guangzhou, China). 100 mL dichloromethane was added into the cell debris for extraction at different pH value (2, 7 and 12). After extraction, the dichloromethane was concentrated using a rotatory evaporator efore being dried in a stream of high purity nitrogen. Derivatization experiment was further processed using Bis (trimethylsilyl) trifluoroacetamide (BSTFA and TMCS, volume ratio: 99/1) to replace the active hydrogen atom in phenanthrene metabolic intermediates by TMS group ($Si(CH_3)_3$) with m/z 73. Then, the derivatized samples and standard compounds (1-hydroxy-2-naphthoic acid, 1,2-dihydroxynaphthalene, catechol, and gentisic acid) were detected using GC-MS (Agilent 7890A GC, Inert MSD with TripleAxis Detector, Agilent, USA) according to previously report (Wang et al., 2019).

2.4 Enzymatic activities assay

Under different salinities (1%, 3%, 5%, and 10%), the activities of ring-hydroxylating dioxygenase (RHD), catechol-2,3-dioxygenase (C23O) and gentisate 1,2-dioxygenase (G12O) were also measured. Consortium 5H was incubated under 5% salinity with 100 mg/L phenanthrene concentration. 100mL cell suspension was collected after incubation and centrifuged at 12000 rpm for 5 minutes. 10 mL distilled water was used for cell resuspension and then disrupted using high pressure homogenizer as described above at 4 ℃. The disrupted solution was used as the crude enzyme for detection of the activities of RHD, C23O and G12O.

The enzymatic activities were measured in 1 mL system with 0.9 mL substrate medium and 0.1 mL crude enzyme. The substrate medium of RHD contained 0.1 mM naphthalene, 50 mM phosphate buffer with pH 7 and different NaCl concentration. The substrate medium of C23O and G12O was similar to the substrate medium of RHD, the substrate was replaced by 0.1 mM catechol and 0.1 mM gentisic acid, respectively. The detection of activities of RHD, C23O and G12O was as previously described (Wang et al., 2017).

2.5 Degradation of phenanthrene degradation rate under different salinities, pH and concentration.

The phenanthrene degradation ability of consortium 5H was measured under different salinity (1%, 3%, 5%, 10% and 20%) and pH (5, 6, 7, 8, 9 and 10) for investigating the environmental tolerance of

consortium 5H. Salt content of different salinity was adjusted by changing the concentration of NaCl, $MgCl_2$ and $MgSO_4$ according to previous report (Wang et al., 2018). The pH was adjusted using 0.5mol/L NaOH and 1:5 (v/v) HCl solution. The detection of residual phenanthrene concentration was detected using HPLC as described above. SSDM with 5% salinity containing different phenanthrene initial concentrations (50mg/L, 100mg/L, 200mg/L and 400mg/L) was also used for investigating the degrading ability of consortium 5H.

2.6 Phytotoxicity assay

In order to estimate the biotoxiciy of metabolic intermediates produced by consortium 5H, 100mL cell suspension was collected 1, 3, 5 and 7 days after incubation. The cell pellet was collected by centrifugation at 12000 rpm, 4℃ and resuspended using 10mL distilled water. Cell disruption and metabolic intermediates extraction were described as previously (Guo et al., 2020). Distilled water was used to dissolve the extracted intermediate metabolism, and a final solution of 100 mg/L was adjusted. 20 mL final solution was added to culture dish with 20 seeds of *Cucumis sativus* and *Oryza sativa*. 7 days after immersion, the generation rate, the length of plumule and radical were recorded as indexes for the evaluation of the phytotoxicity. Treatment using distilled water was set as the control.

3. Result And Discussion

3.1 Community structure of consortium 5H

The halotolerant consortium 5H was enriched by weekly transfer for 3 months to obtain a stable bacteria community. The degradation rate and cell growth curve was shown in Fig. 1. Consortium 5H showed a rapid degradation rate of phenanthrene under 5% salinity. In just 2 days, 84% of the phenanthrene had been degraded, and after 6 days, all of the phenanthrene had been removed from the medium. Consistent with the phenanthrene removal, the OD600 value of consortium 5H increased to 0.095 in day 2 and maintained around 0.11 in the following degradation process. Halotolerant strain *Ochrobactrum* sp. VA1 isolated by Arulazhagan et al. was identified with ability to degrade 3 mg/L phenannthrene in 4 days under 3% salt content (Arulazhagan and Vasudevan, 2011). *Marinobacter* sp. N4 was identified able to degrade 100% phenanthrene in about 7 days with the assistance of *Halomonas* sp. G29 (Wang et al., 2019). Consortium CY-1 enriched by Wang et al. was reported able to degrade PAHs in 7 days under 5% salinity (Wang et al., 2018). Dastgheib et al. reported that halophilic consortium Qphe could eliminate 100 mg/L phenanthrene in 12 day when cultured under 5% salinity (Dastgheib et al., 2012). Compared with degradation rate of reported in both halotolerant isolates and consortiums, consortium 5H showed a relatively high rate in phenanthrene removal under saline condition, especially in the early stage of the degradation.

Through high-throughput sequencing, the community structure of consortium 5H was studied. As shown in Fig. 2, *Methylophaga* (57.5%), *Marinobacter* (18.2%) and *Thalassospira* (15.2%) were identified as the most abundant genus in consortium 5H. To the best of our knowledge, no pure cultures of Methylophaga have been reported to be capable of degrading PAHs on their own. However, some researchers had found

this genera closely related with PAHs degradation in contaminated beach environment (Joaquim et al., 2010), oil contaminated sea water (Mishamandani et al., 2016; Muangchinda et al., 2018), and marine oil spill samples (Jiménez et al., 2011). This is the first report that *Methylophaga* was identified as the most abundant player in a halotolerant PAH-degrading consortium. *Marinobacter* species were discovered to be linked to PAHs polluted marine environments (Hidalgo et al., 2020; Jamal, 2020; Rodrigue et al., 2020). *Marinobacter* isolates able to degrade PAHs was firstly reported by Gauthier et al that was able to degrade low-molecular-weight PAHs (Gauthier et al., 1992). Then, *Marinobacter* had been widely detected or isolated in polluted marine environments with high PAH-removal efficiency (Yu et al., 2005; Joaquim et al., 2010; Guangsu et al., 2013; Cui et al., 2014). *Thalassospira* sp. TSL5-1 reported by Zhou et al. had been identified able to degrade high-molecular-weight PAHs (Zhou et al., 2016). Other genus included in consortium 5H, such as *Halomonas* (1.6%), *Alcanivorax* (0.5%), and *Martelalla* (0.3%), were also found to be closely related with PAH-contamination and degradation. The presence of *Methylophaga*, *Marinobacter*, and other genus with high salt tolerance can explain why consortium 5H has such a high rate of PAH removal during the degradation process.

3.2 Metabolic pathway of phenanthrene degradation in consortium 5H

GC-MS and enzymatic activity detection were used to investigate the metabolic pathway for PAHs degradation in consortium 5H. The metabolic intermediates detected in the PAHs degradation process were shown in Table 1. Metabolite 1 eluted at 38.25 min was detected with the major ions at m/z 336 and major ions fragments at m/z 317, 243, 185, 147, and 73. Compared with the mass spectrum obtained by standard compounds detection, metabolite 1 was identified to be 1-hydroxy-2-naphthoic acid. Metabolite 2 with major ion fragment at m/z 306, 216, 186, 147, 73 was identified as TMS-derived 1,2-dihydroxynaphthalene. Metabolite 3 eluted at 35.11 min was identified with major ions fragments at m/z 443 (M^+), 370, 311, 281, 193, 137, 73. This intermediate was considered as gentisic acid. Metabolite 5 with ion fragment m/z at 254 (M^+), 223, 197, 151, 133, 73 was eluted at 22.56 min, which was considered as catechol. All the detected intermediates were closely related to PAHs biodegradation. 1-hydroxy-2-naphthoic acid was once considered as the major accumulated intermediates in the degradation of phenanthrene by *Martelella* sp. AD-3(Feng et al., 2012) and *Marinobacter* sp. N4 (Wang et al., 2019). 1,2-dihydroxynaphthalene is also an important intermediates that are widely reported in PAH-degrading *Pseudomonas*. Salicylate hydroxylase that encoded by *nahG* like genes in *Pseudomonas* and *Marinobacter* was reported able to transform 1-hydroxy-2-naphthoic acid to 1,2-dihydroxynaphthalene in PAH-degrading process (Sanseverino et al., 1993; Rosselló-Mora et al., 1994; Wang et al., 2019). Gentisic acid and catechol are important intermediates with single benzene ring, that are detected in PAH-degrading halophiles frequently (Huang et al., 2015; Zhou et al., 2016; Wang et al., 2018). Meanwhile, studies indicated that these two compounds were always followed by ring-cleavage process in PAH-degrading process and thus serve as indicators for PAHs downstream degrading pathway.

Table 1
detected intermediates in PAH-degrading process by consortium 5H

Metabolites	Retention Time	m/z of major ions (m/z of major ion fragments)	Identification
1	38.25	336 (317, 243, 185, 147, 73)	1-hydroxy-2-naphthoic acid
2	39.86	306 (289, 216, 186, 147, 73)	1,2-dihydroxynaphthalene
3	35.11	443 (370, 311, 281, 193, 137, 73)	Gentisic acid
4	22.56	254 (223, 197, 151, 133, 73)	Catechol

The produced gentisic acid and catechol could be further ring-cleaved by G12O, C12O and C23O, respectively. As reported by Wang et al., G12O, C12O and C23O pathway were all existed in a halotolerant PAH-degrading consortium and G12O pathway was identified as the dominant downstream pathway (Wang et al., 2018). In order to further investigate the downstream pathways of PAH-degrading in consortium 5H, the activities of G12O, C12O and C23O were investigated under different salinity. As shown in Fig. 3, the activities of G12O and C23O were identified with a wide range of salinity. Both G12O and C23O showed the highest activity at 3% salinity, and their activity was dramatically reduced as salinity rose above 10%. The activity of C12O had not been detected in this experiment, indicating that C12O pathway was not involved in PAH-downstream degrading process in consortium 5H. Meanwhile, in consortium 5H, the behavior of RHD was measured in order to better understand the PAH-upstream degrading pathway. All enzymatic activities shown in Fig. 3 were relative activities using the activity of corresponding enzyme under 5% salinity as 100%. A shown in Fig. 3A, the RHD activity was slightly influenced by salinity from 1–10%. Even at 10%, the RHD can still hold 88% activity compared to the 5% condition, indicating a high salt tolerance of RHD in consortium 5H. The activity of G12O was highest at 3% salinity, which was about 158% compared with that in 5% salinity (Fig. 3B). When the salt content rise to 10%, the G12O activity was greatly reduced, with only 43% remaining. C23O was detected with highest activity at 3% salinity (255%, Fig. 3C). When the salinity increased to 10%, only 24% activity was remained. The trend of enzyme activity was consistent with the change of PAHs degradation rate in consortium 5H and revealed a high tolerance to salinity variation.

The PAH-degrading pathway of consortium 5H was proposed based on the results of intermediate detection and enzymatic activities. As shown in Fig. 4, the first step of phenanthrene degradation is predicted to be catalyzed by RHD which converts phenanthrene to cis-3,4-dihydroxy-3,4-dihydrophenanthrene, a process that has been widely found in PAH-degrading *Pseudomonas* (Natalia et al., 1999), *Sphingomonas* (Cho et al., 2006) and *Martelella* (Feng et al., 2012). Then by several metabolic steps, the cis-3,4-dihydroxy-3,4-dihydrophenanthrene was transferred to 1-hydroxy-2-naphthoic acid and 1,2-dihydroxynaphthalene. Then, as the detection of gentisic acid and catechol, the following pathway was proposed to be separated. Then, using G12O and C23O, the last benzene ring was cleaved. Both G12O and C23O were found in PAH-degrading pure cultures (Liu et al., 2004; Hesham et al., 2014; Huang

et al., 2015). Several studies reported the co-existing of multiple PAH-downstream pathways in one halophilic consortium. As reported by Wang et al., consortium CY-1 was able to degrade PAHs using G12O, C12O, C23O and P34O pathways (Wang et al., 2018). The intermediates 1-hydroxy-2-naphthoic acid and catechol were also detected in halophilic consortium Qphe (Dastgheib et al., 2012). Consortiums are thought to have higher functional redundancy than pure cultures since they own many pathways. Meanwhile, Cooperation was predicted to be the dominant mode of PAH elimination, particularly in hypersaline conditions (Debajyoti et al., 2016; Wang et al., 2018). Different bacteria species participating in the degradation process through a variety of downstream degradation pathways will greatly promote the degradation rate of PAHs and avoid the accumulation of intermediate metabolites.

3.3 Environmental factors affecting the PAH-degrading process in consortium 5H

The effect of salinity and pH on the PAH-degrading process of consortium 5H were also investigated in this study. As shown in Fig. 5A, consortium 5H was able to degrade 100% of phenanthrene under a wide range of salinity (1–10%). Under 1% salinity, consortium 5H showed the highest rate of phenanthrene degradation, 97.78% phenanthrene was removed in 3 days. The degradation ability of phenanthrene showed no significant difference when salinity increased to 3% and 5%. Meanwhile, under 10% salinity, consortium 5H was able to degrade nearly 100% phenanthrene in 7 days. However, only 30% of phenanthrene was removed under 20% salinity after 8 days incubation, indicating that the work of consortium 5H was severely restricted at such a high salinity. Compared with previous studies, Wang et al. enriched a halophilic consortium CY-1 capable of degrading phenanthrene at salinities ranging from 1–15% (Wang et al., 2018). Consortium enriched by Sami et al. was identified able to degrade 41.2% PAHs in 6 days (Mnif et al., 2017). Halotolerant consortium enriched by Arulazhagan was able to degrade about 90% of PAHs in 22 days under 20% salinity (Pugazhendi et al., 2017). Consortium 5H showed a relative good tolerance to salinity variation, especially at 1–5% salinity, the degradation rate was rapid.

As shown in Fig. 5B, no significant degradation of PAHs occurred when pH adjusted to 5. When pH increased to 6 and 7, about 95% of phenanthrene was removed in 5 days. With the increased of pH, the degradation rate of PAHs was slight limited. About 88% and 68% of phenanthrene were degraded when pH increased to 8 and 9. Consortium 5H was identified with no degradation ability when pH increased to 10. Therefore, consortium 5H was suitable for the PAH-degradation under neutral and week alkaline environment.

Furthermore, consortium 5H degraded phenanthrene effectively over a broad range of initial concentrations. As shown in Fig. 5C, consortium 5H completely degraded 50mg/L phenanthrene in 4 days. The degradation rate was about 97.4% when consortium 5H cultured 8 days under 400mg/L initial phenanthrene concentration. When combined with the results of the metabolic pathway, the consortium was able to effectively mineralize PAHs while accumulating no biotoxic intermediates.

3.4 Phytotoxicity of metabolic intermediates produced by consortium 5H

PAHs was investigated with significant biotoxicity to crops (Somtrakoon and Chouychai, 2013). Therefore, it was necessary to evaluate the metabolic intermediates produced by consortium 5H. The biotoxicity of the metabolic intermediates in PAH-degrading process were measured 1, 3, 5 and 7 days after incubation and the result was shown in Table 2. The generation rate, length of plumule and radical of seeds (*Cucumis sativus* and *Oryza sativa*) that immersed in distilled water were used as the control. The results showed that as the degradation process progressed, the phytotoxicity of metabolic intermediates decreased. One day after incubation, the generated metabolic intermediates showed obvious phytotoxicity on both *Cucumis sarivus* and *Oryza sativa* seeds. With the incubation process, the biotoxicity level decreased. The phytotoxicity of intermediates showed no significant difference with the distilled water 7 days after cultivation, indicating that no biotoxic intermediate was accumulated 7 days after degradation. The biotoxicity in the metabolic intermediate showed similar trend with intermediate produced by phenanthrene treatment by laccase (Wulandari et al., 2021) and an azo dye decolorizing consortium reported by Guo et al (Guo et al., 2021). The phytotoxicity text results show that consortium 5H has an environmental friendly potential for PAH elimination in hypersaline environments.

Table 2
Phytotoxicity studies of the formed metabolites 1, 3, 5 and 7 days after incubation.

Parameter	**Distill water**	**Metabolites**			
		1 day	**3 day**	**5 day**	**7 day**
Cucumis sativus					
Germination (%)	100	55	70	100	100
Plumule (cm)	4.49 ± 0.42	2.50 ± 0.32**	3.52 ± 0.30**	4.29 ± 0.33	4.43 ± 0.46
Radical (cm)	5.49 ± 0.39	2.83 ± 0.44**	3.65 ± 0.51**	4.58 ± 0.46**	5.46 ± 0.36
Oryza sativa					
Germination (%)	100	60	70	95	100
Plumule (cm)	3.58 ± 0.35	1.66 ± 0.49**	2.67 ± 0.28**	3.44 ± 0.37	3.63 ± 0.42
Radical (cm)	2.58 ± 0.18	1.71 ± 0.29**	1.97 ± 0.30**	2.37 ± 0.23*	2.67 ± 0.18
The number in each blank is the average ± standard deviation.					
** stands for the number with extremely significant difference with the control (P = 0.01).					
* stands for the number with significant difference with the control (P = 0.05).					

4. Conclusion

Under 5% salinity, the Consortium 5H enriched in this study showed a rapid rate of phenanthrene degradation. By high-throughput sequencing, this consortium was main composed of *Methylophaga*

(57.5%), *Marinobacter* (18.2%) and *Thalassospira* (15.2%). Using GC-MS analysis and enzymatic assay, consortium 5H was able to use RHD for the initial benzene ring activation and finally used C23O and G12O pathways for the mineralization of phenanthrene. Consortium 5H was identified with a high tolerance for salinity (1–10%) and was able to realize the degradation process under neutral to week alkaline (pH 6 to 9) conditions. Meanwhile, after 7 days of degradation, the metabolic intermediates displayed no substantial phytotoxicity as compared to distilled water. Therefore consortium 5H was identified with a high utilization potential of PAH-elimination under hypersaline encironments.

Declarations

Author contribution

This paper's co-first authors are Jiaqi Jin and Zuotao Zhang, who both contributed equally to the research. Jiaqi Jin enriched the consortium and studied the rate of deterioration under various conditions. Zuotao Zhang detected the degrading pathway and enzymatic activities. Lu Han modified the language. Chicheng Yan participated in the detection of phenanrhrene degradation. Xinxing Ding and Jiage Sun designed the whole experiment and helped to contrast the detection methods, Jingyu Cui joined in the experiment and recorded and analyzed the data. Chongyang Wang is the corresponding author. All authors contributed to this study and modified the manuscript.

Funding

This work was supported by Research and Practice Project of Higher Education Teaching Reform in Henan Province (2019SJGLX202) and Teaching Reform Project of Henan University (HDXJJG2020-104).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval: Not applicable.

Consent to participate: The authors have agreed with the content and all have given consent to publish

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests.

References

1. Arulazhagan, P., Vasudevan, N., 2009. Role of a moderately halophilic bacterial consortium in the biodegradation of polyaromatic hydrocarbons. Marine Pollution Bulletin 58, 256-262.

2. Arulazhagan, P., Vasudevan, N., 2011. Biodegradation of polycyclic aromatic hydrocarbons by a halotolerant bacterial strain *Ochrobactrum* sp. VA1. Marine Pollution Bulletin 62, 388-394.
3. Cho, H.Y., Woo, S.H., Park, J.M., 2006. Effects of intermediate metabolites on phenanthrene biodegradation. Journal of Microbiology and Biotechnology 16, 969-973.
4. Crampon, M., Bodilis, J., Portet-Koltalo, F., 2018. Linking initial soil bacterial diversity and polycyclic aromatic hydrocarbons (PAHs) degradation potential. Journal Of Hazardous Materials 359, 500-509.
5. Cui, Z., Xu, G., Gao, W., Li, Q., Yang, B., Yang, G., Zheng, L., 2014. Isolation and characterization of Cycloclasticus strains from Yellow Sea sediments and biodegradation of pyrene and fluoranthene by their syntrophic association with *Marinobacter* strains. International Biodeterioration & Biodegradation 91, 45-51.
6. Dastgheib, S., Amoozegar, M.A., Khajeh, K., Shavandi, M., Ventosa, A., 2012. Biodegradation of polycyclic aromatic hydrocarbons by a halophilic microbial consortium. Applied Microbiology & Biotechnology 95, 789-798.
7. Debajyoti, G., Shreya, G., Dutta, T.K., Youngho, A., 2016. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Frontiers in Microbiology 7, 1369.
8. Feng, T.c., Cui, C.z., Dong, F., Feng, Y.y., Liu, Y.d., Yang, X.m., 2012. Phenanthrene biodegradation by halophilic *Martelella* sp AD-3. Journal Of Applied Microbiology 113, 779-789.
9. Gauthier, M.J., Lafay, B., Christen, R., Fernandez, L., Bertrand, J.C., 1992. *Marinobacterhydrocarbonoclasticus* gen. nov., sp. nov., a New, Extremely Halotolerant, Hydrocarbon-Degrading Marine Bacterium. International journal of systematic bacteriology 42, 568-576.
10. Guangsu, X.U., Cui, Z., Zheng, L., Zang, J., Yang, B., Song, Y., Hou, W., 2013. Bacterial Interaction in the Biodegradation of High Molecular Weight Polycyclic Aromatic Hydrocarbons. Chinese Journal of Applied & Environmental Biology 19, 677-682.
11. Guo, G., Li, X., Tian, F., Liu, T., Yang, F., Ding, K., Liu, C., Chen, J., Wang, C., 2020. Azo dye decolorization by a halotolerant consortium under microaerophilic conditions. Chemosphere 244, 125510.
12. Guo, G., Liu, C., Hao, J., Tian, F., Ding, K., Zhang, C., Yang, F., Liu, T., Xu, J., Guan, Z., 2021. Development and characterization of a halo-thermophilic bacterial consortium for decolorization of azo dye. Chemosphere 272, 129916.
13. Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): a review. Journal of Hazardous Materials 169, 1-15.
14. Hedlund, B.P., Geiselbrecht, A.D., Staley, J.T., *Marinobacter* strain NCE312 has a *Pseudomonas*-like naphthalene dioxygenase. Fems Microbiology Letters, 47-51.
15. Hentati, D., Chebbi, A., Loukil, S., Kchaou, S., Godon, J.-J., Sayadi, S., Chamkha, M., 2016. Biodegradation of fluoranthene by a newly isolated strain of *Bacillusstratosphericus* from Mediterranean seawater of the Sfax fishing harbour, Tunisia. Environmental Science And Pollution Research 23, 15088-15100.

16. Hesham, E.L., Mawad, A., Mostafa, Y.M., Shoreit, A., 2014. Study of enhancement and inhibition phenomena and genes relating to degradation of petroleum polycyclic aromatic hydrocarbons in isolated bacteria. Microbiology 83, 599-607.
17. Hidalgo, K.J., Sierra-Garcia, I.N., Dellagnezze, B.M., de Oliveira, V.M., 2020. Metagenomic Insights Into the Mechanisms for Biodegradation of Polycyclic Aromatic Hydrocarbons in the Oil Supply Chain. Frontiers In Microbiology 11, 561506.
18. Huang, L., Hu, H., Tang, H., Liu, Y., Xu, P., Shi, J., Lin, K., Luo, Q., Cui, C., 2015. Identification and Characterization of a Novel Gentisate 1,2-Dioxygenase Gene from a Halophilic *Martelella* Strain. Scientific Reports 5, 14307.
19. Jamal, M.T., 2020. Enrichment of Potential Halophilic *Marinobacter* Consortium for Mineralization of Petroleum Hydrocarbons and Also as Oil Reservoir Indicator in Red Sea, Saudi Arabia. Polycyclic Aromatic Compounds.
20. Jiménez, N., Vinas, M., Guiu-Aragonés, C., Bayona, J.M., Albaigés, J., Solanas, A.M., 2011. Polyphasic approach for assessing changes in an autochthonous marine bacterial community in the presence of Prestige fuel oil and its biodegradation potential. Applied Microbiology & Biotechnology 91, 823-834.
21. Joaquim, V., María, N., Jelle, M., Dirk, S., Magdalena, G., 2010. Microbial community structure of a heavy fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. Fems Microbiology Ecology 73, 349-362.
22. Liu, Y., Jie, Z., Zhang, Z., 2004. Isolation and characterization of polycyclic aromatic hydrocarbons-degrading *Sphingomonas* sp. strain ZL5. Biodegradation 15, 205-212.
23. Mishamandani, S., Gutierrez, T., Berry, D., Aitken, M., 2016. Response of the bacterial community associated with a cosmopolitan marine diatom to crude oil shows a preference for the biodegradation of aromatic hydrocarbons. Environmental Microbiology 18.
24. Mnif, S., Chebbi, A., Mhiri, N., Sayadi, S., Chamkha, M., 2017. Biodegradation of phenanthrene by a bacterial consortium enriched from Sercina oilfield. Process Safety & Environmental Protection 107, 44-53.
25. Muangchinda, C., Rungsihiranrut, A., Prombutara, P., Soonglerdsongpha, S., Pinyakong, O., 2018. 16S metagenomic analysis reveals adaptability of a mixed-PAH-degrading consortium isolated from crude oil-contaminated seawater to changing environmental conditions. Journal of Hazardous Materials 357, 119-127.
26. Natalia, V., Balashova, and, Irina, A., Kosheleva, and, Nicolai, P., 1999. Phenanthrene metabolism by *Pseudomonas* and *Burkholderia* strains. Process Biochemistry 35, 291-296.
27. Patel, A.B., Shaikh, S., Jain, K.R., Desai, C., Madamwar, D., 2020. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. Frontiers in Microbiology 11, 562813.
28. Pugazhendi, A., Qari, H., Al-Badry Basahi, J.M., Godon, J.J., Dhavamani, J., 2017. Role of a halothermophilic bacterial consortium for the biodegradation of PAHs and the treatment of

petroleum wastewater at extreme conditions. International Biodeterioration & Biodegradation 121, 44-54.

29. Qasemian, L., Billette, C., Guiral, D., Alazard, E., Moinard, M., Farnet, A.-M., 2012. Halotolerant laccases from *Chaetomium* sp., Xylogone sphaerospora, and Coprinopsis sp isolated from a Mediterranean coastal area. Fungal Biology 116, 1090-1098.
30. Rodrigue, M., Elango, V., Curtis, D., Collins, A.W., Pardue, J.H., 2020. Biodegradation of MC252 polycyclic aromatic hydrocarbons and alkanes in two coastal wetlands. Marine Pollution Bulletin 157.
31. Rosselló-Mora, R., Lalucat, J., García-Valdés, E., 1994. Comparative biochemical and genetic analysis of naphthalene degradation among *Pseudomonasstutzeri* strains. Applied & Environmental Microbiology 60, 966.
32. Sanseverino, J., Applegate, B.M., King, J., Sayler, G.S., 1993. Plasmid-mediated mineralization of naphthalene, phenanthrene, and anthracene. Applied & Environmental Microbiology 59, 1931-1937.
33. Somtrakoon, K., Chouychai, W., 2013. Phytotoxicity of single and combined polycyclic aromatic hydrocarbons toward economic crops. Russian Journal of Plant Physiology 60, 139-148.
34. Wang, C., Guo, G., Huang, Y., Hao, H., Wang, H., 2017. Salt Adaptation and Evolutionary Implication of a *Nah*-related PAHs Dioxygenase cloned from a Halophilic Phenanthrene Degrading Consortium. Scientific Reports 7.
35. Wang, C., Huang, Y., Zhang, Z., Hao, H., Wang, H., 2019. Absence of the *nahG*-like gene caused the syntrophic interaction between *Marinobacter* and other microbes in PAH-degrading process. Journal of Hazardous Materials 384, 121387.
36. Wang, C., Huang, Y., Zhang, Z., Wang, H., 2018. Salinity effect on the metabolic pathway and microbial function in phenanthrene degradation by a halophilic consortium. Amb Express 8, 67.
37. Wang, X., Jin, D., Zhou, L., Zhang, Z., 2015. Draft Genome Sequence of *Ochrobactrumanthropi* Strain W13P3, a Halotolerant Polycyclic Aromatic Hydrocarbon-Degrading Bacterium. Microbiology Resource Announcements 3.
38. Wulandari, R., Lotrakul, P., Punnapayak, H., Amirta, R., Prasongsuk, S., 2021. Toxicity evaluation and biodegradation of phenanthrene by laccase from *Trametes polyzona* PBURU 12. 3 Biotech 11.
39. Xu, M., Wu, M., Zhang, Y., Zhang, H., Liu, W., Chen, G., Xiong, G., Guo, L., 2021. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial mixture. International Journal Of Environmental Science And Technology. 2, 1-12.
40. Yu, S.H., Ke, L., Wong, Y.S., Tam, N., 2005. Biodegradation of polycyclic aromatic hydrocarbons by a bacterial consortium enriched from mangrove sediments. Environment International 31, 149-154.
41. Zhou, H., Wang, H., Huang, Y., Fang, T., 2016. Characterization of pyrene degradation by halophilic *Thalassospira* sp strain TSL5-1 isolated from the coastal soil of Yellow Sea, China. International Biodeterioration & Biodegradation 107, 62-69.

Figures

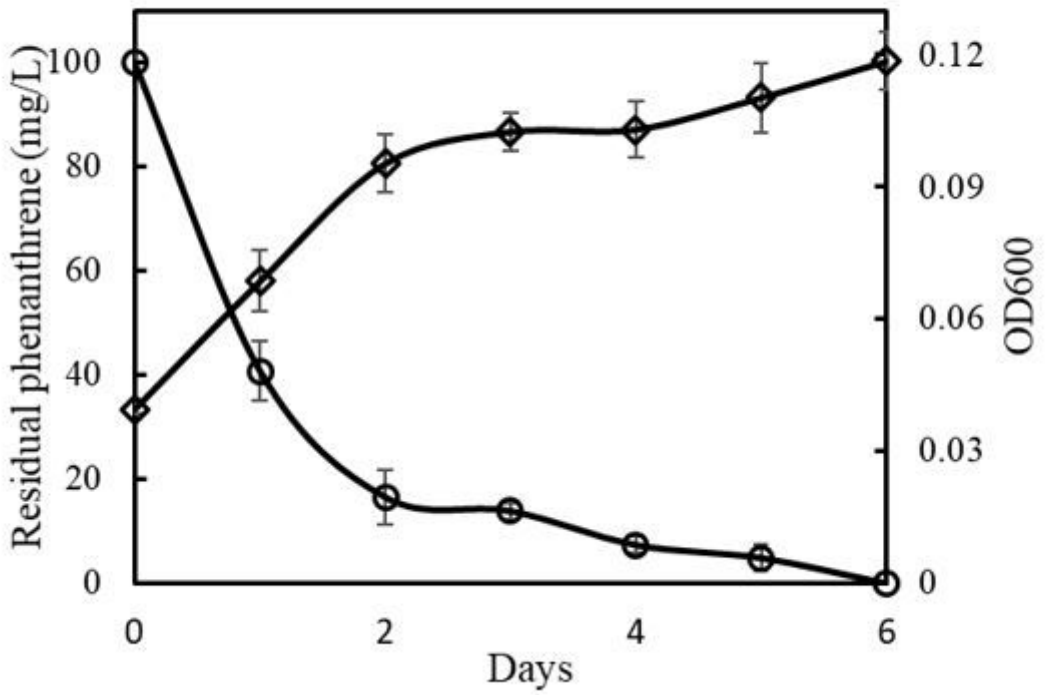

Figure 1

the phenanthrene degradation rate of consortium 5H and the cell growth curve.

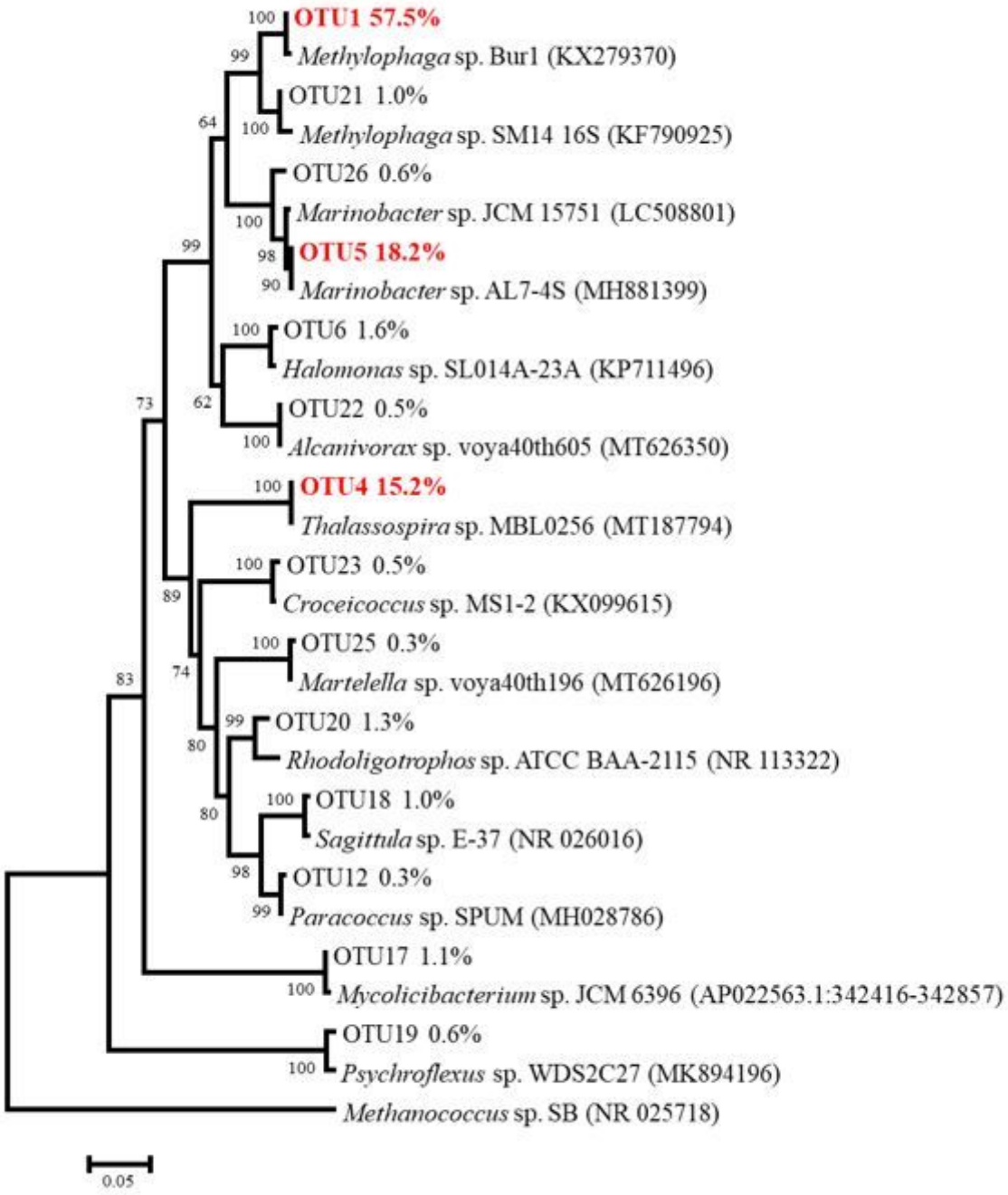

Figure 2

Community structure of consortium 5H in genera level. The physiological tree was established based on the OTUs separated on 97% identities. A reference sequence in NCBI database was selected for each genus that present in the consortium by BLAST. The physiological tree was established by MAGE 6.0 according to neighbor-joining methods in the booktstrap test (1000 replicates).

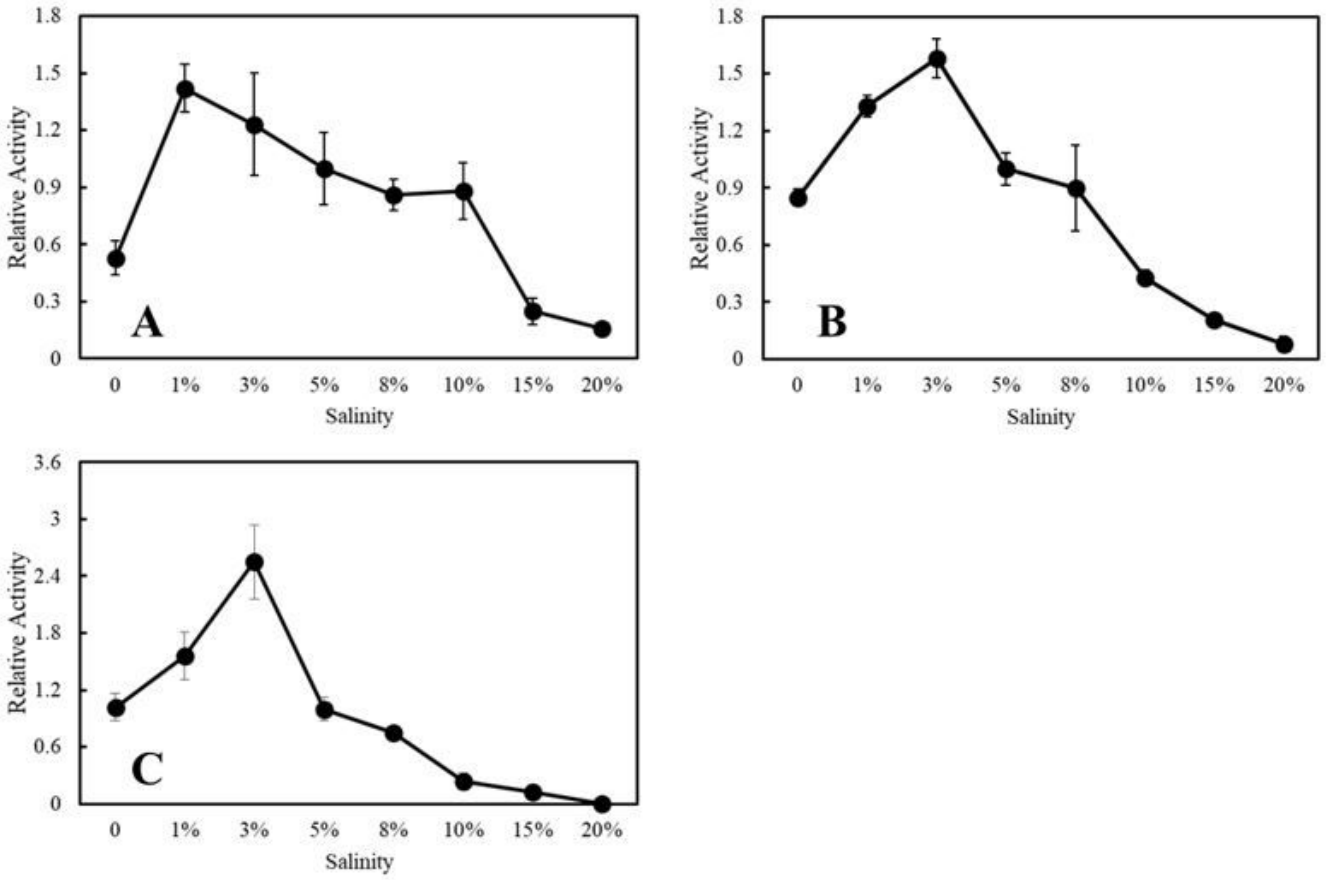

Figure 3

Activities of A RHD, B G12O and C C23O in consortium 5H under different salinities

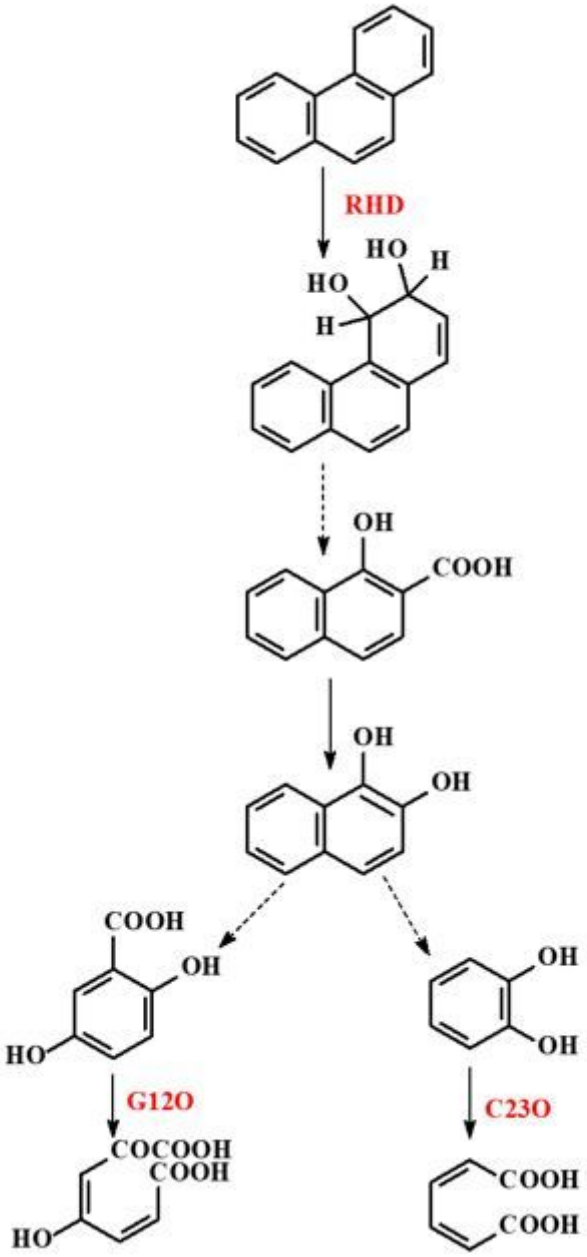

Figure 4

Metabolic pathway of PAHs degradation in consortium 5H. Solid arrows represented only one step in the process. Dotted arrows represented several steps in the process.

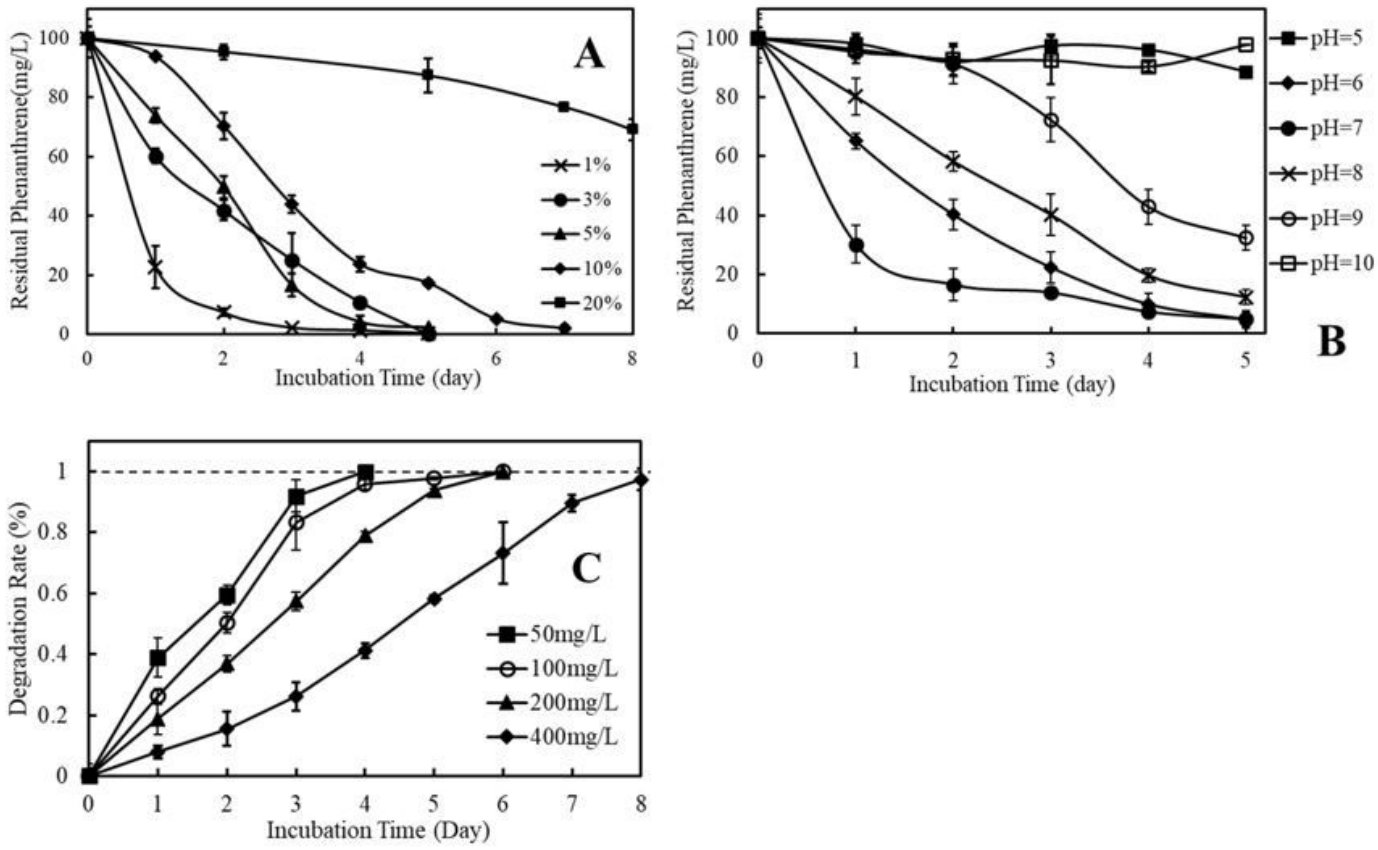

Figure 5

Effect of A salinity, B pH and C initial phenanthrene concentration on the PAHs degradation of consortium 5H